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Safety evaluation of the food enzyme endo-1,4-β-xylanase and β-glucanase from *Disporotrichum dimorphosporum* strain DXL

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Abstract

The food enzyme with xylanases (4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and glucanases active against β-1,4 linkages is produced with the non-genetically modified fungus *Disporotrichum dimorphosporum* strain DXL by DSM Food Specialities B.V. The food enzyme is intended to be used in brewing processes. Based on the maximum use level and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.167 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 (NOAEL) at the highest dose of 199 mg TOS/kg bw per day that, compared with the estimated dietary exposure, results in a high Margin of Exposure of at least 1,100. Similarity of amino acid sequences of the identified xylanases and β-glucanases to those of known allergens was searched. No matches were found for two endo-1,4-β-glucanases and two endo-1,4-β-xylanases. However, for a third endo-β-1,4-glucanase the search resulted in matches with three mite protein sequences. While incidental cases of allergic reactions to endo-1,4-β-xylanases and β-glucanases have been reported after inhalation in respiratory sensitised individuals in the workplace, no allergic reactions to xylanases or β-glucanases have been reported in the literature after oral exposure. The Panel considered that, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this 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, 4-β-D-xylan xylanohydrolase, EC 3.2.1.8, β-glucanases, *Disporotrichum dimorphosporum*

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

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

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Two applications have been introduced by the companies 'Amano Enzyme Inc.' and 'DSM Food Specialties B.V.' for the authorisation of the food enzymes leucyl aminopeptidase from *Rhizopus oryzae* (strain AE-PER), and endo-1,4-β-xylanase and β-glucanase from *Disporotrichum dimorphosporum* (strain DXL).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the 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 assessments on the food enzymes leucyl aminopeptidase from *Rhizopus oryzae* (strain AE-PER), and

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

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

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

endo-1,4-β-xylanase and β-glucanase from *Disporotrichum dimorphosporum* (strain DXL) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme endo-1,4-β-xylanase and β-glucanase from a non-genetically modified microorganism *D. dimorphosporum* strain DXL.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase and β-glucanase from a non-genetically modified microorganism *D. dimorphosporum* strain DXL.

Additional information was requested from the applicant during the assessment process on 28 May 2015, 21 September 2017, 26 March 2019 and 11 September 2019 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 21 September 2017, the applicant requested a clarification teleconference, which was held on 6 November 2017.

2.2. Methodologies

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

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

3. Assessment

The food enzyme under application contains two declared activities:

IUBMB nomenclature:	Endo-1,4-β-xylanase
Systematic name:	4-β-D-Xylan xylanohydrolase
Synonyms:	Xylanase; β-D-xylanase; 4-xylanohydrolase; β-1,4-xylanase
IUBMB No:	EC 3.2.1.8
CAS No:	9025-57-4
EINECS No:	232-800-2.

Xylanases catalyse the random hydrolysis of 1,4-β-D-xylose linkages in xylans (including arabinoxylans) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths.

The applicant recognises from whole genome sequence (WGS) that several genes encoding extracellular glucanases are present, all active in the hydrolysis of β-1,4-glucosidic linkages and all potentially contributing to the technological role of the food enzyme.[4] Consequently, the applicant monitors only total β-glucanase activity using a substrate for the in-house assay relevant to the technological function and bases the specification for the food enzyme on this total value.

The food enzyme is intended to be used in brewing processes.

3.1. Source of the food enzyme[5]

The food enzyme is produced with a non-genetically modified fungus *D. dimorphosporum* strain DXL. The production strain represents a single colony isolate of the strain [redacted][6].

[4] Technical dossier/Additional information, 1 August 2019.
[5] Technical dossier/p. 40–44; Technical dossier/Annex 6 and Annex 7; Technical dossier/Additional information, 3 January 2019; Technical dossier/Additional information, 1 August 2019; Technical dossier/Additional information, 24 September 2019.
[6] Technical dossier/p. 42.

