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Safety evaluation of the food enzyme maltogenic amylase from genetically modified *Escherichia coli* (strain BLASC)

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Abstract

The food enzyme, a maltogenic amylase (glucan 1,4-α-maltohydrolase; EC 3.2.1.133), is produced with a genetically modified *Escherichia coli* strain BLASC by Advanced Enzyme Technologies Ltd. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. This maltogenic amylase is intended to be used in baking and brewing processes and starch processing for the production of glucose syrups. Residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups; consequently, dietary exposure was not calculated for this food process. For baking and brewing processes, based on the maximum use levels recommended for food processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–TOS was estimated to be up to 0.107 mg TOS/kg body weight (bw) per day. 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 at the highest dose tested of 838 mg TOS/kg bw per day that, compared with the estimated dietary exposure, resulted in a sufficiently high margin of exposure (at least 7,800). Similarity of the amino acid sequence to those of known allergens was searched and one match was found with respiratory allergen produced by *Aspergillus oryzae*. The Panel considered that, under the intended conditions of use, the risk for allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood of such reaction to occur is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, maltogenic amylase, glucan, 1,4-α-maltohydrolase, EC 3.2.1.133, *Escherichia coli*, 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[2] 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 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 an 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[2] on food enzymes.

Five applications have been introduced by the companies 'DSM Food Specialties B.V.' for the authorisation of the food enzyme carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG); 'Advanced Enzyme Technologies Ltd.' for the authorisation of the food enzymes maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC) and triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC); 'Danisco US Inc.' for the authorisation of the food enzyme glucan 1,4-α-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24), and 'Amano Enzyme Inc.' for the authorisation of the food enzyme catalase from *Aspergillus niger* (strain AE-CN).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[3], 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 1493/199, 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, p. 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, 11.03.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG), maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC), triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC), glucan 1,4-α-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24) and catalase from *Aspergillus niger* (strain AE-CN) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[2] 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 the food enzyme maltogenic amylase from a genetically modified *Escherichia coli* (strain BLASC).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme maltogenic amylase from a genetically modified *Escherichia coli* (strain BLASC).

Additional information was requested from the applicant during the assessment process on 17 January 2018, 27 June 2018, 16 October 2018 and 28 November 2018, and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) 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 with the methodology described in the 'CEF Panel statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment[4]

IUBMB nomenclature:	Glucan 1,4-α-maltohydrolase
Systematic name:	4-α-D-glucan α-maltohydrolase
Synonyms:	Maltogenic α-amylase
IUBMB No.:	EC 3.2.1.133
CAS No.:	160611-47-2
EINECS No.:	Not available

The enzyme maltogenic amylase catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in starch polysaccharides, to successively remove maltose from the non-reducing chain ends. It is intended to be used in baking and brewing processes and starch processing for the production of glucose syrups.

3.1. Source of the food enzyme[5]

The maltogenic amylase is produced with a genetically modified bacterium *Escherichia coli* strain BLASC.

The production strain *E. coli* BLASC is deposited in the American Type Culture Collection (ATCC) with deposit number [redacted][6] The identity of the strain was confirmed as *E. coli* through [redacted][7]

[4] Technical dossier/p. 3.
[5] Technical dossier/p. 18–25.
[6] Technical dossier/p. 18 and Additional data, 13 April 2018/Annex 5.
[7] Technical dossier/Annex I1.

3.1.1. Characteristics of the parental and recipient microorganisms[8]

The parental microorganism is the bacterium *E. coli* strain [redacted]

The recipient strain [redacted] was derived from the parental strain by [redacted] (see Section 3.1.2. below).

3.1.2. Characteristics of the introduced sequences[8]

[redacted]

3.1.3. Description of the genetic modification process[10]

The purpose of genetic modification was to enable the production strain to synthesise maltogenic amylase from [redacted]

The production strain contains [redacted] The absence of the antimicrobial resistance (AMR) genes used during the genetic modification was confirmed [redacted]

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 *E. coli* BLASC differs from the parental strain in the increased synthesis of maltogenic amylase from [redacted]

The introduced traits do not raise safety concern. No AMR genes are present in the genome of the production strain *E. coli* BLASC.[11]

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

3.2. Production of the food enzyme[12]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004[13], with food safety procedures based on Hazard Analysis and Critical Control Points (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, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation and release of the intracellular enzyme, 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

[8] Technical dossier/Annex M.

[redacted]

[10] Technical dossier/p. 22 and Annex M.

