Safety evaluation of the food enzyme $\alpha$-amylase from<br>Aspergillus oryzae (strain DP-Bzb41)

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Abstract

The food enzyme $\alpha$-amylase (4-$\alpha$-D-glucan glucanohydrolase, EC 3.2.1.1) is produced with a non-
genetically modified Aspergillus oryzae (strain DP-Bzb41) by Danisco US Inc. (USA). The $\alpha$-amylase
food enzyme is intended to be used in baking, brewing, distilled alcohol production and starch
processing for the glucose syrup production. Based on the maximum use levels for baking and brewing
processes and individual data from the EFSA Comprehensive European Food Database, dietary
exposure to the food enzyme-Total Organic Solids (TOS) was estimated to be up to 2.59 mg TOS/kg
body weight (bw) per day. Since residual amounts of TOS are removed during distilled alcohol
production and by the purification steps applied during starch processing, dietary exposure for these
processes was not calculated. Genotoxicity tests did not raise a safety concern. The systemic toxicity
was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no
observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.
Comparison with the estimated dietary exposure, results in a margin of exposure of at least 386.
Similarity of the amino acid sequence to those of known allergens was searched and one match to
respiratory allergen was found (an amylase from another strain of A. oryzae). The Panel considered
that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by
dietary exposure cannot be excluded, but the likelihood is considered to be low. Based on the data
provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the
intended conditions of use.

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Keywords: food enzyme, $\alpha$-amylase, 4-$\alpha$-D-glucan glucanohydrolase, EC 3.2.1.1, Aspergillus oryzae,
non-genetically modified microorganism

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1. **Introduction**

   Article 3 of the Regulation (EC) No 1332/2008\(^1\) provides definition for ‘food enzyme’ and ‘food enzyme preparation’.

   ‘Food enzyme’ means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

   ‘Food enzyme preparation’ means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

   Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008\(^1\) on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008\(^2\) established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

   i) it does not pose a safety concern to the health of the consumer at the level of use proposed;

   ii) there is a reasonable technological need;

   iii) its use does not mislead the consumer.

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

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

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

1.1.1. **Background as provided by the European Commission**

   Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008\(^3\) on food enzymes.

   Five applications have been introduced by the company ‘Danisco US Inc.’ for the authorisation of the food enzymes glucan 1,4-\(\alpha\)-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh63), subtilisin from a genetically modified strain of *Bacillus subtilis* (DP-Ezx62), subtilisin from a genetically modified strain of *Bacillus subtilis* (DP-Ezx42), \(\alpha\)-amylase from *Aspergillus oryzae* (DP-Bzb41), and glucan 1,4-\(\alpha\)-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh38).

   Following the requirements of Article 12.1 of Regulation (EC) No 234/2011\(^3\) implementing Regulation (EC) No 1331/2008,\(^2\) 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.

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1. Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

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

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

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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 glucan 1,4-\(\alpha\)-glucosidase from a genetically modified strain of Trichoderma reesei (strain DP-Nzh63), subtilisin from a genetically modified strain of Bacillus subtilis (strain DP-Ezx62), subtilisin from a genetically modified strain of Bacillus subtilis (strain DP-Ezx42), \(\alpha\)-amylase from Aspergillus oryzae (strain DP-Bzb41), and glucan 1,4-\(\alpha\)-glucosidase from a genetically modified strain of Trichoderma reesei (strain DP-Nzh38) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission’s request to carry out the safety assessment of food enzyme \(\alpha\)-amylase from a non-genetically modified microorganism Aspergillus oryzae (strain DP-Bzb41).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme \(\alpha\)-amylase from a non-genetically modified microorganism A. oryzae (strain DP-Bzb41).

Additional information was requested from the applicant during the assessment process on 23 May 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, 2009) and following the relevant existing guidances of EFSA Scientific Committee.

