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Safety evaluation of the food enzyme maltogenic amylase from a genetically modified *Bacillus subtilis* (strain NZYM-OC)

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Abstract

The food enzyme maltogenic amylase (glucan 1,4-a-maltohydrolase; EC 3.2.1.133) is produced with a genetically modified *Bacillus subtilis* strain NZYM-OC by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production microorganism and recombinant DNA. This maltogenic amylase is intended to be used in baking processes. Based on the maximum use levels recommended, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.649 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity in rats. The Panel identified a no observed adverse effect level at the mid-dose of 371 mg TOS/kg bw per day that, compared with the estimated dietary exposure, results in a sufficiently high margin of exposure (at least 570). Similarity of the amino acid sequence to those of known allergens was searched and three matches were found. 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 to occur 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.

Keywords: food enzyme, glucan 1, 4-α-maltohydrolase, 4-α-D-glucan α-maltohydrolase, EC 3.2.1.133, maltogenic amylase, *Bacillus subtilis*, 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 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 EU 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 the 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 on food enzymes.

Three applications have been introduced by the company "Novozymes A/S" for the authorisation of the food enzymes Lysophospholipase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-LP), Phospholipase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-PP) and Maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-OC), and one application by the company "Puratos NV sa" for the authorisation of the food enzyme Aqualysin 1 from a genetically modified strain of *Bacillus subtilis* (strain LMGS 25520).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the four 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 assessments on the food enzymes Lysophospholipase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-LP), Phospholipase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-PP), Maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-OC) and Aqualysin 1 from a genetically modified strain of *Bacillus subtilis* (strain LMGS 25520) 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 the food enzyme maltogenic amylase from a genetically modified strain of *B. subtilis* (strain NZYM-OC).

1.3. Information on existing authorisation and evaluations

The applicant reports that the Danish and French authorities have evaluated and authorised the use of maltogenic amylase obtained from genetically modified *B. subtilis* strain NZYM-OC in baking processes. The Danish authority specifies the conditions of use, which were up to a level of 200 SDMU/kg of flour for baking.

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 *B. subtilis* (strain NZYM-OC).

Additional information was sought from the applicant during the assessment process in response to a request from EFSA sent on 13 July 2017, 30 April 2018 and 25 June 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), `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 from the 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:	Glucan 1,4-α-maltohydrolase
Systematic name:	4-α-D-glucan α-maltohydrolase
Synonyms:	Maltogenic α-amylase; glucan 1,4-α-maltohydrolase
IUBMB No:	EC 3.2.1.133
CAS No:	160611-47-2

Maltogenic amylase catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in amylose, amylopectin and related glucose polymers, liberating maltose units from the non-reducing end of the polymer chain. It is intended to be used in baking processes.

3.1. Source of the food enzyme

The maltogenic amylase is produced with a genetically modified *B. subtilis* strain NZYM-OC, which is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany) with the deposit number [redacted].[4]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *Bacillus subtilis*, strain [redacted].[5] The identity of the parental strain has been confirmed [redacted].

Lack of toxic potential was demonstrated in a derivative strain of [redacted], which was an intermediate in the construction of the recipient strain, by a cytotoxicity test using Chinese hamster ovary cells [redacted].

The recipient strain, [redacted], has been developed from the parental strain [redacted].

During the development of the recipient strain [redacted].

3.1.2. Characteristics of the introduced sequences

The maltogenic amylase encoding gene [redacted].

3.1.3. Description of the genetic modification process

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

[4] Technical dossier/Annex 4/Annex A2.
[5] Technical dossier/Annex 4/Annex A1.
[6] Technical dossier/Annex 4/Section 1.1.10 and Annexes B1 to B11.
[7] Technical dossier/Annex 4/Section 1.2.1.
[8] Technical dossier/Annex 4/Annex C1.
[9] Technical dossier/Annex 4/Annex 1.3.1
[10] Technical dossier/Annex 4/Annex C2.
[11] Technical dossier/Annex 4/Section 1.3.