[11] Technical dossier/Additional data, 13 April 2018/Annex 6.

[12] Technical dossier/p. 26–31.

[13] Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.

which enzyme protein is retained while low molecular weight material passes the filtration membrane and is discarded. Finally, the food enzyme was spray-dried prior to analysis. 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.

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[14]

The maltogenic amylase produced with the genetically modified *E. coli* strain BLASC is a single polypeptide chain of 686 amino acids.[15] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 75.2 kDa.[16] The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis consistently showed one main protein band corresponding to a molecular mass of about 62 kDa.[17] No other enzymatic side activities were reported.[18]

The in-house determination of enzymatic activity is based on the hydrolysis of maltotriose and expressed in Maltogenic Amylase Unit (MAN U)/g. Hydrolysis of maltotriose results in the release of glucose (reaction conditions: pH = 5.0, T = 37°C, incubation time 30 min) which is measured by a commercial test based on the use of glucose dehydrogenase. One MAN U is defined as the amount of enzyme, which cleaves 1 μmol of maltotriose per minute under the standard assay conditions.[19]

The temperature profile of the maltogenic α-amylase has been measured from 25°C up to 90°C, at pH 5.0, with an optimum between 50 and 70°C. The pH profile has been measured from pH 3 up to 8, at 37°C, with an optimum pH 5.0. After a 2-hour pre-incubation, the enzyme activity was retained at temperatures up to 75°C; thereafter, the enzyme activity decreased resulting in a residual activity of 13% at 95°C.[20]

3.3.2. Chemical parameters[21]

Data on the chemical parameters of the food enzyme were provided for three food enzyme batches used for commercialisation. Batch 1 was used for toxicological tests (Table 1).

The average total organic solids (TOS) content of the three food enzyme batches for commercialisation was 84.73% (Table 1). The three food enzyme batches presented in Table 1 are dried food enzyme concentrates.[22]

The average enzyme activity/mg TOS ratio of the three food enzyme batches for commercialisation is 179.5 U/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches		
		1[(a)]	2	3
Maltogenic amylase activity	MAN U/g[(b)]	144,255	153,647	158,521
Protein	%	43.34	45.75	46.56
Ash	%	8.86	8.21	7.75
Water	%	7.35	6.97	6.68
Total organic solids (TOS)[(c)]	%	83.79	84.82	85.57
Activity/mg TOS	MAN U/mg TOS	172.16	181.14	185.25

(a): Batch used for the toxicological studies.
(b): MAN U/g: Maltogenic Amylase Unit (see Section 3.3.1).
(c): TOS calculated as 100% − % water − % ash.

[14] Technical dossier/p. 4–5 and 10–12.
[15] Technical dossier/p. 5.
[16] Technical dossier/Additional data, 7 November 2018.
[17] Technical dossier/p. 4–5; Additional data, 13 April 2018.
[18] Technical dossier/p. 10.
[19] Technical dossier/Annex A2.
[20] Technical dossier/Annex C, Table 5, p. 9.
[21] Technical dossier/p. 4.
[22] Technical dossier/p. 5 and Annex A3.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg 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). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LODs) of the employed methodologies.[23]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram.[24] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[25]

The presence of mycotoxins (aflatoxin B1, B2, G1, G2, M1, ochratoxin A, fumonisin B1, zearalenone, deoxynivalenol, T2-toxin, HT2-toxin, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine) was examined in three food enzyme batches. All were below the LODs of the applied analytical methods.[26]

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three batches of the enzyme preparation analysed in triplicate.[27] [redacted][28] [redacted][29] No colonies were produced.

The absence of recombinant DNA[30] in the food enzyme product was demonstrated by [redacted][31] [redacted][32] The LOD was [redacted][33] [redacted]

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 1 (Table 1) is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

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) in five strains of *Salmonella* Typhimurium (TA1535, TA97a, TA98, TA100, TA102) both in the presence and absence of metabolic activation.[34] The pre-incubation method was applied and two independent experiments were carried out in triplicate using five different concentrations of the food enzyme (50, 150, 500, 1,500 and 5,000 μg/plate, corresponding to 42, 126, 419, 1,257 and 4,190 μg TOS/plate). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, the numbers of the revertant colonies were comparable

