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Safety evaluation of the food enzyme β-amylase obtained from barley (*Hordeum vulgare*)

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Abstract

The food enzyme considered in this opinion is a 4-α-D-glucan maltohydrolase (EC 3.2.1.2) obtained from grain of barley (*Hordeum vulgare*), by the companies Genencor International B.V. and Senson Oy. This β-amylase is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups. The compositional data provided for the food enzyme were considered sufficient. The manufacturing process did not raise safety concerns. Based on the maximum use levels recommended for the respective food processes, dietary exposure to the food enzyme–total organic solids (TOS) was estimated on the basis of individual data from the EFSA Comprehensive European Food Consumption Database. This exposure estimate is similar to or lower than the exposure to a fraction of barley comparable to the food enzyme–TOS, resulting from the consumption of barley-derived foods. As the food enzyme is derived from edible parts of barley, in line with the requirements of the guidance document on food enzyme assessment, the Panel accepted that there was no need for the provision of toxicological data for this food enzyme and the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use. Considering the potential for allergenicity, the gluten content of the food enzyme was below the detection limit of the analytical method, which is well below the level of 20 mg/kg for 'gluten-free' products. The amino acid sequence of the β-amylase was compared to those of known allergens and no match was found. The food enzyme β-amylase from barley is an occupational respiratory allergen and may contain low levels of other allergenic barley proteins that may trigger adverse reactions upon oral challenges in individuals with an oral sensitisation to cereals. The Panel considers that dietary exposure to the food enzyme β-amylase from barley may result in incidental cases of food allergic reactions.

Keywords: food enzyme, β-amylase, 4-α–D-glucan maltohydrolase, EC 3.2.1.2, *Hordeum vulgare*, barley

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Note: The full opinion will be published in accordance with Article 5 of Regulation (EC) No 1331/2008 once the decision on confidentiality, in line with Article 12(3) of the Regulation, will be received from the European Commission. The following information has been provided under the confidentiality framework and has been redacted awaiting the decision of the Commission: yield factor of the food enzyme, analytical data on gluten content.

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Erratum: Allergenicity assessment (section 3.4) was revised. The amendment is consistent with the NDA panel opinion published in 2009. Other parts of the opinion (Abstract, Discussion and Conclusions) were aligned accordingly. Six references were added. The composition of the CEF panel changed in July 2017. Contributors to this revision are acknowledged. The original version and a version showing the changes made are available on request.

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

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

- 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 approval via an EU Community list.

The 'Guidance on submission of a dossier on a food enzyme for evaluation' (EFSA, 2009b) 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 EU Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes. According to this Regulation, a food enzyme that falls within the scope of Regulation (EC) No 1829/2003[3] on genetically modified food and feed should be authorised in accordance with that Regulation as well as under this Regulation.

An application has been submitted by the companies Genencor International B.V. and Senson Oy for authorisation of the food enzyme β-amylase obtained from barley (*Hordeum vulgare*).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[4] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

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

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

[3] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

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

1.1.2. Terms of Reference

The European Commission requests EFSA to perform a safety assessment of the food enzyme β-amylase obtained from barley (*Hordeum vulgare*) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Information on existing authorisations and evaluations

According to the applicant, the authorities of Australia/New Zealand, Brazil, Canada, China, France, Mexico and the USA have evaluated and authorised the use of β-amylase obtained from barley, including malted barley, and/or other cereals in a number of food- and beverage-manufacturing processes. The conditions of use are not specified.

2. Data and methodologies

2.1. Data

The applicants have submitted a dossier in support of the application for authorisation of the food enzyme β-amylase obtained from barley (*Hordeum vulgare*).[5] The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups.[6]

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, 2009a) and following the relevant existing guidelines from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA, 2009b) has been followed for the evaluation of this application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) 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: β-Amylase
Systematic name: 4-α-D-Glucan maltohydrolase
Synonyms: Saccharogen amylase, glycogenase, 1,4-α-D-glucan maltohydrolase
IUBMB No: EC 3.2.1.2
CAS No: 9000-91-3
EINECS No: 232-566-1
The food enzyme is obtained via aqueous extraction from barley grain.

3.1.2. Chemical parameters

The molecular mass of 59.6 kDa reported in the dossier for the β-amylase from barley *Hordeum vulgare*) in the food enzyme, was calculated from the amino acid sequence deduced from the cDNA sequence (UniProtKB – P16098[7]).

Data on chemical parameters of the food enzyme were provided for three commercial batches (Table 1). The average total organic solids (TOS) content of the three commercial batches was 49.3% (w/w); the values ranged from 47.4% to 51.9%. The TOS content is a calculated value of 100% food enzyme minus % water content minus % ash.

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2014-00731
[6] A EC working document describing the food processes in which food enzymes are intended to be used - not yet published at the time of adoption of this opinion.
[7] Available from: http://www.uniprot.org/uniprot/P16098

Enzyme activity, expressed as degrees diastatic power (°DP), ranged from 1,828 to 2,033 °DP/g batch. The average enzyme activity/TOS ratio of the three commercial food enzyme batches was 3.95 °DP/mg TOS; the values ranged from 3.77 to 4.16 °DP/mg TOS. Considering the low variability of the enzyme activity/TOS ratio of the three batches, the average activity/mg TOS ratio of 3.95 °DP/mg TOS was used for subsequent calculations.