The recipient strain [redacted]

3.1.4. Safety aspects of the production strain

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

The production strain *B. subtilis* NZYM-OC is [redacted] and is not cytotoxic. It differs from the recipient strain [redacted] only in its capacity to produce the maltogenic amylase enzyme from [redacted]. The presence and the location of the enzyme gene were confirmed by Southern analysis.[12] The phenotypic stability of the *B. subtilis* NZYM-OC strain was confirmed by its capacity to produce a constant level of the enzyme maltogenic amylase in relation to the TOS in three independent batches of the food enzyme (Table 1) and its genetic stability was demonstrated by Southern analysis with DNA isolated from end-of-production samples from three different batches.[13]

The absence of antibiotic resistance genes used during the genetic modification was confirmed by Southern analysis of the production strain NZYM-OC [redacted][14]

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[15], 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, 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, and final sterile filtration.[16] 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

The maltogenic amylase is a single polypeptide of 686 amino acids.[17] The molecular mass, derived from the amino acid sequence, was calculated to be 75.1 kDa.[17] The sodium dodecyl sulfate–

[12] Technical dossier/Annex 4/Annex D1.
[13] Technical dossier/Annex 4/Annex D3.
[14] Technical dossier/Annex 4/Annexes D2.1 and D2.2.
[15] 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.
[16] Technical dossier/Section 3.2.1.2.5.
[17] Technical dossier/p. 33 and Annex 1.

polyacrylamide gel electrophoresis (SDS–PAGE) analysis consistently showed one major protein band in all batches, migrating slightly below the 66-kDa reference protein.[18] The food enzyme was tested for lipase, protease and glucoamylase activities, which were not detected. No other enzymatic side activities were reported.[19]

The in-house determination of maltogenic amylase activity is based on the hydrolysis of maltotriose to maltose and glucose (pH 5.0, 37°C, 30 min). Glucose is quantified using a glucose hexokinase assay. The enzyme activity is expressed in Sweet Dough Maltogenic Units (SDMU)/g. One SDMU is defined as the amount of enzyme that catalyses the hydrolysis of 100 μmol maltotriose/minute under the defined assay conditions.[20]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 60°C (pH 5.5) and a pH optimum around pH 5.0 (30°C). Thermostability of the maltogenic amylase was tested after a pre-incubation of the food enzyme for 30 min (pH 5.5) at different temperatures. Under the conditions (pH 5.5) of the applied temperature stability assay, the maltogenic amylase activity decreased rapidly above 70°C, showing no residual activity above 90°C.[21]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological testing (Table 1). The average total organic solids (TOS) content of the three food enzyme batches used for commercialisation was 6.6% (range 5.7–7.3%). The average enzyme activity/TOS ratio of the commercial food enzyme batches was 1.8 SDMU/mg TOS.

Table 1: Compositional data provided of the food enzyme[(d)]

Parameter	Unit	Batches			
		1	2	3	4[(a)]
Maltogenic amylase activity	SDMU/g batch[(b)]	132	111	116	80.9
Protein	%	4.0	4.3	3.9	2.25
Ash	%	0.5	0.6	0.5	2.8
Water	%	92.8	92.1	93.8	86.5
Total organic solids (TOS)[(c)]	%	6.7	7.3	5.7	10.7
Maltogenic amylase activity/mg TOS	SDMU/mg TOS	2.0	1.5	2.0	0.76

(a): Batch used for the toxicological tests.
(b): SDMU/g: Sweet Dough Maltogenic Units/g (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.
(d): Technical dossier/p. 34, p. 59–60 and Additional data August 2018.

3.3.3. Purity

The food enzyme complies with the specification for lead (not more than 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 of the employed methodologies.[22,23]

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 are not more than 30 colony forming units per gram.[24] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[25]

[18] Technical dossier/p. 35.
[19] Technical dossier/p. 40–41.
[20] Technical dossier/p. 38 and Annex 3.01.
[21] Technical dossier/p. 39–40 an Annex 9.
[22] LODs: Pb = 0.5 mg/kg; As = 0.1 mg/kg, Cd = 0.05 mg/kg, Hg = 0.03 mg/kg.
[23] Technical dossier/p. 35–36 and Additional data August 2018.
[24] Technical dossier/p. 37 and Additional data August 2018.
[25] Technical dossier/p. 36 and Additional data August 2018.