The applicant deposited the production strain DXL in the culture collection of [redacted] under number [redacted][7] [redacted] confirmed the identity of the production strain as *D. dimorphosporum* (Arx) Stalpers.[8]

A literature search did not find any indication that *D. dimorphosporum* (synonyms: *Sporotrichum dimorphosporum*, *Chrysosporium dimorphum*) is able to act as a human pathogen.[9] However, it is noted that invasive infections produced by other species of *Chrysosporium* may occur, typically in impaired hosts and can have fatal outcomes (Anstead et al., 2012).

3.2. Production of the food enzyme[10]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch 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 stabilised and then further purified and concentrated, including an ultrafiltration step, in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.

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[12]

The whole genome sequencing and functional gene annotation[4] showed that the genome of the non-genetically modified strain of *D. dimorphosporum* DXL encodes a total of three extracellular endo-1,4-β-glucanases and two endo-1,4-β-xylanases.[4]

The amino acid sequences have been provided.[13] The proteins with endo-1,4-β-D-xylanase activity have calculated molecular masses of [redacted] and [redacted] kDa, and the proteins with endo-1,4-β-glucanase activity have calculated molecular masses of [redacted] and [redacted] kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis.[14] This showed the presence of three major protein bands, corresponding to about 50 kDa, 36 kDa and 25 kDa, in all three commercial batches examined. Other bands of minor intensity were also found. No other enzymatic side activities were reported.[15]

The in-house determination of the endo-1,4-β-xylanase[16] activity is based on the hydrolysis of rye xylan resulting in lowering of the viscosity at pH 4.7 and 42°C, which is measured in a continuous flow viscometer. Xylanase units (XVU) are defined relative to an internal enzyme standard.

The in-house method used for the measurement of total β-glucanase[17] activity is based on the hydrolysis of β-glucan and the concomitant reduction in viscosity. The change in viscosity is measured in a continuous flow viscometer. The enzyme activity is measured relative to an internal enzyme standard. One (total) Fungal β-Glucanase Unit (FBG) is defined as the amount of enzyme per mL reaction mixture that causes a change in viscosity of the substrate solution with a speed giving a slope of 0.147 per minute under the reaction conditions pH 4.7 and 45°C.

[7] Technical dossier/Additional information, 3 January 2019/p. 2 and Annex 2.
[8] Technical dossier/Annex 7; Technical dossier/Additional information, 3 January 2019/p. 3 and Annex 3.
[9] Technical dossier/Annex 6.
[10] Technical dossier/p. 45–52; Technical dossier/Annex 10, Annex 11 and Annex 12.
[11] 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.
[12] Technical dossier/p. 37–40 and Technical dossier/Additional information, 1 August 2019.
[13] Technical dossier/Additional information, 1 August 2019/Annex 3.
[14] Technical dossier/p. 36; Technical dossier/Additional information, 14 July 2015.
[15] Technical dossier/p. 38.
[16] Technical dossier/Annex 2.
[17] Technical dossier/Annex 3.

The endo-1,4-β-xylanases are active at temperatures up to 80°C, with an optimum at 65–70°C. The pH profile has been measured within a pH range of 3.0–7.5 at 30°C, with an optimum at of 4.5. Xylanase activity is completely inactivated at temperatures above 80°C.[18]

The overall β-glucanase temperature profile has been measured from 50°C up to 70°C (with an optimum at 60°C). The pH profile has been measured within a pH range of 3–7 (with an optimum of 4–5). The β-glucanase activity is completely lost at temperatures above 75°C.[18]

3.3.2. Chemical parameters[19]

Data on the chemical parameters of the food enzyme were provided for five food enzyme batches, three batches used for commercialisation and two batches produced for the toxicological tests (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 24.3%. The average enzyme activity/mg TOS of the three food enzyme batches for commercialisation was 13.7 XVU/mg TOS and 2,115 FBG/mg TOS (Table 1).

