Safety evaluation of food enzyme glucan 1,4-α-maltohydrolase produced with a genetically modified Bacillus subtilis (strain MAM)

Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean Pierre J. P. Cravedi, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, et al.

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Safety evaluation of food enzyme glucan 1,4-α-maltohydrolase produced with a genetically modified *Bacillus subtilis* (strain MAM)

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Abstract

The food enzyme considered in this opinion is a glucan 1,4-α-maltohydrolase (maltogenic α-amylase; EC 3.2.1.133) produced with the genetically modified *Bacillus subtilis* strain MAM by the company DSM Food Specialties B. V. The food enzyme contains neither the production microorganism nor recombinant DNA; therefore, no environmental risk assessment is required. However, the Panel emphasises that this conclusion only covers the food enzyme recovered via filter press. The glucan 1,4-α-maltohydrolase is intended for use in baking processes. Based on the maximum use levels recommended and individual consumption data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.175 mg TOS/kg body weight (bw) per day in European populations. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. A no observed adverse effect level (NOAEL) was derived (986 mg TOS/kg bw per day for both males and females), which, compared with the dietary exposure, results in a sufficiently high margin of exposure. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens; one match was found. However, the Panel considered that there are no indications for food allergic reactions to this glucan 1,4-α-maltohydrolase by dietary exposure. No safety concerns were identified in relation to the genetic modifications, the manufacturing process, the compositional data provided, as well as the exposure, allergenicity and systemic toxicity assessments. However, owing to the incompleteness of the genotoxicity data, the Panel is not able to conclude on the safety of the food enzyme.

Keywords: food enzyme, maltohydrolase, glucan 1, 4-α-maltohydrolase, EC 3.2.1.133, maltogenic α-amylase, *Bacillus subtilis*, genetically modified microorganism

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1. Introduction

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

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

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

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

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

All food enzymes currently on the 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 an approval via a Union list.

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union 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.

Two applications have been introduced by the companies Novozymes A/S and DSM Food Specialties B. V. for the authorisation of the food enzymes Xylanase from a genetically modified strain of Aspergillus oryzae (strain NZYM-FA) and Glucan 1,4-α-maltohydrolase from a genetically modified strain of *Bacillus subtilis* (strain MAM), respectively.

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[2] implementing Regulation (EC) No 1331/2008[3], the Commission has verified that the two applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes Xylanase from a genetically modified strain of Aspergillus oryzae (strain NZYM-FA) and Glucan 1,4-α-maltohydrolase from a genetically modified strain of *Bacillus subtilis* (strain MAM) in accordance with the article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[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] 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.

[3] 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.

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 glucan 1,4-α-maltohydrolase produced with *Bacillus subtilis* (strain MAM) submitted by DSM Food Specialties B. V.

1.3. Information on existing authorisations and evaluations

The applicant reports that the Russian food authorities have evaluated and authorised the use of a glucan 1,4-α-maltohydrolase from a genetically modified *B. subtilis* strain MAM 14 for a number of food manufacturing processes.

2. Data and methodologies

2.1. Data

The applicant submitted a dossier supporting the application for authorisation of the food enzyme glucan 1,4-α-maltohydrolase produced with a genetically modified *B. subtilis* strain MAM. The food enzyme is intended to be used in baking processes.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009b) and following the relevant Guidances from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA, 2009a) has been followed by the CEF Panel 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

3.1. Technical data

3.1.1. Identity of the food enzyme

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.

3.1.2. Chemical parameters

The glucan 1,4-α-maltohydrolase (maltogenic α-amylase) produced with the genetically modified *B. subtilis* strain MAM consists of a single polypeptide of 719 amino acids, including a signal peptide of 33 amino acids, which is cleaved off during secretion of the enzyme protein. The molecular mass of the enzyme protein of 66 kDa was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, which was also used to investigate the protein homogeneity of the food enzyme. The gels presented showed one main protein band and some minor protein bands of lower molecular mass. No enzymatic side activities have been reported by the applicant.