The protein pattern of the food enzyme was determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. The protein profiles provided for three food enzyme batches were comparable. In addition to the band assigned to β-amylase by the applicant, i.e. slightly above 50 kDa, the presented gels showed two further major protein bands at ~ 38 and 12 kDa with similar intensities, and several other additional bands. The observed complexity of the protein profiles reflects the fact that the food enzyme is extracted from barley grain without further protein fractionation.

No analytical data on the activities of other enzymes in the food enzyme product were provided. Taking into account that the food enzyme is obtained from an edible part of a plant, the Panel considered this acceptable.

Table 1: Compositional data provided for three commercial batches of the food enzyme

Parameter	Unit	Batches		
		1	2	3
β-Amylase activity	°DP/g batch	1,970	1,828	2,033
Protein	%	17.9	16.6	18.6
Ash	%	2.4	2.3	2.3
Water	%	50.2	49.2	45.8
TOS[(a)]	%	47.4	48.5	51.9
β-Amylase activity/mg TOS	°DP/mg TOS	4.16	3.77	3.92

°DP: diastatic power degrees; TOS, total organic solids.
(a): TOS calculated as 100% – % water – % ash.

In addition to the data presented in Table 1, the applicant provided data on three other batches of the food enzyme, demonstrating that the contents of lead (< 0.05 mg/kg) and several mycotoxins (ochratoxin A, aflatoxins and *Fusarium* toxins) were below the respective limits of quantification.[8]

The applicant also provided analytical data on three other batches of the food enzyme, demonstrating that the gluten content was below the limit of quantification ([redacted]) of the applied enzyme-linked immunosorbent assay (ELISA)-based method.

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 are present at not more than 30 colony forming units per gram.

The Panel considered the compositional data provided for the food enzyme as sufficient.

3.1.3. Properties of the food enzyme

The food enzyme β-amylase catalyses the hydrolysis of 1,4-α-glycosidic linkages in polysaccharides and successively releases maltose units from the non-reducing ends of the chains. No cofactors are required.

The activity of β-amylase is quantified based on measurement of the reducing sugars released from soluble starch via hydrolysis and is expressed in degrees diastatic power (°DP/g). Quantitative measurement of the released reducing sugars is based on titration of Fehling's solution (copper II ions and tartrate ions) with a starch solution hydrolysed by the food enzyme (reaction conditions: pH = 4.6, temperature = 20 ± 0.2°C, incubation time = 60 min) using methylene blue as an indicator.

The β-amylase was characterised regarding its activity dependence on temperature and pH. The pH profile was measured over a range of 3.5–8.0 at 20°C (25% relative activity at pH 3.5 and ~ 60% relative activity at pH 8.0, with an optimum pH between 4.7 and 5.5). The temperature profile was measured from 55°C to 80°C at pH 4.6. The temperature optimum was found to be between 55°C and

[8] The limit of quantification for ochratoxin A is 0.2 μg/kg food enzyme product, for all four aflatoxins (B1, B2, G1 and G2) is 0.1 μg/kg food enzyme product, for two fusarium toxins (deoxynivalenol and nivalenol) is 20 μg/kg food enzyme product, for three fusarium toxins (HT-2, T2 and zearalenone) is 10 μg/kg food enzyme product.

63°C. The thermostability of β-amylase was measured under standard assay conditions after pre-incubation at pH 4.6 and different temperatures for up to 100 min. No enzyme activity could be measured after treatment for > 50 min at 67°C.

3.1.4. Information on the plant source material

Barley grain is routinely eaten in many parts of the world (for example, see Loftas et al., 1995). It is also commonly malted and then used as a key ingredient in alcoholic beverage production (e.g. beer and whisky). According to information provided by the applicant, barley grain used to extract the food enzyme fulfils the requirements of Regulation (EU) No 1881/2006[9] setting maximum levels for certain contaminants in foods and of Regulation (EU) No 396/2005[10] on maximum residue levels of pesticides in or on food and feed of plant and animal origin, and Regulation (EU) No 2015/1940[11] on maximum residue levels of ergot alkaloids in unprocessed cereals.

3.1.5. Manufacturing process

The manufacturing process comprises extraction and downstream processing. A comprehensive dataset related to the manufacturing process including a list of raw materials used and a flow diagram was provided. The food enzyme is manufactured in accordance with Regulation (EC) No 852/2004[12], with food safety procedures based on HACCP principles, and in accordance with current good manufacturing practice.

Prior to extraction, barley grains may be subjected to dehusking and/or malting. The food enzyme is obtained by extraction with water and separated from insoluble barley material by centrifugation and filtration.

After separation, the liquid containing the enzyme is concentrated to reach the desired enzyme activity and/or to increase the enzyme activity/TOS ratio. Concentration may be achieved by ultrafiltration, diafiltration and/or evaporation. At the end of this process, [redacted] of TOS can be obtained from 1 kg of barley malt, which corresponds to a yield factor of [redacted]

The Panel considered the information provided on the raw materials and the manufacturing process as sufficient. The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns.