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

Parameter	Unit	Batches				
		1	2	3	4[(a)]	5[(b)]
Xylanase activity	XVU/g batch[(c)]	3,340	3,350	3,280	3,300	3,098
Glucanase activity	FBG/g batch[(d)]	469,000	646,000	437,000	349,600[(g)]	570,578
Protein	%	16.7	10.4	18.4	14.8	n.a.
Ash	%	0.32	0.48	0.39	1.9	0.44
Water	%	76.7	72.9	76.3	78.2	74.26
Total Organic Solids (TOS)[(e)]	%	23.0	26.6	23.3	19.9	25.3
Xylanase activity/mg TOS	XVU/mg TOS	14.5	12.6	14.1	16.6	12.2
Glucanase activity/ mg TOS	FBG/mg TOS	2,039	2,429	1,876	1,757	2,255

n.a.: not analysed.
(a): Batch used for a bacterial reverse mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, *in vivo* mammalian erythrocyte micronucleus test, and a repeated dose 90-day oral toxicity study in rats.
(b): Batch used for a combined *in vivo* mammalian bone marrow erythrocyte micronucleus test and alkaline Comet assay.
(c): XVU: Xylanase Units (see Section 3.3.1).
(d): FBG: Fungal β-Glucanase Units (see Section 3.3.1).
(e): TOS calculated as 100% – % water – % ash – % diluent.
(f): Technical dossier/p. 35; Technical dossier/Annex 4; Technical dossier/Additional information, 3 January 2019/Annex 1.
(g): Technical dossier/Additional information, 14 July 2015/p. 4.

3.3.3. Purity[20]

The lead content in the three commercial batches was below 2 mg/kg[21] and in batch 4[22] used for toxicological studies 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).

The food enzyme preparation 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.[23] No antimicrobial activity was detected in any of the batches.[24]

[18] Technical dossier/p. 39–40; Technical dossier/Additional information, 14 July 2015.
[19] Technical dossier/Annex 1 and Annex 4; Technical dossier/Additional information, 3 January 2019.
[20] Technical dossier/p. 36–37; Technical dossier/Annex 4 and Annex 5; Technical dossier/Additional information, 14 July 2015/Annex 1; Technical dossier/Additional information, 3 January 2019/Annex 1.
[21] Technical dossier/Additional information, 3 January 2019/Annex 1/the lead content from 0.9 to 2 mg/kg (in different commercial batches); Technical dossier/Additional information, 3 January 2019/Annex 1/LOD: Pb = 5 mg/kg.
[22] Technical dossier/Additional information, 14 July 2015/Annex 1/in batch 4 used for toxicological studies: lead content < 0.08 mg/kg; Technical dossier/Additional information, 14 July 2015/Annex 1.
[23] Technical dossier/Additional information, 3 January 2019/Annex 1.

Many strains of filamentous fungi have the capacity to produce a range of secondary metabolites. The presence of mycotoxins (trichothecenes, aflatoxin B_1, B_2, G_1 and G_2, zearalenone, ochratoxin A, fumonisin B_1, B_2, and B_3)[24] was examined in the fermentation broth. The concentration of these mycotoxins were below the respective limits of detection (LODs) of the applied analytical methods.[25] The applicant did not provide information on other secondary metabolites potentially produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme.

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

3.3.4. Viable cells of the production strain

No experimental data were provided on the number of viable cells of the production strain in the food enzyme. However, the Panel notes that the manufacture of the food enzyme involves a final membrane filtration step intended to remove viable cells.[10]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, an *in vivo* mammalian erythrocyte micronucleus test, a combined *in vivo* mammalian bone marrow erythrocyte micronucleus test and alkaline Comet assay, and a repeated dose 90-day oral toxicity study in rats have been provided. The batches used for the toxicological assays are described in Table 1 (batches 4 and 5) and were considered to be representative of the food enzyme.