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

3. Assessment

IUBMB nomenclature: alpha-Amylase
Systematic name: 4-\(\alpha\)-D-glucan glucanohydrolase
Synonyms: Amylase; Glycogenase; Endo-amylase; Taka-amylase A; 1,4-alpha-D-glucan glucanohydrolase
IUBMB No.: 3.2.1.1
CAS No.: 9000-90-2
EINECS No.: 232-565-6

The \(\alpha\)-amylase catalyses the hydrolysis of (1\(\rightarrow\)4)-\(\alpha\)-D-glucosidic linkages in polysaccharides (amylose and amylopectin), resulting in the generation of oligosaccharides. It is intended to be used in baking, brewing, distilled alcohol production, and starch processing for the glucose syrup production.

3.1. Source of the food enzyme

The food enzyme \(\alpha\)-amylase is produced with a non-genetically modified filamentous fungus A. oryzae (strain DP-Bzb41). It is deposited in the collection of [Redacted] with deposition number [Redacted].

The production strain has an extended genealogy, arising from an initial isolate made in Japan in the 1900s. This initial isolate has, in subsequent years, been subjected to [Redacted] intended to improve its fermentation characteristics and enzyme yield.

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4 Technical dossier/2nd submission/p. 36.
5 Technical dossier/2nd submission/p. 43–48.
6 Technical dossier/Additional data, 23 August 2019/Annex U.
7 Technical dossier/Additional data, 23 August 2019/Annex Y.
Evidence of the identity of the fungal strain used in the production of the food enzyme based on a phylogenomic approach was provided. A maximum likelihood phylogenetic tree based on the showed that the production strain is firmly located within the A. flavus and A. oryzae clade.

3.2. Production of the food enzyme

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

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is 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 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 food enzyme α-amylase consists of a single polypeptide chain of amino acids. The molecular mass of the food enzyme, calculated from its amino acid sequence is kDa. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis for the three batches for commercialisation and the batch used for toxicological testing showed a single major protein band corresponding to an apparent molecular mass of about kDa protein. No other enzymatic side activities were reported.

The in-house determination of α-amylase activity is based on the hydrolysis of a non-reducing-end blocked p-nitrophenyl maltoheptaoside (BPNPG7) substrate in the presence of excess levels of α-glucosidase and glucoamylase and is expressed in Sandstedt Kneen Blish Units (SKBU)/g. One SKBU is defined as the amount of enzyme required to release 1 l mol of p-nitrophenol per minute from BPNPG7 under the conditions described for the assay (pH 5.6, 25°C, reaction time 5 min).

The food enzyme has a temperature maximum around 50°C (pH 5.6) and a pH maximum around pH 5 (40°C). Thermostability was tested by the pre-incubation of the food enzyme at 55°C and at a pH of 4.6 for periods up 140 min. Enzyme activity was lost after incubation for 20 min under these conditions.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 29.73%. The average enzyme activity/mg TOS ratio of the three food enzyme batches for commercialisation is 304 SKBU/mg TOS.

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8 Technical dossier/Additional data, 23 August 2019/Annex X.
9 Technical dossier/2nd submission/p. 49–57; Technical dossier/1st submission/Annex K; Technical dossier/Additional data, 23 August 2019/Annex T.
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 J.
12 Technical dossier/1st submission/Annex H.
13 Technical dossier/Additional data, 23 August 2019/Annex 1/p. 1.
14 Technical dossier/2nd submission/p. 37; Technical dossier/1st submission/Annex E; Technical dossier/Additional data, 23 August 2019/Annex W.
15 Technical dossier/2nd submission/p. 40–41.
16 Technical dossier/1st submission/Annex D.
17 Technical dossier/2nd submission/p. 42.
18 Technical dossier/2nd submission/p. 41.
19 Technical dossier/2nd submission/p. 37; Technical dossier/2nd submission/Annex G; Technical dossier/1st submission/Annex F; Annex O; Annex C.
### 3.3.3. Purity

The lead content\textsuperscript{20} in the three commercial batches and in the batch 4 used for toxicological studies 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). In addition, the levels of arsenic, mercury and cadmium in the batch used for toxicological testing were each below the limits of detection (LODs) of the employed methodologies.\textsuperscript{21}

The food enzyme $\alpha$-amylase complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species\textsuperscript{22} are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram. No antimicrobial activity was detected in any of these batches\textsuperscript{23} (FAO/WHO, 2006).