[23] Technical dossier/p. 6 and Annex D; LODs: Pb = 0.1 mg/kg; As and Cd = 0.1 mg/kg; Hg = 0.25 mg/kg and Annex A1 (analytical method) and Additional data, 13 April 2018/Annex 1.
[24] Technical dossier/Annex E2.
[25] Technical dossier/p. 6; Annex E2 and Annex A1 (analytical method).
[26] Technical dossier/p. 6; Annex E1: LODs: aflatoxin B1, B2, G1 and G2 and ochratoxin A = 1 μg/kg; zearalenone = 5 μg/kg; deoxynivalenol = 25 μg/kg; T2-toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine = 100 μg/kg, Annex A1 (analytical method) and Additional data, 13 April 2018.
[27] Technical dossier/Annex N.
[28] Technical dossier/Annex N/p. 5.
[29] Technical dossier/Annex N/p. 6.
[30] Technical dossier/Additional data, 13 April 2018/Annex 7.
[31] Technical dossier/Additional data, 13 April 2018/Annex 7; Additional data, 7 November 2018/Annexure A.
[32] Technical dossier/Annex M/p. 120.
[33] Technical dossier/Additional data/23 May 2019/Annexure 1 and 2.
[34] Technical dossier/Annex J; Additional data, 13 April 2018/Annex 9 (certificate of S9).

to the values observed in the vehicle control group in any tested strain, both in the presence and absence of metabolic activation. The Panel concluded that the food enzyme maltogenic amylase did not induce gene mutations in bacteria under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Whole blood cultures were treated with 500, 1,500 and 5,000 μg food enzyme/mL (corresponding to 419, 1,257 and 4,190 μg TOS/mL), applying a short-term treatment (3 hours followed by 21 hours recovery) in the presence and absence of S9-mix, and a continuous treatment (24 hours) in the absence of S9-mix.[35] Cytotoxicity at the highest concentration, detected as a reduction in the mitotic index in relation to the vehicle control, was not higher than 40% and was observed in the short treatment in the presence of S9-mix. In all the tested conditions, no statistically significant increases in the frequency of structural chromosomal aberrations were observed in the treated cultures compared to the negative controls. No significant increase in polyploid or endoreplicated cells was observed. The Panel concluded that the food enzyme maltogenic amylase did not induce structural and numerical chromosomal aberrations in cultured human peripheral blood lymphocytes when tested up to 5,000 μg food enzyme/mL (corresponding to 4,190 μg TOS/mL) under the experimental conditions employed for this study.

The Panel concluded on the basis of the *in vitro* studies that there is no concern for genotoxicity for the food enzyme tested.

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

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD Test Guideline 408 (OECD, 1998) and following GLP.[35] Groups of 10 male and 10 female Wistar rats received via gavage the food enzyme at the doses of 210, 419, and 838 mg TOS/kg body weight (bw) per day with a dose volume of 5 mL/kg bw per day. Controls received the vehicle (water). Additionally, groups of 5 rats per sex which had received the vehicle or the test article at the high dose level (838 mg TOS/kg bw per day) were further observed for a period of 28 days following 90 days treatment, for assessment of reversibility, persistence and delayed occurrence of toxicity (recovery period).

No mortality was observed.

No significant haematological changes were seen in the main study. However, haematological examination after the 4-week recovery period revealed a statistically significant increase in white blood cells and lymphocytes and a decrease in neutrophils and eosinophils in the male recovery group as compared to the recovery vehicle controls. In the female recovery group, a statistically significant increased number of red blood cells was observed. These findings were considered by the Panel as incidental.

In clinical chemistry parameters, a statistically significant increase in albumin was observed in the mid-dose females on day 91 as compared to vehicle controls. In the female recovery group, a statistically significant increase in alkaline phosphatase and triglycerides and a decrease in sodium were observed in comparison with recovery vehicle controls. These changes were considered by the Panel as incidental and as such not treatment related due to lack of a dose–response relationship.

In the male recovery group, absolute and relative spleen weights and absolute liver weights were statistically significantly increased. In the female recovery group, absolute adrenal and thymus weights were statistically significantly decreased. These findings were considered by the Panel as not treatment related because of lack of dose–response relationship, they were not accompanied by histopathological findings and no changes were recorded at the end of treatment.

No other significant effects were observed.

Overall, the Panel identified a no observed adverse effect level (NOAEL) of 838 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[36]

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

[35] Technical dossier/Annex J.
[36] Technical dossier/Annex L.

The potential allergenicity of maltogenic amylase produced with the genetically modified *E. coli* strain BLASC 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 the criterion, one match was found. The matching allergen is Asp o 21, an α-amylase produced by *Aspergillus oryzae*, known as a respiratory, occupational allergen (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002).