Data on the chemical parameters and the protein homogeneity of the food enzyme were provided for four food enzyme batches, three batches to be used for commercialisation and one batch used for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 6.3%; the values ranged from 4.7% to 8.4%.

The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation was 205 New Maltogenic Amylase Units (NMAU)/mg TOS; the values ranged from 176 to 249 NMAU/mg TOS (Table 1).

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Glucan 1,4-α-maltohydrolase activity	NMAU/g batch[(b)]	14,800	14,200	8,950	10,455
Protein	%	3.8	3.4	2.6	3.8
Ash	%	2.0	1.1	1.2	1.0
Water	%	89.6	93.2	94.1	91.9
Total organic solids (TOS)[(c)]	%	8.4	5.7	4.7	7.1
Activity/mg TOS	NMAU/mg TOS	176	249	190	147

(a): Batch used for the toxicological studies.
(b): NMAU: New Maltogenic Amylase Units (see Section 3.1.3).
(c): TOS calculated as 100% – % water – % ash – % diluents.

The lead content on the three commercial batches and the batch used for toxicological studies was below 2 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).

No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).

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 *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram.

The applicant has provided information on the identity of the antifoam agent used and the method used for analysis. Taking into account the nature and properties of the antifoam agent, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers its use as of no safety concern.

The compositional data provided for the food enzyme batches are considered sufficient.

3.1.3. Properties of the food enzyme

The food enzyme catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides like starch, removing successively maltose units from the non-reducing ends of the chains.

The enzymatic activity is determined on the basis of an in-house testing using a maltotriose standard and expressed in NMAU/g (reaction conditions: pH = 5.0, T = 37°C, incubation time 5 min). The enzymatic hydrolysis of maltotriose results in release of glucose, which is quantitatively measured using the Ecoline S + Glucose Hexokinase kit. The released glucose is converted into gluconate-6-P in two steps, in which NADH is formed. The resulting increase in absorbance at 340 nm is a measure for the amount of released glucose and, accordingly, a measure of the maltogenic α-amylase activity. One NMAU is defined as the amount of enzyme that produces 1 μmol glucose per minute using maltotriose substrate under the defined assay conditions.

The food enzyme has been characterised regarding its temperature and pH profiles. The glucan 1,4-α-maltohydrolase is active up to 90°C (with an optimum around 65°C) and within a pH range of 3.5–6.5 (with an optimum around pH 5). The enzyme shows 75% residual activity at approximately 80°C after 30 min, and no activity at temperatures above 90°C.

3.1.4. Information on the source material

3.1.4.1. Information on the genetically modified microorganism

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

According to the CEF Guidance, the certificate of deposit of the production strain in a public validated culture collection should be provided. The applicant deposited the glucan 1,4-α-maltohydrolase production strain *B. subtilis* MAM only in the [redacted] under code [redacted]. The Panel noted that this would not allow a verification of the strain independently of the company.

3.1.4.2. Characteristics of the parental and recipient microorganisms

[redacted]

3.1.4.3. Characteristics of the donor microorganisms

[redacted]

3.1.4.4. Description of the genetic modification process

[redacted]

3.1.4.5. Safety aspects of the genetic modification

[redacted]

3.1.5. Manufacturing process

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[4] and in accordance with current Good Manufacturing Practice (GMP). The manufacturing process is certified according to Food Safety Systems Certification 22000 (FSSC 22000). A data set related to the manufacturing process including a list of raw materials used and a production flow process from fermentation and downstream processes was provided.

The food enzyme is produced by a pure culture of *B. subtilis* MAM in a contained, submerged, fed-batch fermentation system with conventional process controls in place. The identity and purity of the culture are checked at each transfer step from frozen vials to the end of fermentation.

The downstream processing includes a pretreatment, recovery, purification and concentration. The pretreatment comprises killing of the cells [redacted]. Upon request by the Panel, the applicant provided data on the use of these agents in the fermentation broth. However, their concentrations in the food enzyme were not given. Therefore, a conclusion cannot be reached on compliance of the food enzyme with the requirements of Regulation (EC) No 1333/2008[5] [redacted]

According to the applicant, only the filter press procedure is currently applied in regular production. However, the applicant emphasises that the use of microfiltration is maintained as an option for further production. Data for this recovery route were not provided.