3.1.6. Reaction and fate in food

β-Amylase catalyses the cleavage of 1,4-α-glycosidic linkages in the starch polysaccharides amylose and amylopectin, resulting in the successive removal of maltose units from the non-reducing ends of the starch chains.

β-Amylase is specific in its action and is not known to catalyse reactions other than the hydrolysis of the starch polysaccharides amylopectin and amylose. The reaction products, i.e. maltose and the remaining oligosaccharides, are naturally present in starch-containing foods.

The data and the information provided indicate that β-amylase is inactivated and/or removed during the food manufacturing processes under the intended conditions of use, e.g. via thermal treatment or distillation.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups (Table 2).

[9] Commission Regulation (EU) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

[10] Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/4141/EEC.

[11] Commission Regulation (EU) 2015/1940 of 28 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia in certain unprocessed cereals and the provisions on monitoring and reporting. OJ L283, 29.10.2015, p. 4–6.

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

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

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	10–80 DP°/kg flour, corresponding to 2–20 mg TOS/kg flour
Brewing processes (beer)	Cereals	200–1,000 DP°/kg malted barley or other cereals, corresponding to 50–250 mg TOS/kg malted barley or other cereals
Distilled alcohol production	Cereals	200–1,000 DP°/kg cereals, corresponding to 50–250 mg TOS/kg cereals
Starch processing for the production of glucose syrups	Starch	60–1,600 DP°/kg cereals, corresponding to 15–400 mg TOS/kg starch

DP°: diastatic power degrees; TOS, total organic solids.
(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

In baking, the food enzyme is added to the raw materials during preparation of the dough. It is used to partially hydrolyse starch, resulting in a starch backbone with shorter chains, in non-branched glucan chains of lower molecular mass and in maltose. Its use provides maltose for yeast fermentation, improves the dough structure, ensures a uniform volume and better crumb structure, reduces viscosity and thus facilitates handling of the dough.

In brewing processes, the food enzyme is added during the mashing step. β-Amylase is used to convert liquefied starch into a maltose-rich solution, improving the amounts of fermentable sugars and thus increasing brewing yield.

In beverage alcohol (distilling) processes, the food enzyme is added during the slurry mixing step, in the liquefaction step and/or in the presaccharification step. β-Amylase is intended to be used to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars and the concentration of solids, which potentially results in higher alcohol yields.

In starch processes, β-amylase is added after the secondary liquefaction during the saccharification step, to convert liquefied starch into a maltose-rich solution, to ensure smooth and efficient processing, to facilitate separation and to ensure high-quality maltose syrup, resulting in energy savings in production and less waste water.

According to the applicant, the food enzyme is used at the minimum dosage necessary to achieve the desired reaction according to good manufacturing practice. In practice, the dosage applied by a food manufacturer will depend on the particular process (Table 2).

3.2. Dietary exposure

Following the EFSA Guidance Document on food enzymes (EFSA, 2009b), a comparison was made between:

- dietary exposure to the food enzyme–TOS, resulting from the intended use as proposed by the applicant (herein referred to as 'FE–TOS'); and
- dietary exposure to a fraction of barley comparable to the food enzyme-TOS, resulting from the consumption of barley-derived foods (herein referred to as source material TOS equivalent, 'SMT–Equivalent').

In both cases, food consumption data from the EFSA Comprehensive European Food Consumption Database (hereafter the EFSA Comprehensive Database[13]) were used.

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 processes covered in this opinion involved selection of relevant food groups and application of process and technical conversion factors (Appendix B), as appropriate (Section 3.2.3). These input data were subject to a stakeholder consultation through open calls,[14] and adjusted in accordance with feedback received.

[13] Available from: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[14] http://www.efsa.europa.eu/en/data/call/161110

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the Comprehensive Database 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 interpreted with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to possible subjects' underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe.

Food consumption data from the following 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).

Exposure to the FE–TOS was calculated by multiplying values reported in Appendix B for each food category by their respective consumption amount per kilogram of body weight (kg bw) separately for each individual in the database.

The dietary exposure to the SMT–Equivalent was calculated by first estimating the intake of barley grain from all dietary sources (applying recipe and conversion fractions reported in Appendix C). Second, the so-derived intake of barley grain was converted into a barley fraction comparable to the food enzyme–TOS via application of a factor provided by the applicant to take into account the yield of the FE–TOS from the barley source (Section 3.1.5).

In both cases, the exposure per FoodEx category (Appendices B and C) 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 group, resulting in distributions of individual average exposure per survey and population group. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per population group.

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

Exposure to the FE–TOS was estimated based on intended uses and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Table 2). The FE–TOS exposure was calculated from foods produced involving baking and brewing processes.

Foods/ingredients derived through alcohol (distillation) processes and starch processing, i.e. spirits and glucose syrups, were excluded from the analysis. Experimental data on the significant removal (> 99%) of protein in the course of these processes have been provided (Documentation provided to EFSA, No.6). The Panel considered this evidence as sufficient to conclude that the presence of residual amounts of TOS after distilling or filtration and purification during processing is negligible.