3.4.1. Genotoxicity

3.4.1.1. *In vitro* studies

3.4.1.1.1. Bacterial reverse mutation test

The Ames test was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537, TA98) and *Escherichia coli* strain CM891 (WP2*uvrA*/pKM101), in the presence and absence of metabolic activation (S9 mix).[26] The treat and plate assay was applied and two experiments were carried out using five different concentrations of the food enzyme (100, 300, 1,000, 3,000 and 10,000 μg dry matter/plate, corresponding to 91.3, 274, 913, 2,739 and 9,128 μg TOS/plate; Batch 4[27]). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no increase in revertant colony numbers. Therefore, the Panel concluded that the food enzyme did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.4.1.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[28] The food enzyme was tested for its ability to induce chromosomal aberrations in cultured human peripheral blood lymphocytes in the presence and absence of metabolic activation (S9 mix) at concentrations up to 5,000 μg food enzyme dry matter/mL. Two experiments were performed. In the first experiment, the cultures were exposed to 1,250, 2,500 and 5,000 μg food enzyme dry matter/mL, corresponding to 1,141, 2,282 and 4,564 μg TOS/mL, for 3 h followed by a 17 h recovery period (short treatment) either in the presence or absence of S9-mix. In the second experiment, two treatment conditions were applied: continuous treatment (20 + 0 h in the absence of the S9 mix) where the cultures were exposed to 750, 3,000 and 5,000 μg food enzyme dry matter/mL (corresponding to 685, 2,739 and 4,564 μg TOS/mL), and short treatment (3 + 17 h in the presence of S9-mix) where the cultures were exposed to 1,250, 2,500 and 5,000 μg

[24] Technical dossier/Annex 8 and Annex 9; Technical dossier/Additional information, 14 July 2015; LODs: trichothecenes: 10 μg/kg each toxin; aflatoxin B_1, B_2, G_1 and G_2: 0.1 μg/kg each toxin; zearalenone: 3 μg/kg; ochratoxin A: 0.1 μg/kg; fumonisin B_1, B_2, and B_3: 10 μg/kg each toxin.
[25] Technical dossier/Additional information, 14 July 2015.
[26] Technical dossier/Annex 18.
[27] Batch 4 used for toxicological studies: dry matter: 21.8%, TOS: 19.9%.
[28] Technical dossier/Annex 19.

food enzyme dry matter/mL (corresponding to 1,141, 2,282 and 4,564 μg TOS/mL). After short treatment in the presence and absence of S9 mix in both experiments, the food enzyme did not induce a significant increase in structural or numerical chromosome aberrations in cultured human blood lymphocytes. Following continuous treatment in the absence of S9-mix, a statistically significant increase in chromosomal aberrations was observed at the highest concentration tested (5,000 μg food enzyme dry matter/mL, corresponding to 4,564 μg TOS/mL) showing 58% cytotoxicity; values were slightly above the upper 99% limit of historical negative control data. In order to clarify this result, an *in vivo* mouse erythrocyte micronucleus test was performed by the applicant.

3.4.1.2. *In vivo* studies

3.4.1.2.1. *In vivo* mammalian erythrocyte micronucleus test

The *in vivo* mammalian erythrocyte micronucleus test in mice was carried out according to the OECD Test Guideline 474 (OECD, 1997c) and following GLP.[29] Five CD-1 outbred albino mice of Swiss origin per group (males) were treated with a single oral administration (intragastric gavage) of the food enzyme dissolved in purified water at doses of 500, 1,000 and 2,000 mg dry matter/kg body weight (bw), corresponding to 456, 913 and 1,826 mg TOS/kg bw (Batch 4[30]). Mice were sacrificed 24 h after dosing. In addition, a group of animals from the negative controls (purified water) and from high level treatment group was sacrificed 48 h after dosing. No mortalities and clinical signs of toxicity were reported after treatment with the test item. The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was assessed by examination of at least 1,000 erythrocytes per animal. For each animal, 2,000 PCE were scored for the presence of micronuclei (micronucleated polychromatic erythrocytes (MNPCE)). The incidence of MNNCE was also noted. No statistically significant increases in the frequency of MNPCE and no substantial decrease in the proportion of immature erythrocytes were observed in animals treated with the food enzyme, compared with vehicle control values. The Panel concluded that the food enzyme did not induce micronuclei in bone marrow when tested up to 2,000 mg dry matter/kg bw (corresponding to 1,826 mg TOS/kg bw) under the experimental conditions employed, however, considered this study of limited validity because no data on bone marrow exposure were provided.

3.4.1.2.2. Combined *in vivo* mammalian bone marrow erythrocyte micronucleus test and alkaline Comet assay

The genotoxic potential of the food enzyme endo-1,4-β-xylanase and β-glucanase from *D. dimorphosporum* strain DXL was assessed *in vivo* using the bone marrow erythrocyte micronucleus assay combined with the Comet assay in liver, duodenum and glandular stomach of rats.[30] The study was conducted in accordance with GLP, OECD Test Guideline 474 (OECD, 2016a) and 489 (OECD, 2016b).