The presence of a number of mycotoxins (total aflatoxin, aflatoxin B1, zearalenone, ochratoxin A, sterigmatocystin and fumonisins B1 + B2) was examined in the commercial batches\textsuperscript{23} and batch used for toxicological testing\textsuperscript{24}. All were found to be below the limits of detection\textsuperscript{25} of the applied analytical methods.

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Blumenthal, 2004; Frisvad et al., 2018). The applicant did not provide information on other secondary metabolites which maybe produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

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

### 3.3.4. Absence of the production strain\textsuperscript{26}

The absence of the production strain in the food enzyme was demonstrated in three independent batches of the food enzyme. No colonies were produced.

### 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 used in these studies is considered representative of the batches intended for commercialisation and thus suitable as a test item.

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\textsuperscript{20} Technical dossier/2nd submission/p. 39; Technical dossier/1st submission/Annex F and G.

\textsuperscript{21} Technical dossier/1st submission/Annex O; LOD: As = 3 mg/kg, Hg = 0.5 mg/kg, Cd = 0.5 mg/kg.

\textsuperscript{22} Technical dossier/2nd submission/p. 39; Technical dossier/1st submission/Annex O.

\textsuperscript{23} Technical dossier/2nd submission/Annex G_updated.

\textsuperscript{24} Technical dossier/1st submission/Annex O.

\textsuperscript{25} Technical dossier/1st submission/Annex G-updated; LOD for total aflatoxins and aflatoxin B1 < 5 µg/kg; LOD for zearalenone < 250 µg/kg; LOD for ochratoxin A < 10 µg/kg; LOD for sterigmatocystin < 100 µg/kg; LOD for fumonisin B1 + B2 < 2,000 µg/kg.

\textsuperscript{26} Technical dossier/1st submission/Annex F and Annex G.
3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a), and following Good Laboratory Practice (GLP) in four strains of *Salmonella Typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA, in the presence or absence of metabolic activation (S9). The treat and plate method was applied. Two experiments were carried out in triplicate using five different concentrations of the food enzyme (50, 150, 500, 1,500 and 5,000 μg total protein per plate, corresponding to 50, 151, 502, 1,507, 5,022 μg TOS/plate). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no increase in revertant colony numbers. Therefore, the Panel concluded that the food enzyme α-amylase did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in cultured human peripheral blood lymphocytes. Two experiments were performed. In the first experiment, applying 4 h treatment + 16 h recovery, the cultures were exposed to concentrations of 1,000, 2,500 and 5,000 μg total protein/mL (corresponding to 1,004, 2,511 and 5,022 μg TOS/mL), either in the presence or the absence of the S9. In the second experiment, applying continuous 20 h treatment without metabolic activation, the concentrations tested were 1,000, 2,500 and 5,000 μg total protein/mL (corresponding to 1,004, 2,511 and 5,022 μg TOS/mL). For all food enzyme concentrations used, the frequency of cells with chromosomal aberrations was similar to that of negative controls. No significant increase in polyploid or endoreplicated cells was observed. The Panel concluded that the food enzyme α-amylase did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested up to 5,000 μg total protein/mL (corresponding to 5,022 μg TOS/mL) under the experimental conditions employed for this study. Therefore, the Panel concluded that on the basis of the *in vitro* studies there is no concern for genotoxicity for the α-amylase tested.

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

A 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 Crl:CD(SD) rats received by gavage the food enzyme in doses of 250, 500 and 1,000 mg TOS/kg body weight (bw) per day. Controls received the vehicle (deionised water).