[redacted]

[4] 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, p. 3–21.

[5] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

The absence of DNA only relates to the recovery route involving the application of filter press. The respective data referring to the use of microfiltration or centrifugation have not been provided by the applicant.

The production strain could not be detected in samples from three fermentations taken after the chemical killing process. No recombinant DNA was detected by PCR in three batches from the filter press recovery route.

The Panel considered the information provided on the raw materials as sufficient. The Panel also considered the information on the manufacturing process as sufficient as long as the filter press is used. The Panel noted that for the manufacturing processes involving microfiltration, the absence of the recombinant DNA in de food enzyme cannot be assessed due to absence of data.

3.1.6. Safety for the environment

The production strain and its recombinant DNA were not detected in the final product, when obtained via the filter press recovery route. Therefore, no environmental risk assessment is required (EFSA GMO Panel, 2011). Due to the lack of analytical data provided, the Panel is not able to conclude on the final product if obtained by microfiltration.

3.1.7. Case of need and intended conditions of use

As proposed by the applicant, the food enzyme is intended to be used in baking processes at an intended use level of up to 15.6 mg TOS/kg flour.

The food enzyme is added to the raw materials during the preparation of dough. It is used to hydrolyse starch and related polysaccharides, thus contributing to reduce the viscosity of the dough. The decrease in dough viscosity facilitates the handling of the dough, resulting in more uniform products with improved crumb resilience and cohesiveness.

3.1.8. Reaction and fate in food

The enzyme maltogenic α-amylase catalyses the hydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides like starch and glycogen, thus removing successively maltose residues from the non-reducing ends of the chains, resulting in the production of maltose and oligosaccharides of different lengths. These reaction products are naturally present in starch-containing foods. Based on the substrate specificity of the maltogenic amylase, no unintended reaction products are to be expected in foods.

According to the data provided on the thermostability, it is anticipated that the maltogenic α-amylase is inactivated during baking processes under the intended conditions of use.

3.2. Dietary exposure

Exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment of the food process, covered in this opinion, involved selection of relevant food groups and application of process and technical conversion factors (Appendix B). These input data were subjected to a stakeholder consultation through open calls,[6] and adjusted in accordance with feedback received.

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (hereafter the EFSA Comprehensive Database[7]) has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011a).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be made with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing

[6] http://www.efsa.europa.eu/en/data/call/161110
[7] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

to subjects possibly underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database is the best available source of food consumption data across Europe.

Food consumption data from the population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Appendix A).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

3.2.2. Exposure assessment methodology

Chronic exposure was calculated based on individual consumption, averaged over the total survey period, excluding surveys with only one day per subject. 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, 2011a).

The exposure per FoodEx category (Appendix B) was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of survey days and normalised for individual body weight (bw), resulting in an individual average exposure/day per kg bw for the survey period. This was done for all individuals in the survey and per age class, resulting in distributions of individual average exposure per survey and age class. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class.

3.2.3. Exposure to food enzyme–TOS according to the intended use proposed by the applicant

Exposure to the food enzyme–TOS was based on intended use and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Section 3.1.8). Food enzyme–TOS exposure was calculated from foods produced involving a baking process.

Relevant food groups and/or individual foods were selected from the Comprehensive Database and were assumed to always contain the food enzyme–TOS at the maximum recommended use level. This will result in an overestimation of exposure to food enzyme–TOS.

To facilitate matching of the reported use levels for baking processes with foods identified in the Comprehensive Database, the selected foods were disaggregated to ingredient level as appropriate, and converted into the corresponding raw material, i.e. flour, via the application of conversion factors (Appendix B). For example, consumption of 100 g of bread was converted into an intake of 70 g flour (recipe fraction of 0.7) and then multiplied by 15.6 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 1.09 mg TOS/100 g bread.