In the dose-range finding study, groups of three male and three female Wistar Han rats were given once daily doses of 2,000 mg TOS/kg bw via oral gavage for 3 days. No mortality and no treatment related clinical signs after dosing were observed. Since there were no differences in toxicity between sexes, only males were used in the main study.

In the main study, five male rats were dosed once daily by oral gavage with vehicle (water, UltraPure Elix®) or 500, 1,000 and 2,000 mg TOS/kg bw per day for three consecutive days. Five animals of positive control group for micronucleus assay were dosed with 30 mg cyclophosphamide (CP)/kg bw once on day 1. Five animals of positive control group for Comet assay were dosed once daily with 200 mg ethyl methanesulfonate (EMS)/kg bw per day for two consecutive days (on day 2 and 3). The bone marrow, liver, duodenum and glandular stomach were collected 3–4 h after the last treatment.

No mortality or treatment-related clinical signs were observed in any animal group.

Micronucleus assay

Bone marrow from the femurs was prepared for micronucleus scoring. A total of at least 1,000 PCE and NCE were scored to calculate the degree of bone marrow toxicity by the relative decrease in PCE. Four thousand PCE per animal were scored for the presence of micronuclei (MN).

No decrease in the ratio of PCE to NCE compared to the concurrent vehicle control was recorded in treated animals.

[29] Technical dossier/Annex 20.
[30] Technical dossier/Additional information, 3 January 2019/Annex 4.

Rats treated with the food enzyme exhibited mean frequencies of MNPCE that were similar to and not statistically different from those observed in the concurrent vehicle control. The positive control (CP) induced a clear, statistically significant increase in the incidence of MNPCEs.

The Panel concluded that the food enzyme did not induce micronuclei in bone marrow when tested up to 2,000 mg TOS/kg bw under the experimental conditions employed, however, considered this study of limited validity because no evidence of bone marrow exposure was provided.

Comet assay

Liver, duodenum and glandular stomach analysis

Measurements of tail intensity (% DNA in tail) were obtained from 150 cells/animal. No statistically significant increase in mean tail intensity values for animals treated with food enzyme were observed in liver, duodenum and glandular stomach of any treated group compared to the concurrent vehicle control group.

The positive control (EMS) induced a statistically significant increase in the mean tail intensity in liver, duodenum and glandular stomach, within the 95% control limit of the distribution of the historical positive control database.

The food enzyme did not induce DNA damage in liver, duodenum and glandular stomach of rats, administered via oral gavage, as analysed by the Comet assay. The Panel considered the results on liver as limited because the exposure was not demonstrated, however the negative results obtained at the first sites of contact allow to rule out the concern for clastogenicity.

The Panel concluded on the basis of the *in vitro* and *in vivo* 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

The repeated dose 90-day oral toxicity study was performed in accordance with of the Food and Drug Administration of the USA (Red Book 1, USA, 1982[31]) and following GLP.[32] Groups of 20 male and 20 female CD rats received by gavage the food enzyme in doses corresponding to 12.9, 49.8 and 199 mg TOS/kg bw per day. Controls received the vehicle (water obtained by reverse osmosis).

No mortality was observed.

Several animals from the high-dose group exhibited salivation after dosing in the last three weeks of treatment (11 week: 2/20 males; 12 and 13 weeks: 2/20 males and 4/20 females). The Panel considered this effect is possibly due to repeated use of gavage.

Haematological examination revealed several statistically significant differences from controls. In males, a decrease in prothrombin time at the high dose (9%) and an increase in mean cell haemoglobin at the mid-dose (3.4%) were recorded. In females, a lower haematocrit at the mid- (5%) and high doses (2.6%), a decrease in haemoglobin at the mid-dose (4%) and decrease in red blood cells in the low- (3.3%) and mid-doses (5%), a decrease in platelet count (9.7%) and in prothrombin time (6%) at the low dose were observed. As no dose response was observed and these findings were not consistent between the sexes, they were considered not of toxicological significance.