3.3.4. Viable cells and DNA of the production strain

The absence of the production microorganism in the food enzyme was demonstrated [redacted] Three independent production batches were analysed in triplicate.[26]

No recombinant DNA was detected in three independent batches in triplicate by polymerase chain reaction (PCR) test for amplification of a [redacted][27]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian micronucleus test and a repeated dose 90-day toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern[28] as the batches used for commercialisation, but has lower purity, and thus 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, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2 uvrA pKM 101 in the presence or absence of metabolic activation, applying the treat-and-plate assay.[29] Two experiments were carried out using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/plate, corresponding to ca. 124, 248, 495, 991, 1,981 and 3,963 μg TOS/plate). No increase in the mean number of revertant colonies was observed at any tested concentration in any test strain with or without S9-mix.

The Panel concluded that the food enzyme maltogenic amylase did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2008) and following GLP in peripheral blood human lymphocytes.[30] Two experiments were performed applying a 3 + 21 h treatment in the presence and absence of S9-mix, and a continuous 24 + 24 h treatment without S9-mix. The cultures were exposed to the food enzyme at concentrations of 3,000, 4,000 and 5,000 μg food enzyme/mL (corresponding to ca. 321, 428 and 535 μg TOS/mL). No significant cytotoxicity was observed after treatments both in the presence and absence of S9-mix. No statistically significant increase in the frequency of binucleated cells with micronuclei (MNBN) was observed, except for 3,000 μg food enzyme/mL (corresponding to ca 321 μg TOS/mL) in the 3 + 21 h treatment without S9-mix. Although the effect was observed in both replicate slides it fell within historical vehicle control (normal) ranges and it is not considered biologically relevant.

The Panel concluded that the food enzyme maltogenic amylase did not induce an increase in the frequency of MNBNs in cultured human lymphocytes under the test conditions employed for 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.[31] Groups of 10 male and 10 female Crl:CD Sprague–Dawley rats received by gavage the food enzyme in doses corresponding to 112, 371 and 1,124 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

[26] Technical dossier/Annex 4/Annex E1.
[27] Technical dossier/Annex 4/Annex E2.
[28] Technical dossier/p. 59.
[29] Technical dossier/Annex 7.01.
[30] Technical dossier/Annex 7.02.
[31] Technical dossier/Annex 7.03.

There were two prematured sacrifices for welfare reasons in the control female group due to misdosing in week 1 and week 9, respectively. Only a female killed in the first week of the study was replaced.

Transient overactivity was observed immediately after dosing after week 8 in some high-dose animals, in a lesser extent, in the mid-dose group. Transient chin rubbing on the cage bedding occurred in four high-dose males on day 5, and on few occasions after week 8 in mid- and high-dose animals. These findings were considered not-adverse by the Panel.

The haematological investigation revealed a dose-dependent decrease in counts of total leucocytes, and of lymphocytes, eosinophils, monocytes and large unstained cells which reached statistical significance in high-dose females as compared to controls. Differential leucocyte counts of the high-dose male group showed a similar tendency but were not statistically significant. The Panel considered that these changes should be regarded as a treatment-related effect and the high dose considered a lowest observed adverse effect level (LOAEL).

Other statistically significant differences to controls in haematological parameters were increased mean corpuscular haemoglobin concentration and decreased platelet counts in high-dose males, and shortened activated partial thromboplastin times in low-dose females. As these changes were recorded in one sex and with no apparent dose relationship, they were considered by the authors of the study to present a normal biological variation. The Panel concurred with this view.

Among clinical chemistry parameters differences to controls included a statistically significant increased plasma glucose concentration in the mid- and high-dose males, plasma potassium concentrations in high-dose males, plasma creatinine concentrations in all female groups. Furthermore, in all female groups, a lower activity of aspartate amino transferase was observed and the difference to controls reached statistical significance in the high-dose. As no dose relationship was observed, the most of individual values were within the background range and the differences were small when compared to controls these findings were considered by the authors of the study to be of no toxicological concern. The Panel agreed with this view.

For organ weights the only difference to controls was a statistically significant increase in relative kidney weight in the high-dose males. This finding was considered by the Panel of no toxicological concern, since it was not accompanied by any gross or microscopic changes and similar difference was not observed in females.

No other effects attributable to the treatment were observed.

Overall, the Panel identified a no observed adverse effect level (NOAEL) at the mid dose of 371 mg TOS/kg bw per day, based on the reduction of total white blood cell counts in the high-dose females.