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

To facilitate matching of the reported use levels for baking and brewing 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 or grain, 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 20 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 1.4 mg TOS/100 g bread.

Exposure to the FE–TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight separately for each individual in the database. Table 3 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 E – Table 1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix E – Table 2.

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

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)	0.0–0.06 (6)	0.05–0.13 (10)	0.06–0.12 (18)	0.03–0.10 (17)	0.06–0.36 (17)	0.04–0.19 (14)
Min–max 95th percentile (number of surveys)	0.07–0.17 (5)	0.11–0.21 (7)	0.10–0.22 (18)	0.06–0.42 (17)	0.19–1.48 (17)	0.09–0.70 (14)

bw: body weight.

3.2.4. Dietary exposure to SMT–Equivalent resulting from the consumption of barley-derived foods

The FoodEx categories likely to contain barley or be produced from barley were selected from the EFSA Comprehensive Database, at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b). The process of selecting relevant food groups was aided by obtaining qualitative information from the Mintel's Global New Products Database.[15]

Major FoodEx categories identified as containing barley or being derived from barley comprise beer, coffee imitates, breakfast cereals, barley-derived distilled spirits and malted beverages. Barley was found as a minor ingredient in some FoodEx categories, but due to a lack of detailed recipe information, these categories were not taken into account in the assessment (see Appendix D). Exclusion of these food categories results in a minor underestimation of intake. Appendix B provides an overview of the categories selected following this approach.

The selected FoodEx categories were converted from food as consumed (including ingredients in composite foods) to the SMT–Equivalent via application of the correction factors described in Appendix C. For example, consumption of a 100 g cereal bar was converted into an intake of 5 g of malted barley (5% of malted barley used as an ingredient), and the malted barley intake was further converted via application of the FAO conversion of 1.37, into an intake of raw barley grain of 6.85 g. Finally, the 6.85 g intake of raw barley grain was converted into 137 mg of SMT–Equivalent/100 g cereal bar by application of the yield factor described in Section 3.1.5.

Table 4 provides an overview of the estimated exposure to the SMT–Equivalent. The average and 95th percentile exposure to the SMT–Equivalent per age class, country and survey are reported in Appendix E – Table 3. The contribution of the SMT–equivalent from each FoodEx category to the total dietary exposure is indicated in Appendix E – Table 4.

Table 4: Summary of estimated dietary exposure to the SMT–Equivalent, resulting from the consumption of barley-derived foods, in six population groups

Estimated intake (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)	0.0–4.88 (6)	0.08–14.72 (10)	0.14–8.93 (18)	0.38–7.09 (17)	2.88–28.23 (17)	1.17–13.85 (14)
Min–max 95th percentile (number of surveys)	0.0–25.57 (5)	0.0–35.16 (7)	0.0–29.68 (18)	1.92–40.90 (17)	15.28–125.27 (17)	8.98–58.04 (14)

bw: body weight.

[15] Available online: http://www.mintel.com/global-new-products-database

3.2.5. Comparison of the exposure estimates

Exposure to the food enzyme, calculated on the basis of food enzyme–TOS (Table 3), was similar to or lower than the exposure to the SMT–Equivalent (Table 4).

3.2.6. 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 5.

Table 5: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact	
	Exposure to FE–TOS	Exposure to SMT–equivalent
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	+	NA
Exposure to food enzyme–TOS was always calculated based on the maximum recommended use level	+	NA
Selection of broad FoodEx categories for the exposure assessment	+	NA
Selection of FoodEx categories likely to contain barley for the intake assessment of a fraction of barley comparable to the food enzyme–TOS, based on the national food descriptors, recipe databases and Mintel Global New Products Database	NA	+/–
Use of FAO technical conversion factors: whenever a range is reported the median value was used	+/–	+/–
Range of yield factors provided by applicant, the mean value was used	NA	+/–
Yield factor of the food enzyme was available only for barley malt	NA	+/–
Use of recipe fractions in disaggregation FoodEx categories likely to contain barley and/or food likely to contain the food enzyme	+/–	+/–

+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure; NA: not applicable.

Both estimates are derived using the same food consumption data and exposure model, and hence share a number of uncertainties, which do not have an effect on the comparison of the two estimates. 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. For the estimation of intake of the SMT-Equivalent, uncertainties taken into account do not indicate either over- or underestimation of intake.

Overall, the Panel noted that exposure to the food enzyme–TOS is likely to be considerably lower than estimated, and consequently the margin between the estimate of exposure to the food enzyme–TOS and the SMT-Equivalent is likely to be higher than estimated.

3.3. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012[16], an application for the safety evaluation of a food enzyme does not need to include toxicological data if the food enzyme is obtained from edible parts of a plant intended or reasonably expected to be ingested by humans.

[16] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes. OJ L 168, 28.6.2012, p. 21–23.