Among clinical chemistry parameters, statistically significant differences from controls included for mid-dose treated males decreases in alanine aminotransferase activity (14%), creatinine (6%) and sodium (0.7%) and an increase in glucose (22%). The albumin to globulin ratio was also reduced for mid- (4.7%) and high-dose (5.8%) males. For treated females, decreases in alanine aminotransferase activity at mid- and high doses (17.9% and 20.5%), in gamma glutamyl transpeptidase (66.7%) and in sodium (0.3%) at the mid-dose, and in blood urea nitrogen (BUN) at mid- and high doses (14% and 22.5%) were observed. Furthermore, an increase in total triglycerides at the high dose (53.3%) and in total protein and albumin (6% and 6%) at the mid-dose were reported.

The Panel noted that the changes of prothrombin time, triglycerides and BUN might have indicated a change in hepatic metabolism/hepatic damage. However, these changes were not associated with any changes in absolute and relative liver weights and macroscopic or microscopic findings in the organ. Furthermore, the changes in clinical chemistry parameters lacked dose-response relationship (except for BUN) and consistency between sexes. As such, all of these changes were considered by the Panel to be of no toxicological significance.

[31] Technical dossier/Additional information, 3 January 2019/p. 5.
[32] Technical dossier/Annex 21.

Statistically significant increases in relative kidney weights of mid- and high-dose males (6.1% and 6.4%) and in relative ovary weight of high-dose females (16%) were reported. In absence of any significant morphological changes in the kidneys and in ovaries and in other reproductive organs, the increase in the relative weights of these organs was considered of no toxicological significance.

No other statistically significant differences to controls were observed.

The Panel identified a no observed adverse effect level (NOAEL) of 199 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the endo-1,4-β-xylanases and the β-glucanases[33] produced with the non-genetically modified *D. dimorphosporum* strain DXL was assessed by comparing their amino acid sequences with those of known allergens according to the 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no matches were found for two extracellular endo-1,4-β-glucanases and two endo-1,4-β-xylanases. For the third endo-β-1,4-glucanase, the search resulted in matches with three protein sequences from the mite [redacted].

No information is available on oral sensitisation or elicitation reactions of endo-1,4-β-xylanases or β-glucanases from *D. dimorphosporum.*

Cases of occupational allergy following exposure by inhalation of aerosols containing xylanase have been reported in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) as well in case reports (Baur et al., 1998; Merget et al., 2001; O'Connor et al., 2001). Several studies have shown that adults with occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). No food allergic reactions to xylanase and β-glucanase have been reported in the literature.

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 endo-1,4-β-xylanase and β-glucanase produced with *D. dimorphosporum* strain DXL 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[34]

The food enzyme is intended for use in brewing processes at the maximal recommended use levels of 36.5 mg TOS/kg cereals.

In the brewing process, the food enzyme is added during the mashing step in order to decrease viscosity, improve filterability, improve yield and contribute to consistent product quality.

3.5.2. Dietary exposure estimation

For 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) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-

[33] Technical dossier/Additional information, 1 August 2019/Annex 4.
[34] Technical dossier/p. 54–56.

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 A.1 and A.2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

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

Population group	Estimated exposure (mg TOS/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	0	0–0.001	0–0.007	0.003–0.037	0.001–0.018
	(10)	(14)	(19)	(18)	(19)	(18)
Min–max 95th percentile (number of surveys)	0	0	0	0–0.044	0.021–0.167	0.005–0.077
	(8)	(12)	(19)	(17)	(19)	(18)

TOS: Total Organic Solids; bw: body weight.

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 3.

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

FoodEx: food classification system; 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.

3.6. Margin of exposure

A comparison of the NOAEL (199 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0–0.037 mg TOS/kg bw per day at the mean and from 0–0.167 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 1,192.

4. Conclusions

Based on the data provided, and the derived margin of exposure for brewing processes, the Panel concluded that the food enzyme with endo-1,4-β-xylanase and β-glucanase activities from *D. dimorphosporum* strain DXL does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Technical dossier 'Application for authorisation of endo-1,4-ß-xylanase and β-glucanase activities from *Disporotrichum dimorphosporum*'. 27 February 2014. Submitted by DSM Food Specialties B.V.
2) Additional information. 14 July 2015. Submitted by DSM Food Specialties B.V.
3) Additional information. 3 January 2019. Submitted by DSM Food Specialties B.V.
4) Additional information. 1 August 2019. Submitted by DSM Food Specialties B.V.
5) Additional information. 24 September 2019. Submitted by DSM Food Specialties B.V.
6) Summary report on technical data and dietary exposure. Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy). 28 April 2015.