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 maltogenic amylase produced with the genetically modified *B. subtilis* strain NZYM-OC was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, three matches were found. The matching allergens are: Asp o 21, an α-amylase produced by *Aspergillus oryzae*; Asp f 13, a serine protease produced by *Aspergillus fumigatus*; and Sch c 1, a glucoamylase produced by *Schizophyllum commune*.[32]

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

Alpha-amylase from *A. oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002), serine protease from *A. fumigatus* (Kurup et al., 2002) and glucoamylase from *S. commune* (Toyotome et al., 2014) are known as occupational respiratory allergens associated with asthma. However, 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). Taking into account the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in literature that focused on allergic reactions upon oral exposure to α-amylase in individuals

[32] Technical dossier/Annex 8.

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 glucoamylase and serine protease. Therefore, it can be concluded that an allergic reaction upon oral ingestion of maltogenic amylase, produced by the genetically modified B. subtilis strain NZYM-OC, in individuals respiratory sensitised to α-amylase, serine protease produced by *A. fumigatus* or glucoamylase produced by *S. commune* cannot be excluded, but the likelihood is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011[33]) are used as raw materials ([redacted]) in the growth medium of the production organism. However, during the fermentation process, these substances will be degraded and utilised by the bacterium for cell growth, cell maintenance and production of enzyme. 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 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 baking processes at recommended use levels up to 100 SDMU/kg flour for cakes and 25 SDMU/kg flour for bread, corresponding to 54.5 and 13.6 mg TOS/kg flour, respectively.[34,35]

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, thus contributing to slow down staling of the bakery product.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is expected that the maltogenic amylase is inactivated during baking processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1) 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 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 2 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).

[33] 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.

[34] Technical dossier/Additional data September 2017

[35] The original intended uses proposed by the applicant were: 'Baking processes' and 'Other cereal-based processes'. In the course of the evaluation, the applicant informed EFSA about withdrawal of the intended use in 'Other cereal-based processes'. In addition, the applicant changed the use level of the food enzyme in 'Baking process' from up to 100 SDMU/kg flour to up to 100 SDMU/kg flour for cakes and 25 SDMU/kg flour for bread.

Table 2: Summary of estimated dietary exposure to the food enzyme–TOS in six population groups

Population group	Estimated exposure (mg/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.010–0.152 (10)	0.115–0.326 (14)	0.131–0.315 (19)	0.071–0.201 (18)	0.054–0.125 (19)	0.053–0.111 (18)
Min–max 95th percentile (number of surveys)	0.059–0.649 (8)	0.287–0.555 (12)	0.257–0.591 (19)	0.160–0.409 (17)	0.118–0.245 (19)	0.106–0.194 (18)

TOS: total organic solid.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA Opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact Exposure to food enzyme–TOS
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 from baking processes, including bread, was calculated using the TOS indicated for cakes	+
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 likely to contain the food enzyme	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solid.
+: uncertainty with potential to cause over-estimation 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 over-estimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (371 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.010–0.326 mg TOS/kg bw per day at the mean and from 0.059 to 0.649 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) of at least 572.

4. Conclusions

Based on the data provided, the Panel concludes that the food enzyme maltogenic amylase produced with the genetically modified *B. subtilis* strain NZYM-OC does not give rise to safety concerns under the intended conditions of use.

The 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 produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-OC)". December 2014. Submitted by Novozymes A/S.
2) Additional information, September 2017. Submitted by Novozymes A/S.
3) Additional information, May 2018. Submitted by Novozymes A/S.
4) Additional information, August 2018. Submitted by Novozymes A/S.
5) Summary report on GMM part for maltogenic amylase produced by *Bacillus subtilis* strain NZYM-OC. January 2016. Delivered by DTU (Copenhagen, Denmark).
6) Summary report on genotoxicity and subchronic toxicity study related to Maltogenic amylase produced with a strain of *Bacillus subtilis* (strain NZYM-OC) by Novozymes A/S. November 2015. Delivered by FoBiG (Freiburg, Germany).

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Abbreviations

bp	base pairs
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes, Processing Aids
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EC	Enzyme Commission
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	lowest observed adverse effect level
LOD	limit of detection
MNBN	binucleated cells with micronuclei

MOE	margin of exposure
[redacted]	[redacted]
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SDMU	Sweet Dough Maltogenic Units
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 here).

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: The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure

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).