According to the EFSA Guidance on the submission of a dossier on food enzymes for safety evaluation, the justification for not supplying toxicological data may include a documented history on the safety of the source of the food enzyme, the composition and the properties of the food enzyme, as well as its use in foods, demonstrating no adverse effects on human health when consumed in a comparable way (EFSA, 2009b).

The Panel considers that these requirements are fulfilled, because:

i) Barley grain is routinely eaten in many parts of the world and malted barley grain is a key ingredient in the production of alcoholic beverages.
ii) Barley used for the production of the food enzyme complies with the legal requirements regarding limits of potential contaminants and residues. The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns.
iii) The compositional data provided on the food enzyme are considered sufficient.
iv) Exposure to the food enzyme, calculated on the basis of food enzyme–TOS, is similar to or lower than the exposure to the SMT–Equivalent.

3.4. Allergenicity

Barley is a source of allergenic proteins (respiratory and food allergens) and a source of gluten proteins that can elicit coeliac disease – a form of gluten intolerance.

3.4.1. β-Amylase and other proteins extracted from barley grain

Potential allergenicity of β-amylase from barley was assessed by comparing the amino acid sequence (Kreis et al., 1987) 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 > 35% identity in a sliding window of 80 amino acids as the criterion, no matches were found.

With regard to the allergenicity of β-amylase from barley, conflicting data are presented in various allergen databases. In the AllergenOnline database,[17] β-amylase from barley is not listed as an allergen, whereas in the WHO/IUIS Allergen Nomenclature database[18] β-amylase from barley is indicated as Hor v 17[19] and indicated as a food allergen with no further information. In the Allergome database,[20] β-amylase from barley is also listed as allergen Hor v 17, but according to the allergenicity scoring it is only found positive in non-functional testing like immunoglobulin (Ig)E/IgE immunoblot testing. Moreover, it is indicated that no data on oral-, bronchial-, nasal- and conjunctival-provocation testing and skin testing, as well as on functional testing, such as the basophil degranulation/activation test, are available.

β-Amylase from barley is described as an occupational respiratory allergen: sensitization to barley can induce asthma in bakers and millers (Sandiford et al., 1994; Tatham and Shewry, 2008). In the various allergen databases, several other barley proteins (e.g. Hor v 12, Hor v 15, Hor v 16 and Hor v 20) are also listed as being associated with mainly baker's asthma (Barber et al., 1989; Mena et al., 1992; Sanchez-Monge et al., 1992; Armentia et al., 1993).

Several studies have shown that adults suffering from occupational asthma due to food enzymes as described for α-amylase can commonly ingest the corresponding enzymes without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). However, incidental cases have been described where ingestion of α-amylase led to adverse reactions in patients sensitized through the respiratory route (Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information for β-amylase has not been reported. Oral challenges with protein extracts from barley have shown to induce clinical symptoms of allergy in individuals orally sensitised to cereals (wheat, rye and barley) (Armentia et al., 2002).

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed (e.g. in distilled alcohol production). In the starch processing for the production of glucose syrups, although experimental data showed a significant removal (> 99%) of protein, traces amount of protein, estimated to be up to 0.5 mg/kg, could be present in glucose syrup. Products such as candy and ice creams can contain

[17] Available from: www.allergenonline.org
[18] Available from: www.allergen.org
[19] Allergen nomenclature database: Hor v 17: Hor(deum) v(ulgare), the species name of barley and allocated index number.
[20] Available from: http://allergome.org

about 50% and 40% glucose syrup, respectively, and therefore proteins could be present in quantities sufficient to elicit an allergic reaction.

As the food enzyme β-amylase from barley may also contain traces of other allergenic barley proteins, as indicated by SDS-PAGE analysis showing several other protein bands, it might trigger adverse reactions in cereal allergic individuals. This is in line with the conclusions of the EFSA Panel on Dietetic products, Nutrition and Allergies (EFSA, 2009c).

3.4.2. Gluten proteins extracted from barley grain

In Regulation (EU) No 1169/2011[21], barley is listed as gluten-containing cereal which therefore can elicit coeliac disease, a form of gluten intolerance. However, because gluten is poorly soluble in water, the food enzyme obtained via extraction of barley grain using an aqueous solution is expected not to contain gluten. The applicant provided analytical data on three batches of the food enzyme, demonstrating that the gluten content was below the limit of quantification ([redacted] food enzyme) of the applied ELISA-based method.

Based on the estimated dietary exposure to the food enzyme TOS in six population groups (Table 3), the highest potential exposure to gluten from the food enzyme was calculated (for adults) and amounts to [redacted] per day, which is several orders of magnitude lower than the exposure from the gluten-free foods with the threshold value of 20 mg/kg set for the labelling of foods as 'gluten-free' specified in European Commission Regulation (EC) 41/2009[22].

Several clinical studies performed in patients with coeliac disease indicated the safe thresholds for gluten content in 'gluten-free' products in a range of 20–100 mg/kg (Collin et al., 2004; Catassi et al., 2007). The threshold value of gluten in 'gluten-free' foods is set as 20 mg/kg in Commission Regulation (EC) No 41/2009)[13] for European population.