Two deaths were recorded; one low-dose male (day 57, gross and microscopic findings revealed severe lung and thymus haemorrhage) and one high-dose female (day 87, gross and microscopic findings demonstrated severe lung haemorrhage and pleural haemorrhage) died due to misdosing. Statistically significant decrease in body weight gain in high-dose males (days 29–36) as compared to controls was recorded. As no other statistically significant differences in body weight or body weight gain in either sex at any interval or time point were observed, the Panel considered this body weight gain change to reflect normal biological variation.

Statistically significant differences in food intake were recorded on several occasions in all treated groups as compared to controls. These differences occurred in opposite directions in males and females, were transient, not reflected in overall food intake (days 1–90) and food efficiency, and did not affect statistically significantly body weight gains. Therefore, these findings were considered by the Panel of not toxicological significance.

Statistically significant differences in food intake were recorded on several occasions in all treated groups as compared to controls. These differences occurred in opposite directions in males and females, were transient, not reflected in overall food intake (days 1–90) and food efficiency, and did not affect statistically significantly body weight gains. Therefore, these findings were considered by the Panel of not toxicological significance.

Among haematology parameters, a statistically significant decrease in absolute reticulocyte count in high-dose males (14.3%) and in mean corpuscular volume in low-dose females (3.6%) were recorded. These findings were not consistent among genders and considered by the Panel as not treatment-related because of lack of an apparent dose dependency.

There was a statistically significant increase in the prostate weight relative to body weight (29.5%) and relative to brain weight (29.1%) in low-dose males and in relative to body weight (28%) in

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27 Technical dossier/1st submission/Annex P; Technical dossier/Additional data, 23 August 2019/Annex V.
28 Technical dossier/1st submission/Annex Q.
29 Technical dossier/1st submission/Annex R.
high-dose males. The Panel considered these findings as incidental in the light of lack of the apparent dose response and microscopic changes in the prostate in these groups. No other statistically significant differences to controls were observed. Overall, the Panel identified a no observed adverse effect level (NOAEL) of 1,000 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 the food enzyme α-amylase produced with non-genetically modified microorganism A. oryzae strain DP-Bzb41 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, one match was found. The matching allergen was TAKA-amylase-A, an α-amylase from A. oryzae, which is also referred to as Asp o 21. The sequence identity to this respiratory allergen was 100%.

α-Amylase from A. oryzae is not identified as a food allergen by both the AllergenOnline and the WHO/IUIS allergen nomenclature sub-committee database. α-Amylase from A. oryzae (Brisman and Belin, 1991; Brisman, 2002) is described as an occupational respiratory allergen associated with baker’s asthma. However, several studies have shown that adults with occupational asthma to a food enzyme, as described for α-amylase, can commonly ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase, only a low number of case reports have been described focussed 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). The Panel noted that an allergic reaction upon oral ingestion of this α-amylase, produced with non-genetically modified A. oryzae (strain DP-Bzb41), in individuals respiratory sensitised to α-amylase cannot be ruled out, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU No 1169/2011) are used as raw materials in the growth medium of the production organism. However, during the fermentation process, these products will be degraded and utilised by the fungus for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids will be removed. Considering 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 considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions occurring is considered to be low.

### 3.5. Dietary exposure

#### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in four food processes. Intended uses and the recommended use levels are summarised in Table 2.
In baking processes, the food enzyme is added to flour during the preparation of dough. The α-amylase hydrolyses starch from granules that have been damaged during milling and releases fermentable sugars and dextrins. This reaction shortens the processing time and decreases dough viscosity. The latter facilitates the handling of the dough, resulting in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness).

In brewing processes, the food enzyme is added during the mashing and cereal cooking steps. The α-amylase is used to convert liquefied starch into a maltose-rich solution, improving the amounts of fermentable sugars and thus increasing brewing yield.

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. α-Amylase is intended to be used to convert liquefied starch into a maltose-rich solution, 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 typically added after the liquefaction step where it degrades gelatinised starch into dextrins.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production and starch processing for the production of glucose syrups (Documentation provided to EFSA No. 3). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation. In addition, taking into account the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon, the Panel also considers that the amount of TOS in the final glucose syrup will be removed to a similar degree.