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

Several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens 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 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). Such information has not been reported for maltogenic amylase. Overall, the likelihood of an allergic reaction upon oral ingestion of this maltogenic amylase, produced with the genetically modified *E. coli* strain BLASC, in individuals respiratory sensitised to α-amylase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. In the starch processing for the production of glucose syrups, experimental data showed a significant removal (> 99%) of protein. However, traces of proteins could be present in glucose syrup.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food manufacturing processes at the recommended use levels summarised in Table 2.

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

Food manufacturing process	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	8.47 mg TOS/kg flour
Starch processing for the production of glucose syrups	Starch	33.89 mg TOS/kg starch
Brewing processes	Malted barley	16.95 mg TOS/kg malted barley

TOS: total organic solids.

During baking, the maltogenic amylase food enzyme is added to the raw materials during the preparation of the dough. It is used to shorten the branched part of the amylopectin molecules during dough handling.

In starch processing for the production of glucose syrups, the maltogenic amylase food enzyme is added to the feed tank/mixing and secondary liquefaction steps. The hydrolysis of starch results in faster and improved processing, improved yields of high maltose syrup, and hydrolysis of maltotriose to maltose and glucose.

In brewing processes, the food enzyme is added to different steps of the process (cooking/liquefaction, mashing, before lautering or mash filtration, after fermentation). The benefits from use of maltogenic amylase are improved yields due to release of high amounts of maltose, decreased production time and wider choice of raw materials.

The data and information provided indicate that the food enzyme maltogenic amylase may not be fully inactivated during baking and brewing processes under the conditions of use.

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for the production of glucose syrups (Documentation provided to EFSA No. 7). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups (by > 99%). Consequently, the Panel considered it not necessary to estimate exposure arising from the use of the food enzyme in starch processing.

Owing to the substrate specificity of maltogenic amylase, no unintended reaction products in foods are to be expected under the proposed conditions of use.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups, a dietary exposure was not calculated.

For 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 all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

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

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

Population group	Estimated exposure (mg/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max of means (number of surveys)	0.002–0.024 (10)	0.018–0.051 (14)	0.021–0.049 (19)	0.012–0.032 (18)	0.011–0.037 (19)	0.011–0.023 (18)
Min–max of 95th percentiles (number of surveys)	0.009–0.101 (8)	0.045–0.086 (12)	0.040–0.092 (19)	0.025–0.065 (17)	0.027–0.107 (19)	0.023–0.051 (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 survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

+: uncertainty with potential to cause over-estimation of exposure; –: uncertainty with potential to cause underestimation of exposure.
FoodEx: a standardised food classification and description system; TOS: total organic solids.

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

3.6. Margin of exposure

A comparison of the NOAEL (838 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.002–0.051 mg TOS/kg bw per day at the mean and from 0.009–0.107 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure of at least 7,832.

4. Conclusions

Based on the data provided, the Panel concluded that this food enzyme maltogenic amylase produced with the genetically modified *E. coli* strain BLASC does not give rise to safety concerns under the intended conditions of use.

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) considers the food enzyme free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

1) Technical dossier 'Maltogenic amylase [redacted] produced by GM *Escherichia coli* (strain BLASC)'. 21 February 2015. Submitted by Advanced Enzyme Technologies, Ltd.
2) Additional information, 13 April 2018. Submitted by Advanced Enzyme Technologies, Ltd.
3) Additional information, 16 July 2018. Submitted by Advanced Enzyme Technologies, Ltd.
4) Additional information, 7 November 2018. Submitted by Advanced Enzyme Technologies, Ltd.
5) Additional information, 23 May 2019. Submitted by Advanced Enzyme Technologies, Ltd.
6) Summary report on GMM part. Delivered by Technical University of Denmark (DTU), Copenhagen, Denmark. 2017.
7) AMFEP (Association of Manufacturers and Formulators of Enzyme Products), 2017. Food enzyme carry-over in glucose syrups. 22 February 2017. Unpublished document.

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Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
AMR	antimicrobial resistance genes
ATCC	American Type Culture Collection
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
DTU	Technical University of Denmark
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO/WHO	Food and Agriculture Organization of the United States/World Health Organization
[redacted]	[redacted]
FoodEx	a standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
MAN U	Maltogenic Amylase Unit
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid
S9	metabolic activation
SDS–PAGE	sodium dodecyl sulfate–poly acrylamide gel electrophoresis
TOS	total organic solids

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

Information provided in this appendix is shown in an excel file (downloadable http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2019.5769/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).