3.4.3. Conclusions on allergenicity potential

The Panel concluded that dietary exposure to the food enzyme β-amylase derived from barley might trigger clinical symptoms of food allergy in susceptible cereal allergic individuals.

4. Discussion

The food enzyme is obtained from barley grain, i.e. an edible plant source. The Panel considered the information provided on the source material, the manufacturing process and the composition of the food enzyme sufficient to conclude on its safety.

The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups. The use levels recommended for these food processes have been provided.

Exposure to the food enzyme–TOS was estimated using individual data from the EFSA Comprehensive Database. This exposure was similar to or lower than the exposure to a fraction of barley comparable to the food enzyme–TOS, resulting from the consumption of barley-derived foods.

In line with the requirements of the guidance document, the Panel accepted that there was no need for the provision of toxicological data for this food enzyme.

The gluten content of the food enzyme β-amylase derived from barley, which is listed as gluten-containing cereal in Regulation (EU) No 1169/2011, did not exceed the level of 20 mg/kg for gluten-free foods. The amino acid sequence of β-amylase lacked similarity with those of other known allergens. However, the food enzyme β-amylase from barley is described as an occupational respiratory allergen and in addition contained several other proteins. Upon ingestion, the risk of an allergic reaction in individuals sensitised by inhalation to cereals cannot be ruled out. It should be noted that in individuals with an oral sensitisation to cereals, oral challenges with barley protein extracts resulted in clinical symptoms.

[21] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18–63.

[22] Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

Taken together, the CEF Panel considers that dietary exposure to the food enzyme β-amylase from barley may result in incidental cases of food allergic reactions in susceptible cereal allergic individuals.

Conclusions

Based on the origin of the food enzyme from edible parts of barley, the enzyme-manufacturing process, the compositional and biochemical data provided, and dietary exposure assessment, the Panel considers that this food enzyme β-amylase obtained from barley by the companies Genencor International B.V. and Senson Oy does not raise safety concerns under the intended conditions of use.

However, the CEF Panel considers that dietary exposure to the food enzyme β-amylase from barley may result in incidental cases of food allergic reactions in susceptible cereal allergic individuals.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of β-amylase from barley (*Hordeum vulgare*)'. September 2014. Submitted by Genencor International B.V. and Senson Oy.
2) Summary report on technical data and dietary exposure related to β-amylase from the edible seed of barley (*Hordeum vulgare*). March 2015. Delivered by Hylobates Consulting (Rome, Italy) and BiCTm (Lodi, Italy).
3) Summary report on allergenicity related to β-amylase extracted from Barley (*Hordeum vulgare*). June 2015. Delivered by FoBiG GmbH (Freiburg, Germany).
4) Additional information on raw material, thermos-stability and use level. July 2015. Provided by Genencor International B.V. and Senson Oy.
5) Additional information on the yield factor of the food enzyme. May 2016. Provided by Genencor International B.V. and Senson Oy.
6) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry-over in glucose syrups'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
°DP	diastatic power degree
EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization of the United Nations
FE–TOS	food enzyme–TOS
HACCP	Hazard Analysis and Critical Control Points
IgE	immunoglobulin E
IUBMB	International Union of Biochemistry and Molecular Biology
SMT–Equivalent	source material TOS equivalent
TOS	total organic solids
WHO	World Health Organization

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	20
A.01.03	Grain milling products (unspecified)	1	1	20
A.01.03.001	Wheat milling products (unspecified)	1	1	20
A.01.03.001.001	Wheat flour, brown	1	1	20
A.01.03.001.002	Wheat flour, Durum	1	1	20
A.01.03.001.003	Wheat flour, white	1	1	20
A.01.03.001.004	Wheat flour, wholemeal	1	1	20
A.01.03.001.005	Graham flour	1	1	20
A.01.03.001.006	Wheat flour, gluten free	1	1	20
A.01.03.001.014	Wheat starch	1.2	1	20
A.01.03.002	Rye milling products (unspecified)	1	1	20
A.01.03.002.001	Rye flour, gluten free	1	1	20
A.01.03.002.002	Rye flour, light	1	1	20
A.01.03.002.003	Rye flour, medium	1	1	20
A.01.03.002.004	Rye flour, wholemeal	1	1	20
A.01.03.003	Buckwheat milling products (unspecified)	1	1	20
A.01.03.003.001	Buckwheat flour	1	1	20
A.01.03.004	Corn milling products (unspecified)	1	1	20
A.01.03.004.001	Corn flour	1	1	20
A.01.03.004.003	Corn starch	1.3	1	20
A.01.03.005	Oat milling products (unspecified)	1	1	20
A.01.03.005.002	Oat flour	1	1	20
A.01.03.005.004	Oat starch	1.2	1	20
A.01.03.006	Rice milling products (unspecified)	1	1	20
A.01.03.006.001	Rice flour	1	1	20
A.01.03.006.002	Rice flour white	1	1	20
A.01.03.006.003	Rice flour, instant	1	1	20
A.01.03.006.004	Rice starch	1.2	1	20
A.01.03.007	Spelt milling products	1	1	20
A.01.03.008	Other milling products (unspecified)	1	1	20
A.01.03.008.001	Amaranth flour	1	1	20
A.01.03.008.002	Barley flour	1	1	20
A.01.03.008.003	Chapatti flour	1	1	20
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	20
A.01.03.008.005	Millet flour	1	1	20
A.01.03.008.007	Sorghum flour	1	1	20
A.01.04	Bread and rolls (unspecified)	1	0.7	20
A.01.04.001	Wheat bread and rolls	1	0.7	20
A.01.04.002	Rye bread and rolls	1	0.7	20
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	20
A.01.04.004	Multigrain bread and rolls	1	0.7	20
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	20
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	20