Exposure to the food enzyme–TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight (kg bw) separately for each individual in the database. Table 2 provides an overview of the derived exposure estimates. The average and 95th percentile exposure to the food enzyme-TOS per age class, country and survey are reported in Appendix C – Table C.1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix C – Table C.2.

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

Population group	Estimated exposure (mg/kg bw 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)	0.011–0.048 (6)	0.041–0.099 (10)	0.043–0.093 (18)	0.026–0.062 (17)	0.019–0.038 (17)	0.018–0.033 (14)
Min–max 95th percentile (number of surveys)	0.063–0.134 (5)	0.093–0.166 (7)	0.080–0.175 (18)	0.046–0.123 (17)	0.036–0.074 (17)	0.034–0.059 (14)

bw: body weight.

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

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

3.3. Toxicological data

Table 1 shows that the food enzyme batch 4 used for the toxicological assays has the lowest specific activity (enzyme activity/mg TOS), which indicates a slightly lower purity than the commercial batches, and thus can be considered as a 'worst-case' scenario. Consequently, on the basis of the data provided, batch 4 is considered cruder than the three batches for commercialisation and its use for toxicological testing is considered suitable.

3.3.1. Genotoxicity

3.3.1.1. Bacterial reverse mutation test

To investigate the potential of the maltogenic α-amylase to induce gene mutations in bacteria, a bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP). Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the direct plate incorporation method. Two experiments were carried out using eight different concentrations of the food enzyme, appropriate positive control chemicals and sterile deionised water as a negative control. The highest concentration tested was 5,000 μg/plate, corresponding to 355 μg TOS/plate. All positive controls induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative controls were within the historical control ranges. Upon treatment with the food enzyme, there was no increase in revertant colony numbers above control values in any of the *S.* Typhimurium strains, with or without metabolic activation.

In *E. coli* WP_2*uvrA*, a reproducible, dose-related increase in the number of revertants was observed in the absence of S9-mix, with values above the historical control data range at 3,330 μg/plate,

corresponding to 236 μg TOS/plate. Compared to the solvent control, 1.9- and 2.1-fold increase in the number of revertants was recorded at this concentration in two experiments.

These results could be due to the concentrations of tryptophan in the test item, exceeding the critical concentrations used in the plate incorporation assay and thus leading to a false positive outcome. To rule out this possibility, the applicant was invited to repeat the test applying the 'treat and plate' assay.

The applicant did not submit the requested additional data. The Panel considered the data available as not sufficient to conclude on genotoxicity.

3.3.1.2. *In vitro* mammalian chromosome aberration test

The *in vitro* chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Duplicate cultures of whole blood were treated with culture medium (vehicle control), the food enzyme or appropriate positive controls (mitomycin C and cyclophosphamide, in the absence or presence of S9-mix, respectively). Based on the results of a dose range finding test, two independent experiments were performed 48 h after mitogen stimulation, following three treatment schedules: 3 + 21 h in the presence and absence of S9-mix, 24 + 0 and 48 + 0 h without S9-mix. The cultures were exposed to three concentrations of the food enzyme (1,000, 3,300 and 5,000 μg food enzyme/mL, corresponding to ca. 71, 234 and 355 μg TOS/mL). Two hundred metaphases per concentration were analysed for chromosomal aberrations. The positive controls induced statistically significant increases in the frequency of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S9-mix. No cytotoxicity was observed after short treatment in the presence or absence of S9-mix; in both continuous treatment experiments a dose-dependent decrease in mitotic index (MI) was observed, up to 56% and 44% of the negative control at the highest dose (at 24 + 0 and 48 + 0 h, respectively). Frequencies of numerical and structural chromosomal aberrations were comparable to the negative controls at any dose tested; The Panel concluded that the food enzyme did not induce chromosomal aberrations under the experimental conditions applied in this study.

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

The repeated dose 90-day oral toxicity study with the food enzyme (batch 4 in Wistar (WU) rats) was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Doses of 1,394, 4,169 or 13,887 mg food enzyme/kg bw per day, equivalent to 99, 296 or 986 mg TOS/kg bw per day, at a volume of 13.9 mL/kg bw per day were given to 10 rats/sex by oral gavage. The vehicle MilliQ-water served as a negative control.