### 3.5.2. Dietary exposure estimation

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

For the baking and brewing processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1/Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

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

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

| Food manufacturing process(a) | Raw material | Recommended dosage of the food enzyme |
|------------------------------|-------------|--------------------------------------|
| Baking process               | Flour       | 1–10 mg TOS/kg flour                 |
| Brewing process              | Cereals     | 55.8–558 mg TOS/kg cereal            |
| Distilled alcohol production | Cereals     | 23.2–232 mg TOS/kg cereal            |
| Starch processing for the production of glucose syrup | Starch     | 55.8–558 mg TOS/kg starch |

TOS: total organic solids.
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.

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.

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

| Population group | 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     | 0.002–0.028 (10) | 0.022–0.060 (14) | 0.027–0.058 (19) | 0.016–0.127 (18) | 0.055–0.591 (19) | 0.028–0.297 (18) |
| Min–max 95th percentile | 0.011–0.119 (8) | 0.053–0.102 (12) | 0.047–0.109 (19) | 0.029–0.697 (17) | 0.324–2.585 (19) | 0.085–1.184 (18) |

TOS: total organic solids.

3.6. Margin of exposure

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of up to 0.59 mg TOS/kg bw per day at the mean and 2.59 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 386.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production and starch processing for the glucose syrup production and the derived margin of exposure for baking and brewing processes, the Panel concluded that the food enzyme α-amylase produced with a non-genetically modified A. oryzae (strain DP-Bzb41) does not give rise to safety concerns under the intended conditions of use.
Documentation provided to EFSA

1) Technical dossier ‘Application for authorisation of Alpha-amylase from Aspergillus oryzae (DP-Bzb41) in accordance with Regulation (EC) No 1331/2008’ (the first and second submission). 10 March 2015. Submitted by Danisco US Inc. (USA).

2) Additional information. 22 August 2019. Submitted by Danisco US Inc. (USA).

3) Additional information on ‘Food enzyme removal during the production of cereal based distilled alcoholic beverages’ and ‘Food enzyme carry-over in glucose syrups’. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP). Unpublished document.

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Abbreviations

AMFEP Association of Manufacturers and Formulators of Enzyme Products
BPNPG7 p-nitrophenyl maltoheptaoside
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
CFU colony forming units
EINECS European Inventory of Existing Commercial Chemical Substances
FAO Food and Agricultural Organization of the United Nations
FoodEx The food classification and description system
HACCP Hazard Analysis and Critical Control Points
GLP Good Laboratory Practice
GM genetically modified
GMO genetically modified organism
GMP Good Manufacturing Practice
IUBMB International Union of Biochemistry and Molecular Biology
IUIS International Union of Immunological Societies
JECA Joint FAO/WHO Expert Committee on Food Additives
LOD limit of detection
MOE margin of exposure
NOAEL no observed adverse effect level
OECD Organisation for Economic Cooperation and Development
SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis
S9 metabolic activation
SKBU Sandstedt Kneen Blish Unit
TOS Total Organic Solids
WHO World Health Organization
Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2019.5899/suppinfo).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.
### Appendix B – Population groups considered for the exposure assessment

| Population   | Age range                                      | Countries with food consumption surveys covering more than one day |
|--------------|------------------------------------------------|------------------------------------------------------------------|
| Infants      | From 12 weeks on up to and including 11 months of age | Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom |
| Toddlers     | From 12 months up to and including 35 months of age     | Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom |
| Children\(^{(a)}\) | From 36 months up to and including 9 years of age       | Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom |
| Adolescents  | From 10 years up to and including 17 years of age       | Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom |
| Adults       | From 18 years up to and including 64 years of age       | Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom |
| The elderly\(^{(a)}\) | From 65 years of age and older            | Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom |

\(^{(a)}\): The terms ‘children’ and ‘the elderly’ correspond, respectively, to ‘other children’ and the merge of ‘elderly’ and ‘very elderly’ in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011).