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	20
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	20
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	20
A.01.04.005.005	Rusk, light	1	0.9	20
A.01.04.005.006	Rusk, wholemeal	1	0.9	20
A.01.04.005.007	Pita bread	1	0.7	20
A.01.04.005.008	Matzo	1	0.9	20
A.01.04.005.009	Tortilla	1	0.7	20
A.01.04.006	Other bread	1	0.7	20
A.01.04.007	Bread products	1	0.7	20
A.01.07	Fine bakery wares (unspecified)	1	0.5	20
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	20
A.01.07.001.001	Beignets	1	0.15	20
A.01.07.001.002	Buns	1	0.7	20
A.01.07.001.003	Cake from batter	1	0.25	20
A.01.07.001.004	Cheese cream cake	1	0.24	20
A.01.07.001.005	Cheese cream sponge cake	1	0.24	20
A.01.07.001.006	Chocolate cake	1	0.24	20
A.01.07.001.007	Chocolate cake with fruits	1	0.24	20
A.01.07.001.008	Cream cake	1	0.24	20
A.01.07.001.009	Cream cheese cake	1	0.24	20
A.01.07.001.010	Cream custard cake	1	0.24	20
A.01.07.001.011	Cream custard sponge cake	1	0.24	20
A.01.07.001.012	Croissant	1	0.5	20
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	20
A.01.07.001.014	Croissant, filled with cream	1	0.5	20
A.01.07.001.015	Croissant, filled with jam	1	0.5	20
A.01.07.001.016	Croquembouche	1	0.15	20
A.01.07.001.017	Doughnuts	1	0.24	20
A.01.07.001.018	Clair	1	0.15	20
A.01.07.001.019	Flan	1	0.5	20
A.01.07.001.020	Fruit cake	1	0.6	20
A.01.07.001.021	Fruit pie	1	0.15	20
A.01.07.001.022	Cheese pie	1	0.15	20
A.01.07.001.023	Fruit tart	1	0.15	20
A.01.07.001.024	Gingerbread	1	0.6	20
A.01.07.001.025	Gougere	1	0.15	20
A.01.07.001.026	Kringles	1	0.25	20
A.01.07.001.027	Nut cream cake	1	0.24	20
A.01.07.001.028	Pancakes	1	0.25	20
A.01.07.001.029	Profiterole	1	0.15	20
A.01.07.001.030	Pyramid cake	1	0.25	20
A.01.07.001.031	Rhubarb flan	1	0.15	20
A.01.07.001.032	Scone	1	0.5	20
A.01.07.001.033	Sponge dough	1	0.25	20
A.01.07.001.034	Sponge cake	1	0.25	20
A.01.07.001.035	Sponge cake roll	1	0.25	20

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	20
A.01.07.001.037	Waffles	1	0.25	20
A.01.07.001.038	Apple strudel	1	0.15	20
A.01.07.001.039	Cream-cheese strudel	1	0.24	20
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	20
A.01.07.001.041	Croissant from puff pastry	1	0.6	20
A.01.07.001.042	Brioche	1	0.5	20
A.01.07.001.044	Lebkuchen	1	0.6	20
A.01.07.001.045	Dumpling	1	0.5	20
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	20
A.01.07.001.047	Marzipan pie	1	0.25	20
A.01.07.001.048	Baklava	1	0.15	20
A.01.07.002	Biscuits (cookies)	1	0.9	20
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	20
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	20
A.01.07.002.003	Biscuits, cream filling	1	0.81	20
A.01.07.002.004	Biscuits, fruit filling	1	0.81	20
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	20
A.01.07.002.006	Butter biscuits	1	0.81	20
A.01.07.002.007	Biscuit, iced	1	0.81	20
A.01.07.002.008	Speculaas	1	0.9	20
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	20
A.01.07.002.010	Biscuits, oat meal	1	0.9	20
A.01.07.002.011	Biscuits, spelt meal	1	0.9	20
A.01.07.002.012	Biscuits, salty	1	0.9	20
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	20
A.01.07.002.014	Sticks, salty	1	0.81	20
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	20
A.18.04.001	Find bakery products for diabetics	1	0.5	20
A.19.01.002	Pizza and pizza-like pies	1	0.3	20
A14.01	Beer and beer-like beverage	1.37	0.19	250
A.14.01.001	Beer, strong	1.37	0.265	250
A.14.01.002	Beer, regular	1.37	0.19	250
A.14.01.003	Beer, light (reduced alcohol content)	1.37	0.135	250
A.14.01.004	Beer, alcohol-free	1.37	0.135	250
A.14.01.005	Beer-like beverages (malt drink)	1.37	0.19	250

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 the Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).