References

Anstead GM, Sutton DA and Graybill JR, 2012. Adiaspiromycosis causing respiratory failure and a review of human infections due to *Emmonsia* and *Chrysosporium* spp. Journal of Clinical Microbiology, 50, 1346–1354. https://doi.org/10.1128/JCM.00226-11

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

Baur X, Sander I, Posch A and Raulf-Heimsoth M, 1998. Baker's asthma due to the enzyme xylanase - a new occupational allergen. Clinical and Experimental Allergy, 28, 1591–1593.

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

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

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

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

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids. EFSA Journal 2009;1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305. Updated on May 2013.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Fowler P, Franz R, Grob K, Gurtler R, Husøy T, Karenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Pocas MF, Tlustos C, Wolfle D, Zorn H, Zugravu C-A, Arcella D, Liu Y and Engel K-H, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and FernandezDumont A, 2017. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmental Medicine, 60, 802–804. https://doi.org/10.1136/oem.60.10.802

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, 63–67. Available online: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.en-75

Merget R, Sander I, Raulf-Heimsoth M and Baur X, 2001. Baker's asthma due to xylanase and cellulase without sensitization to alpha-amylase and only weak sensitization to flour. International Archives of Allergy and Immunology, 124, 502–505.

O'Connor TM, Bourke JF, Jones M and Brennan N, 2001. Report of occupational asthma due to phytase and β-glucanase. Occupational and Environmental Medicine, 58, 417–419.

OECD (Organisation for Economic Co-operation and Development), 1997a. Bacterial Reverse Mutation Test. Guideline 471, adopted 21.07.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-operation and Development), 1997b. *In vitro* Mammalian Chromosomal Aberration Test. Guideline 473, adopted 21.07.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Co-operation and Development), 1997c. OECD Guideline 474: Guideline for testing of chemicals. Mammalian Erythrocyte Micronucleus Test, adopted 21.07.1997. Available online: http://www.oecd.org/chemicalsafety/risk-assessment/1948442.pdf

OECD (Organisation for Economic Co-Operation and Development), 2016a. OECD Guideline 474. Mammalian Erythrocyte Micronucleus Test, (adopted 29 July 2016). Available online: https://read.oecd-ilibrary.org/environment/test-no-474-mammalian-erythrocyte-micronucleus-test_9789264264762-en#page1

OECD (Organisation for Economic Co-Operation and Development), 2016b. OECD Guideline 489. *In Vivo* Mammalian Alkaline Comet Assay, (adopted 29 July 2016). Available online: https://read.oecd-ilibrary.org/environment/test-no-489-in-vivo-mammalian-alkaline-comet-assay_9789264264885-en#page1

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

Red Book USA, 1982. US Food and Drug Administration. Guidance for Industry and Other Stakeholders. Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook, 2000, 1–286 Available online: https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf

Abbreviations

[redacted]	[redacted]
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
[redacted]	[redacted]
CFU	colony forming units
CP	cyclophosphamide
EC	Enzyme Commission
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
EMS	ethyl methanesulfonate
FAO	Food and Agricultural Organization
FBG	Fungal β-Glucanase Units
FoodEx	food classification system
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
[redacted]	[redacted]
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MN	micronucleus
MNNCE	micronucleated normochromatic erythrocytes
MNPCE	micronucleated polychromatic erythrocytes
MOE	Margin of Exposure
n.a.	not analysed
NCE	normochromatic erythrocytes

NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCE	polychromatic erythrocytes
[redacted]	[redacted]
S9 mix	metabolic activation
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization
WGS	whole genome sequence
XVU	Xylanase Unit

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

Information provided in this appendix is shown in an excel file (downloadable https://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2020.5975/suppinfo).

The file contains two sheets, corresponding to two tables.

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

Table A.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).