The food enzyme did not have any effect on general health and growth, body weight and food consumption. No treatment-related changes were observed in haematology, clinical chemistry, urinalysis, organ weights and organ weight ratios. Gross and histopathology examination did not reveal any treatment-related changes. In the functional observation tests, a number of endpoints of the neurological examination showed statistically significant changes (hind limb foot splay, hind limb grip strength, horizontal counts and time, ambulatory counts and stereotypical time). However, on the basis of historical control data the Panel concluded that the changes in functional observations were incidental.

Overall, the Panel derived a no observed adverse effect level (NOAEL) at the high dose level of 986 mg TOS/kg bw per day for both males and females.

A comparison of the NOAEL (986 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates in six human population groups of 0.004–0.098 mg TOS/kg bw per day at the mean and from 0.033 to 0.175 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) of 5,634, indicating that there is no concern.

3.4. Allergenicity

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

The allergenicity of glucan 1,4-α-maltohydrolase produced with the genetically modified *B. subtilis* strain MAM was assessed by comparing its amino acid sequence with those of known allergens according to the EFSA Scientific opinion on the assessment of allergenicity of GM 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 criterion, one match was found with Asp o 21, an α-amylase from *A. oryzae*.

α-amylase from *A. oryzae* is described as an occupational respiratory allergen associated with baker's asthma (Brisman and Belin, 1991; Brisman, 2002). However, several studies have shown that adults with occupational asthma to a food enzyme (like 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). Taking into account the wide use of α-amylase as a food additive, only a low number of case reports have been described in literature focusing on allergic reactions upon oral exposure to α-amylase in individuals' respiratory sensitised to α-amylase (Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of glucan 1,4-α-maltohydrolase, produced with the genetically modified strain of *B. subtilis* strain MAM, in individuals respiratory sensitised to α-amylase cannot be excluded, but the likelihood is considered to be low. In addition, no information is available on oral sensitisation or elicitation reactions of this glucan 1,4-α-maltohydrolase.

The potential cross-reactivity of food enzymes was studied by Bindslev-Jensen et al. (2006) There were no indications of cross reactivity between 19 different commercial food enzymes and the main allergens represented by 400 patients (allergic to inhalation allergens, food allergens, allergens of bee or wasp or drugs) included in this study.

Taken together, the Panel considers that there are no indications for allergic reactions by dietary exposure to the food enzyme glucan 1,4-α-maltohydrolase produced with the genetically modified *B. subtilis* strain MAM.

Conclusions

No safety concerns were identified in relation to the genetic modifications, the manufacturing process, the compositional data, the dietary exposure, as well as the allergenicity assessment. Regarding the toxicological studies, the repeated dose 90-day oral toxicity study also did not raise a safety concern. However, owing to the incompleteness of the genotoxicity data, the Panel is not able to conclude on the safety of the food enzyme glucan 1,4-α-maltohydrolase produced with *B. subtilis* strain MAM by the company DSM Food Specialties B. V. In addition, the Panel emphasises that the assessment covers only the food enzyme recovered via filter press.

The Panel noted that data are lacking to conclude on compliance of the food enzyme with the requirements of Regulation 1333/2008[8] [redacted]

Documentation provided to EFSA

1) Dossier 'Glucan 1,4-alpha-maltohydrolase from a genetically modified strain of *Bacillus subtilis* (MAM)', October 2013. Submitted by DSM Food Specialties B.V.
2) Additional information was received from DSM in June 2014.
3) Additional information was received from DSM in August 2014.
4) Additional information was received from DSM in August 2015.
5) Additional information was received from DSM in May 2017.
6) Additional information was received from DSM in October 2017.