Appendix C – FoodEx categories used to derive intake estimates for the raw barley grain and the respective conversion factors

FoodEx code	FoodEx name	FAO conversion factor from FoodEx food group to raw barley grain[(a)]	Proportion of barley used as ingredient or in production of food[(b)] (%)
A.01.02.002	Barley grain	1	100
A.01.02.002.001	Barley grain, whole	1	100
A.01.02.002.002	Barley, pearled	2.525	100
A.01.03.008.002	Barley flour	3.09	100
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	3.09	25
A.01.04.006	Other bread[(c)]	3.09	35
A.01.04.006.004	Muesli bread	3.09	0.2
A.01.05.008	Pasta, mixed cereal flour	3.09	15
A.01.06.001.001	Barley flakes	1.39	100
A.01.06.001.008	Mixed cereal flakes	1.39	30
A.01.06.002	Muesli	1	9
A.01.06.003	Cereal bars	1.37	5
A.01.06.006.001	Barley grits	2.525	100
A.02.11.003	Cocoa beverage-preparation, powder	1.7	73
A.01.06.007.005	Barley porridge	2.525	7.4
A.02.13.005	Barley coffee	1	100
A.02.13.008	Mixture of coffee imitates	1	30
A.06.09.003.001	Blood sausage	1	5
A.13.04	Coffee imitates beverage	1	5
A.13.05.001	Hot chocolate	1.37	8
A.14.01	Beer and beer-like beverage	1.37	19
A.14.01.001	Beer, strong		26.5
A.14.01.002	Beer, regular		19
A.14.01.003	Beer, light (reduced alcohol content)		13.5
A.14.01.004	Beer, alcohol-free		13.5
A.14.01.005	Beer-like beverages (malt drink)		19
A.16.04.014	Malt extract	1.72	100
A.19.10.002	Grain soup	1	3.2

(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 the Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).
(c): Only if barley is clearly indicated in the original food name were surveys included in the food category 'other bread'.

Appendix D – Food groups excluded from the intake assessment of barley grain

FoodEx code	FoodEx name	Reasons for exclusion[(a)]
One reason common to all food groups listed in this table is that the barley component cannot be identified in the FoodEx nomenclature at the same or higher hierarchy level		
A.01.04.005	Unleavened bread, crispbread and rusk	'Melba toast, malted barley' from Italy, 'crispbread wheat or rye barley fruit' from Sweden Further checking against the descriptor could not identify barley
A.06.09.004	Cooked smoked sausage	'Sausage stuffed with pearl barley' from Finland A single item under this FoodEx might be relevant, however, many others certainly do not contain barley
A08.02.001.002	Chocolate and fruit-flavoured milk	Neither the food descriptor nor the food labels can identify barley or barley malt
A.10.04.001	Candies, with sugar	'Boiled sweets barley sugar butterscotch glacier mints hard c' from UK, Barley is a natural flavouring on food labels
A.12.02.005	Juice concentrate, currants (black)	'Conc barley water low cal not blackcurrant' from UK It contains barley malt extract, the amount is not known, but is assumed to be negligible, based on recipe information
A.13	Non-alcoholic beverage	'Barley water, concentrated' from Ireland It contains barley malt extract, the amount is not known, but is assumed to be negligible, based on recipe information
A.17.05	Cereal-based food for infants and young children	Barley component cannot be identified in the ingredient list of several corresponding foods
A.17.06.001	Ready-to-eat meal for children, vegetable based	
A.17.06.002	Ready-to-eat meal for children, cereal based	
A.17.06.005	Fruit pure, for children	
A.18.03.001	Carbohydrate-rich energy food products for sports people	Containing small amount of malt extract-based on food labels
A.19.01.003.006	Pasta, cooked, meat and vegetable filling	'Barley-shaped pasta oven with lamb meat in tomato sauce and butter' from Greece The original language showed that it contains soya not barley
A.19.05	Meat-based meals	'Hash made with EG barley offal' from Sweden A single item under this FoodEx might be relevant, however, many others certainly do not contain barley
A.14.06.002	Whisky	The amount of dry matter is negligible
A.19.10.001	Vegetable/herb soup	'Stock bouillon with pot barley' or 'vegetable soup with pot barley' from Latvia Barley is a natural flavouring on food labels

(a): Based on publically available recipe information, and/or food label information (such as the Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).
'Food descriptor' refers to the names of foods used by national dietary surveys included in the EFSA Comprehensive Database.

Appendix E – Dietary exposure estimates to the food enzyme–TOS and the SMT–Equivalent in details

Information provided in this appendix is shown in an excel file (downloadable http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2017.4756/suppinfo).

The file contains four sheets, corresponding to four tables.

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

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Table 3: Average and 95th percentile exposure to the SMT–Equivalent per age class, country and survey.

Table 4: Contribution of food categories to the dietary exposure of the SMT–Equivalent per age class, country and survey.