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[8] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

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Abbreviations

Bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CFU	colony Forming Units
CHO	Chinese Hamster Ovary
FAO	Food and Agricultural Organization
FSSC	Food Safety Systems Certification
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
MOE	Margin of exposure
[redacted]	[redacted]
NADH	nicotinamide adenine dinucleotide-hydrogen (reduced)
NMAU	New Maltogenic Amylase Units
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
rRNA	ribosomal ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate-poly acrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization
WU	Wistar

Appendix A – 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, Finland, Germany, Italy, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, 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, 2011a).

Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01	Grains and grain-based products (unspecified)	0.8	1	15.6
A.01.03	Grain milling products (unspecified)	1	1	15.6
A.01.03.001	Wheat milling products (unspecified)	1	1	15.6
A.01.03.001.001	Wheat flour, brown	1	1	15.6
A.01.03.001.002	Wheat flour, Durum	1	1	15.6
A.01.03.001.003	Wheat flour, white	1	1	15.6
A.01.03.001.004	Wheat flour, wholemeal	1	1	15.6
A.01.03.001.005	Graham flour	1	1	15.6
A.01.03.001.006	Wheat flour, gluten free	1	1	15.6
A.01.03.001.014	Wheat starch	1.2	1	15.6
A.01.03.002	Rye milling products (unspecified)	1	1	15.6
A.01.03.002.001	Rye flour, gluten free	1	1	15.6
A.01.03.002.002	Rye flour, light	1	1	15.6
A.01.03.002.003	Rye flour, medium	1	1	15.6
A.01.03.002.004	Rye flour, wholemeal	1	1	15.6
A.01.03.003	Buckwheat milling products (unspecified)	1	1	15.6
A.01.03.003.001	Buckwheat flour	1	1	15.6
A.01.03.004	Corn milling products (unspecified)	1	1	15.6
A.01.03.004.001	Corn flour	1	1	15.6
A.01.03.004.003	Corn starch	1.3	1	15.6
A.01.03.005	Oat milling products (unspecified)	1	1	15.6
A.01.03.005.002	Oat flour	1	1	15.6
A.01.03.005.004	Oat starch	1.2	1	15.6
A.01.03.006	Rice milling products (unspecified)	1	1	15.6
A.01.03.006.001	Rice flour	1	1	15.6
A.01.03.006.002	Rice flour white	1	1	15.6
A.01.03.006.003	Rice flour, instant	1	1	15.6
A.01.03.006.004	Rice starch	1.2	1	15.6
A.01.03.007	Spelt milling products	1	1	15.6
A.01.03.008	Other milling products (unspecified)	1	1	15.6
A.01.03.008.001	Amaranth flour	1	1	15.6
A.01.03.008.002	Barley flour	1	1	15.6
A.01.03.008.003	Chapatti flour	1	1	15.6
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	15.6
A.01.03.008.005	Millet flour	1	1	15.6
A.01.03.008.007	Sorghum flour	1	1	15.6
A.01.04	Bread and rolls (unspecified)	1	0.7	15.6
A.01.04.001	Wheat bread and rolls	1	0.7	15.6
A.01.04.002	Rye bread and rolls	1	0.7	15.6
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	15.6
A.01.04.004	Multigrain bread and rolls	1	0.7	15.6
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	15.6
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	15.6

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01.04.005.002	Crisp bread, rye, light	1	0.9	15.6
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	15.6
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	15.6
A.01.04.005.005	Rusk, light	1	0.9	15.6
A.01.04.005.006	Rusk, wholemeal	1	0.9	15.6
A.01.04.005.007	Pita bread	1	0.7	15.6
A.01.04.005.008	Matzo	1	0.9	15.6
A.01.04.005.009	Tortilla	1	0.7	15.6
A.01.04.006	Other bread	1	0.7	15.6
A.01.04.007	Bread products	1	0.7	15.6
A.01.07	Fine bakery wares (unspecified)	1	0.5	15.6
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	15.6
A.01.07.001.001	Beignets	1	0.15	15.6
A.01.07.001.002	Buns	1	0.7	15.6
A.01.07.001.003	Cake from batter	1	0.25	15.6
A.01.07.001.004	Cheese cream cake	1	0.24	15.6
A.01.07.001.005	Cheese cream sponge cake	1	0.24	15.6
A.01.07.001.006	Chocolate cake	1	0.24	15.6
A.01.07.001.007	Chocolate cake with fruits	1	0.24	15.6
A.01.07.001.008	Cream cake	1	0.24	15.6
A.01.07.001.009	Cream cheese cake	1	0.24	15.6
A.01.07.001.010	Cream custard cake	1	0.24	15.6
A.01.07.001.011	Cream custard sponge cake	1	0.24	15.6
A.01.07.001.012	Croissant	1	0.5	15.6
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	15.6
A.01.07.001.014	Croissant, filled with cream	1	0.5	15.6
A.01.07.001.015	Croissant, filled with jam	1	0.5	15.6
A.01.07.001.016	Croquembouche	1	0.15	15.6
A.01.07.001.017	Doughnuts	1	0.24	15.6
A.01.07.001.018	Clair	1	0.15	15.6
A.01.07.001.019	Flan	1	0.5	15.6
A.01.07.001.020	Fruit cake	1	0.6	15.6
A.01.07.001.021	Fruit pie	1	0.15	15.6
A.01.07.001.022	Cheese pie	1	0.15	15.6
A.01.07.001.023	Fruit tart	1	0.15	15.6
A.01.07.001.024	Gingerbread	1	0.6	15.6
A.01.07.001.025	Gougere	1	0.15	15.6
A.01.07.001.026	Kringles	1	0.25	15.6
A.01.07.001.027	Nut cream cake	1	0.24	15.6
A.01.07.001.028	Pancakes	1	0.25	15.6
A.01.07.001.029	Profiterole	1	0.15	15.6
A.01.07.001.030	Pyramid cake	1	0.25	15.6
A.01.07.001.031	Rhubarb flan	1	0.15	15.6
A.01.07.001.032	Scone	1	0.5	15.6
A.01.07.001.033	Sponge dough	1	0.25	15.6
A.01.07.001.034	Sponge cake	1	0.25	15.6
A.01.07.001.035	Sponge cake roll	1	0.25	15.6

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01.07.001.036	Muffins	1	0.25	15.6
A.01.07.001.037	Waffles	1	0.25	15.6
A.01.07.001.038	Apple strudel	1	0.15	15.6
A.01.07.001.039	Cream-cheese strudel	1	0.24	15.6
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	15.6
A.01.07.001.041	Croissant from puff pastry	1	0.6	15.6
A.01.07.001.042	Brioche	1	0.5	15.6
A.01.07.001.044	Lebkuchen	1	0.6	15.6
A.01.07.001.045	Dumpling	1	0.5	15.6
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	15.6
A.01.07.001.047	Marzipan pie	1	0.25	15.6
A.01.07.001.048	Baklava	1	0.15	15.6
A.01.07.002	Biscuits (cookies)	1	0.9	15.6
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	15.6
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	15.6
A.01.07.002.003	Biscuits, cream filling	1	0.81	15.6
A.01.07.002.004	Biscuits, fruit filling	1	0.81	15.6
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	15.6
A.01.07.002.006	Butter biscuits	1	0.81	15.6
A.01.07.002.007	Biscuit, iced	1	0.81	15.6
A.01.07.002.008	Speculaas	1	0.9	15.6
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	15.6
A.01.07.002.010	Biscuits, oat meal	1	0.9	15.6
A.01.07.002.011	Biscuits, spelt meal	1	0.9	15.6
A.01.07.002.012	Biscuits, salty	1	0.9	15.6
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	15.6
A.01.07.002.014	Sticks, salty	1	0.81	15.6
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	15.6
A.18.04.001	Find bakery products for diabetics	1	0.5	15.6
A.19.01.001	Sandwich and sandwich-like meal	1	0.32	15.6
A.19.01.002	Pizza and pizza-like pies	1	0.3	15.6

TOS: total organic solids.
(a): Available at see http://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf
(b): Derived from publically available recipe information, and/or food label information (such as Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).

Appendix C – 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.2018.5168/suppinfo).

The file contains two sheets, corresponding to two tables.

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

Table C.2: The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure.