09/02/2015

European Union comments on

Codex Circular Letter CL 2014/13-FA

Priority list of substances proposed for evaluation by JECFA

Mixed Competence.

Member States Vote.

The European Union and its Member States are proposing to add the following substances to the priority list of substances proposed for evaluation by JECFA:

1) Alpha-amylase in Aspergillus niger

2) Alpha-amylase in Bacillus licheniformis

3) Asparaginase in Bacillus subtilis

4) Xylanase in Bacillus licheniformis

Enclosures:

The forms containing information on the substances mentioned above.

INFORMATION ON THE SUBSTANCE TO BE EVALUATED BY JECFA

1. ***Proposal for inclusion submitted by:***

Danish Veterinary and Food Administration.

2. ***Name of substance; trade name(s); chemical name(s):***

Substance: Alpha-amylase from *Rhizomucor pusillus* expressed in *Aspergillus niger*

Chemical name: 1,4-alpha D-glucan glucanohydrolase; CAS 9000-90-2, EC 3.2.1.1

3. ***Names and addresses of basic producers:***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

4. ***Has the manufacturer made a commitment to provide data?***

Novozymes A/S commits to provide data to support the proposal for inclusion of the alpha-amylase in the list of substances to be evaluated by JECFA.

5. ***Identification of the manufacturer that will be providing data (Please indicate contact person):***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

Attn.: Tine Vitved Jensen
tvit@novozymes.com
+45 4446 0804

6. ***Justification for use:***

The alpha-amylase enzyme preparation is used as a processing aid during food manufacture for hydrolysis of starch during processing of starch containing foods. The alpha-amylase is typically used in the following food processes:

- Starch processing

- Beverage alcohol (distilling) processes
- Baking and other cereal based processes

7. ***Food products and food categories within the GSFA in which the compound is used as a food additive or as an ingredient, including use level(s):***

The enzymes preparation is not added to final foodstuffs but used as a processing aid during food manufacturing. The typical food processes in which the alpha-amylase is used are listed in above section.
The alpha-amylase is used at the minimum dosage necessary to achieve the desired enzymatic reaction.
The ranges of dosage recommended for the alpha-amylase are as follows (expressed in enzyme activity units).

Starch processing:
Up to 200 FAU(F) per kilogram of starch dry matter.

Beverage alcohol (distilling) processes:
Up to 200 FAU(F) per kilogram of starch dry matter.

Baking and other cereal based processes:
Up to 200 FAU(F) per kilogram of starch dry matter.

8. ***Is the compound currently used in food that is legally traded in more than one country? (please identify the countries); or, has the compound been approved for use in food in one or more country? (please identify the country(ies))***

The enzyme was approved under the trade name Novozym 27470 in Denmark in 2014 and was positively evaluated by the regulatory authorities in Mexico and included in the positive list. Novozymes has also applied for approval of the enzyme in France and Brazil. The approvals are expected in 2015.

9. ***List of data available (please check, if available)***
   Toxicological data
   (i) Metabolic and pharmacokinetic studies
   (ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies
   (iii) Epidemiological and/or clinical studies and special considerations
   (iv) Other data

The following food toxicity program according to the EFSA Guideline[i] has been performed:

- Test for mutagenic activity (Ames Test)
- *In vitro* micronucleus
- 13 weeks oral toxicity study in rats

The main conclusions of the safety studies can be summarized as follows:

The alpha-amylase preparation is considered non-cytotoxic and showed no mutagenic activity by testing in a bacterial reverse mutation assay (Ames Test) and by showing no induction of micronuclei in cultured human peripheral blood lymphocytes *in vitro*.

The alpha-amylase preparation was well tolerated at all dose levels when administered to rats for 13 weeks, and the overall No Observed Adverse Effect Level (NOAEL) is considered to be the highest administered dose, corresponding to 1400 mg TOS/kg body weight (bw)/day or 687.6 FAU(F)/kg bw/day.

The safety studies described above were all performed on liquid alpha-amylase enzyme concentrate produced in accordance with ordinary production procedure, omitting stabilization and standardization.

Aspergillus niger is generally considered to be a safe production organism with a long history of safe use for food ingredients. Furthermore, the production strain lacks the ability to produce relevant mycotoxins.

Technological data

(i) Specifications for the identity and purity of the listed compounds (specifications applied during development and toxicological studies; proposed specifications for commerce)

(ii) Technological and nutritional considerations relating to the manufacture and use of the listed compound

The alpha-amylase enzyme preparation complies with the purity criteria recommended for enzyme preparations by Food Chemicals Codex (VIII online edition, 2012). In addition to this, the enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006) as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Combined Compendium of Food Additive Specifications.

Intake assessment data

(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used

(ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

The exposure assessment is performed according to the Budget Method (ILSI, 1997).
Overall, the human exposure to the alpha-amylase will be negligible because the enzyme preparation is used as a processing aid and in low dosages.
The Budget Method assumptions represent a "maximum worst case" situation of human consumption, in which the enzyme would be used at its maximum recommended dosages in all processed food and all processed beverages and not only in those food and drink processes described above.
It is also supposed that the totality of the food enzyme will end up in the final food. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps.
Therefore the safety margin calculation derived from this method is highly conservative.

Assumptions in the Budget Method

Solid food	The maximum energy intake over the course of a lifetime is 50 kcal/kg bw/day. 50 kcal corresponds to 25 g foods. Therefore, adults ingest 25 g foods per kg bw per day. Assuming that 50% of the food is processed food, the daily consumption will be 12.5 g processed foods per kg bw. It is further assumed that, in average, all processed food contains 25% starch (or starch-derived) dry matter = 3.12 g starch derived dry matter per kg bw per day.
Liquids	The maximum intake of liquids (other than milk) is 100 ml/kg bw/day. Assuming that 25% of the non-milk beverages is processed, the daily consumption will be 25 ml processed beverages per kg bw. It is further assumed that all processed beverages contain 10% starch hydrolysates = 2.50 g starch derived dry matter per kg bw per day. It is assumed that the densities of the beverages are ~ 1.

Theoretical Maximum Daily Intake (TMDI) calculation

Solid Food:
The highest dosage for solid food is 200 FAU(F) per kg starch based raw material. 200 FAU(F) correspond to 407 mg TOS. Based on this, 3.12 gram starch-derived dry matter in solid food will maximally contain 1.27 mg TOS.

Liquid Food:
The highest dosage for liquid food (excluding distilled beverage spirits vide supra) is 200 FAU(F) per kg starch based raw material. 200 FAU(F) corresponds to 407 mg TOS. Based on this, 2.50 gram starch-derived dry matter in liquids will maximally contain 1.02 mg TOS.

The theoretical maximum daily intake (TMDI) of consumers of the enzyme is:
1.27 + 1.02 = 2.29 mg TOS/kg bw/day.

Other information as necessary

10. ***Date on which data could be submitted to JECFA:***

November 2015

[i] Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26

INFORMATION ON THE SUBSTANCE TO BE EVALUATED BY JECFA

1. ***Proposal for inclusion submitted by:***

Danish Veterinary and Food Administration.

2. ***Name of substance; trade name(s); chemical name(s):***

Substance:
Alpha-amylase from *Bacillus stearothermophilus* expressed in *Bacillus licheniformis*.

Chemical name: 1,4-alpha D-glucan glucanohydrolase; CAS 9000-90-2, EC 3.2.1.1

3. ***Names and addresses of basic producers:***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

4. ***Has the manufacturer made a commitment to provide data?***

Novozymes A/S commits to provide data to support the proposal for inclusion of the alpha-amylase in the list of substances to be evaluated by JECFA.

5. ***Identification of the manufacturer that will be providing data (Please indicate contact person):***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

Attn.: Tine Vitved Jensen
tvit@novozymes.com
+45 4446 0804

6. ***Justification for use:***

The alpha-amylase enzyme preparation is used as a processing aid during food manufacture for hydrolysis of starch during processing of starch containing foods. The alpha-amylase is typically used in the following food processes:

- Starch processing
- Beverage alcohol (distilling) processes
- Brewing processes and other cereal based beverage processes
- Cereal based processes
- Removal of starch in sugar processing
- Fruit and vegetable processing

7. ***Food products and food categories within the GSFA in which the compound is used as a food additive or as an ingredient, including use level(s):***

The enzymes preparation is not added to final foodstuffs but used as a processing aid during food manufacturing. The typical food processes in which the alpha-amylase is used are listed in above section.
The alpha-amylase is used at the minimum dosage necessary to achieve the desired enzymatic reaction.
The ranges of dosage recommended for the alpha-amylase are as follows (expressed in enzyme activity units).

Starch processing:
Up to 80 KNU(S) per kilogram of starch dry matter.

Beverage alcohol (distilling) processes:
Up to 120 KNU(S) per kilogram of starch dry matter.

Brewing processes and other cereal based beverage processes:
Up to 240 KNU(S) per kilogram of starch dry matter.

Cereal based processes:
Up to 240 KNU(S) per kilogram of starch dry matter.

Removal of starch in sugar processing:
Up to 2 KNU(S) per kilogram of sugar dry matter.

Fruit and vegetable processing:
Up to 4 KNU(S) per litre of juice. This corresponds to approximately 400 KNU(S) per kilogram of starch dry matter.

8. ***Is the compound currently used in food that is legally traded in more than one country? (please identify the countries); or, has the compound been approved for use in food in one or more country? (please identify the country(ies))***

The enzyme was approved under the trade name Termamyl®SC in Denmark in 2006. The enzyme has been positively evaluated by a number of regulatory authorities and included in relevant positive lists in various countries, e.g. Australia, Brazil, France and Mexico.

9. ***List of data available (please check, if available)***
Toxicological data
(i) Metabolic and pharmacokinetic studies

(ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies
(iii) Epidemiological and/or clinical studies and special considerations
(iv) Other data

The production organism is from a safe strain lineage as described in the decision tree in Pariza and Johnson, 2001[i]. The safety of the enzyme product is covered by safety studies according to the EFSA Guideline[ii] conducted on the actual production strain or a previous production strain in the same strain lineage.

The following studies were performed:

- Test for mutagenic activity (Ames Test)
- *In vitro* micronucleus
- 13 weeks oral toxicity study in rats.

The main conclusions of the safety studies can be summarized as follows:

The alpha-amylase preparation is considered non-cytotoxic and showed no mutagenic activity by testing in a bacterial reverse mutation assay (Ames Test) and by showing no induction of micronuclei in cultured human peripheral blood lymphocytes *in vitro*.

Oral administration to rats of up to 10 ml/kg body weight/day (16689 KNU(S)/kg bw/day or 660 mg Total Organic Solids (TOS) /kg bw/day) for 13 weeks did not reveal any significant toxic effects attributable to the test substance and is considered the No Ob-served Adverse Effect Level (NOAEL).

The safety studies described above were all performed on liquid alpha-amylase enzyme concentrate produced in accordance with ordinary production procedure, omitting stabilization and standardization.

Technological data
(i) Specifications for the identity and purity of the listed compounds (specifications applied during development and toxicological studies; proposed specifications for commerce)
(ii) Technological and nutritional considerations relating to the manufacture and use of the listed compound

The alpha-amylase enzyme preparation complies with the purity criteria recommended for enzyme preparations by Food Chemicals Codex (VIII online edition, 2012). In addition to this, the enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006) as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Combined Compendium of Food Additive Specifications.

Intake assessment data
(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used
(ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

The alpha-amylase enzyme preparation is to be used for starch hydrolysis during food processing.
The exposure assessment is performed according to the Budget Method (ILSI, 1997).
Overall, the human exposure to the alpha-amylase will be negligible because the enzyme preparation is used as a processing aid and in very low dosages.

In order to demonstrate a worst case calculation, an exaggerated human intake is estimated using the following assumptions.

a) The food enzyme would be used at its maximum recommended dosage in all processed food and all processed beverages.

b) According to the Budget method, a conservative estimate for the food intake is 25 g per kg body weight (bw) per day of which processed food is 50% of the food intake or 12.5 g per kg bw per day. It is further assumed that in average all processed food contains 25% starch (or starch derived) dry matter = 3.12 g starch derived dry matter per kg bw per day.

c) Also according to the Budget method, a conservative estimate for the beverage (non-milk) intake is 100 ml per kg bw per day of which processed beverages (soft drink) is 25% of the non-milk beverage intake or 25 ml per kg bw per day. It is further assumed that all processed beverages contain 10% starch hydrolysates = 2.50 g starch derived dry matter per kg/bw/day.
It is assumed that the densities of the beverages are ~ 1.

d) The calculation is made assuming that all TOS remains in the final product. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps.

The highest dosage recommended in above applications is 240 KNU(S) per kg starch based raw material. 240 KNU(S) correspond to 9.48 mg TOS. Based on this, 3.12 gram starch-derived dry matter in solid food will maximally contain 0.0296 mg TOS. Furthermore, 2.50 gram starch-derived dry matter in liquids will maximally contain 0.0237 mg TOS.

The theoretical maximum daily intake (TMDI) of consumers of the food enzyme is therefore 0.0533 mg TOS/kg bw/day.

However, this does not include the special applications ‘Removal of starch in sugar processing and ‘Fruit and vegetable processing’ where the aim is to remove small amounts of starch.

For sugar processing, the worst case intake was calculated to be 0.001 TOS /kg bw/day. Since this amount is less than the 0.0296 mg TOS /kg bw/day as estimated by the Budget Method for solid foods, use of the alpha-amylase for starch removal in sugar processing is covered by the Budget Method calculation above.

For fruit and vegetable processing, the worst case intake was calculated to be 0.0040 TOS /kg bw/day. Since this amount is less than the 0.0237 mg TOS /kg bw/day as estimated by the Budget Method for liquids, use of the alpha-amylase in production of fruit and vegetable juices for starch removal is covered by the Budget Method calculation above.

Other information as necessary

10. ***Date on which data could be submitted to JECFA:***

November 2015

[i] Pariza, M.W. and Johnson, E.A.. Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century. Reg. Tox and Pharm 33: 173-186, 2001.

[ii] Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26

INFORMATION ON THE SUBSTANCE TO BE EVALUATED BY JECFA

1. ***Proposal for inclusion submitted by:***

Danish Veterinary and Food Administration.

2. ***Name of substance; trade name(s); chemical name(s):***

Substance: *Pyrococcus furiosus* asparaginase expressed in *Bacillus subtilis*

Chemical name: L-asparagine amidohydrolase, CAS 9015-68-3, EC 3.5.1.1

3. ***Names and addresses of basic producers:***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

4. ***Has the manufacturer made a commitment to provide data?***

Novozymes A/S commits to provide data to support the proposal for inclusion of the asparaginase in the list of substances to be evaluated by JECFA.

5. ***Identification of the manufacturer that will be providing data (Please indicate contact person):***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

Attn.: Tine Vitved Jensen
tvit@novozymes.com
+45 4446 0804

6. ***Justification for use:***

The asparaginase enzyme preparation is used as a processing aid during food manufacture to convert asparagine to aspartic acid in order to reduce the risk for acrylamide formation. The enzyme is thermotolerant and therefore well suited for use in high temperature processing like production of breakfast cereals.

The asparaginase is typically used in the following food processes:

- Baking processes
- Other cereal based processes
- Fruit and vegetable processing
- Coffee and cocoa processing

7. ***Food products and food categories within the GSFA in which the compound is used as a food additive or as an ingredient, including use level(s):***

The asparaginase enzyme preparation is intended to be used to convert asparagine to aspartic acid in order to reduce the risk of formation of acrylamide during food production of products such as breakfast cereals, fabricated potato chips and french fries, and wheat dough based products such as cookies and crackers.

The asparaginase is used at the minimum dosage necessary to achieve the desired enzymatic reaction. Below table lists the typical food processes in which the asparaginase is used and ranges of dosage recommended (expressed in enzyme activity units).

Applications	**Conditions of use**
Baking processes	Up to 15000 TASU/kg final food
Other cereal based processes	Up to 7500 TASU/kg final food
Potato flakes and granules process as ingredients for dough-based potato snacks	Up to 10000 TASU/kg final food
Sliced potato chips process	Up to 15000 TASU/kg final food
French fries process	Up to 15000 TASU/kg final food
Coffee and cocoa processing	Up to 12500 TASU/kg final roasted coffee or cocoa

8. ***Is the compound currently used in food that is legally traded in more than one country? (please identify the countries); or, has the compound been approved for use in food in one or more country? (please identify the country(ies))***

A US GRAS (Generally Recognized As Safe) notification was submitted to FDA and the agency did not question Novozymes' conclusion that the asparaginase enzyme preparation is GRAS under the intended conditions of use. The enzyme was approved under the trade name Acrylaway® HighT in Denmark in 2013. Furthermore, the enzyme has been positively evaluated and included in the positive lists in Brazil and Mexico.

9. ***List of data available (please check, if available)***
   Toxicological data
   (i) Metabolic and pharmacokinetic studies

(ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies
(iii) Epidemiological and/or clinical studies and special considerations
(iv) Other data

The asparaginase enzyme preparation has been subjected to a standard package of toxicological tests according to the EFSA Guideline[i]. The following studies were performed:

- Test for mutagenic activity (Ames Test)
- *In vitro* micronucleus
- 13 weeks oral toxicity study in rats

The main conclusions of the safety studies can be summarized as follows:

The asparaginase preparation is considered non-cytotoxic and showed no mutagenic activity by testing in a bacterial reverse mutation assay (Ames Test) and by showing no induction of micronuclei in cultured human peripheral blood lymphocytes *in vitro*.

The asparaginase preparation was well tolerated at all dose levels when administered to rats for 13 weeks, and the overall No Observed Adverse Effect Level (NOAEL) is considered to be the highest administered dose, corresponding to 1.207 g TOS/kg/day or 584568 TASU/kg body weight/day.

The safety studies described above were all performed on liquid asparaginase enzyme concentrate, produced in accordance with ordinary production procedure, omitting stabilization and standardization.

Technological data

(i) Specifications for the identity and purity of the listed compounds (specifications applied during development and toxicological studies; proposed specifications for commerce)
(ii) Technological and nutritional considerations relating to the manufacture and use of the listed compound

The asparaginase enzyme preparation complies with the purity criteria recommended for enzyme preparations by Food Chemicals Codex (VIII online edition, 2012). In addition to this, the enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006) as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Combined Compendium of Food Additive Specifications.

Intake assessment data

(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used
(ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

The asparaginase is used in the manufacture of a wide variety of foods, food ingredients, and beverages. Due to this wide variety of applications the most appropriate way to estimate the human consumption is using the Budget Method (ILSI, 1997). This method enables to calculate the theoretical maximum daily intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data.

Overall, the human exposure to the food enzyme will be negligible because the enzyme preparation is used as a processing aid and in very low dosages. In order to demonstrate a worst case calculation, an exaggerated human intake is estimated using the following assumptions.

It is assumed that the totality of the food enzyme will end up in the final food. This assumption is highly exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps. Furthermore, far from all processed food and beverages are produced with the enzyme, and those that are, are not always produced with the maximum recommended dosage.

Solid food	The maximum energy intake over the course of a lifetime is 50 kcal/kg body weight (bw)/day. 50 kcal corresponds to 25 g foods. Therefore, adults ingest 25 g foods per kg bw per day. Assuming that 50% of the food is processed food the daily consumption will be 12.5 g processed foods per kg bw.
Liquid food	The maximum intake of liquid food (other than milk) is 100 ml/kg bw/day. Assuming that 25% of the non-milk beverages is processed the daily consumption will be 25 ml processed beverages per kg bw. It is assumed that the densities of the beverages are 1 g/ml.

Processed food
The processed food applications are “Baking and other cereal based processes” and “Fruit and vegetable processing”. The highest dosage is 15000 TASU/kg final food corresponding to 27.8 mg TOS.
Based on this 12.5 gram final solid food will maximally contain 0.348 mg TOS.

Processed beverages
The highest dosage given above is 12500 TASU per kg final roasted coffee or cocoa beans. It is assumed that:

- 1000 ml coffee or cocoa is made from 60 gram roasted (and milled) beans
- 60 gram roasted beans is dosed with 12500 x 60/1000 = 750 TASU corresponding to 1.39 mg TOS

Based on this, 25 ml coffee will contain 1.39 mg TOS x 25 ml/1000 ml = 0.035 mg TOS.

The theoretical maximum daily intake (TMDI)
The TMDI of consumers of the xylanase is 0.348 + 0.035 = 0.383 mg TOS/kg bw/day.

10. ***Date on which data could be submitted to JECFA:***

November 2015

[i] Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26

INFORMATION ON THE SUBSTANCE TO BE EVALUATED BY JECFA

1. ***Proposal for inclusion submitted by:***

Danish Veterinary and Food Administration.

2. ***Name of substance; trade name(s); chemical name(s):***

Substance: *Bacillus licheniformis* Xylanase expressed in *Bacillus licheniformis*

Chemical name: Endo-1,4-beta-xylanase; CAS 9025-57-4, EC 3.2.1.8

3. ***Names and addresses of basic producers:***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

4. ***Has the manufacturer made a commitment to provide data?***

Novozymes A/S commits to provide data to support the proposal for inclusion of the xylanase in the list of substances to be evaluated by JECFA.

5. ***Identification of the manufacturer that will be providing data (Please indicate contact person):***

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd
Denmark

Attn.: Tine Vitved Jensen
tvit@novozymes.com
+45 4446 0804

6. ***Justification for use:***

The xylanase enzyme preparation is used as a processing aid during food manufacture. The enzyme catalyzes the endo-hydrolysis of 1,4-beta-D-xylosidic linkages in xylans, including arabinoxylans (also called pentosans) in various plant materials including the cell walls and endosperm of cereals, such as wheat, barley, oats and malt. The xylanase is typically used in baking processes and other cereal based processes where it

improves handling of the dough, the dough structure and behavior during the baking step in addition to characteristics of the final bread.

7. ***Food products and food categories within the GSFA in which the compound is used as a food additive or as an ingredient, including use level(s):***

The enzyme preparation is not added to final foodstuffs but used as a processing aid during food manufacturing. The xylanase is used at the minimum dosage necessary to achieve the desired enzymatic reaction. The recommended dosage for the xylanase in baking and other cereal based processes is up to 60 NXU per kg of flour.

8. ***Is the compound currently used in food that is legally traded in more than one country? (please identify the countries); or, has the compound been approved for use in food in one or more country? (please identify the country(ies))***

A US GRAS (Generally Recognized As Safe) notification was submitted to FDA and the agency did not question Novozymes' conclusion that the xylanase enzyme preparation is GRAS under the intended conditions of use. The enzyme was approved under the trade name Panzea® in Denmark in 2013. Furthermore, the enzyme has been positively evaluated and included in the positive lists in Brazil and Mexico.

9. ***List of data available (please check, if available)***

Toxicological data

(i) Metabolic and pharmacokinetic studies
(ii) Short-term toxicity, long-term toxicity/carcinogenicity, reproductive toxicity, and developmental toxicity studies in animals and genotoxicity studies
(iii) Epidemiological and/or clinical studies and special considerations
(iv) Other data

The following food toxicity program according to the EFSA Guideline[i] has been performed:

- Test for mutagenic activity (Ames Test)
- *In vitro* micronucleus
- 13 weeks oral toxicity study in rats

The main conclusions of the safety studies can be summarized as follows:
The xylanase preparation is considered non-cytotoxic and showed no mutagenic activity by testing in a bacterial reverse mutation assay (Ames Test) and by showing no induction of micronuclei in cultured human peripheral blood lymphocytes *in vitro*.

The xylanase preparation was well tolerated at all dose levels when administered to rats for 13 weeks, and the overall No Observed Adverse Effect Level (NOAEL) is considered to be the highest administered dose, corresponding to 1.02 g TOS/kg body weight (bw)/day or 38608 NXU/kg bw/day.

The safety studies described above were all performed on liquid xylanase enzyme concentrate produced in accordance with ordinary production procedure, omitting stabilization and standardization.

Technological data

(i) Specifications for the identity and purity of the listed compounds (specifications applied during development and toxicological studies; proposed specifications for commerce)

(ii) Technological and nutritional considerations relating to the manufacture and use of the listed compound

The xylanase enzyme preparation complies with the purity criteria recommended for enzyme preparations by Food Chemicals Codex (VIII online edition, 2012). In addition to this, the xylanase enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006) as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Combined Compendium of Food Additive Specifications.

Intake assessment data

(i) Levels of the listed substance used in food or expected to be used in food based on technological function and the range of foods in which they are used

(ii) Estimation of dietary intakes based on food consumption data for foods in which the substance may be used.

In order to demonstrate a worst case calculation, an exaggerated human intake is estimated using the following method and assumptions.

The xylanase is used at its maximum recommended dosage in baking and other cereal based processes combined with the human intake of the foods produced with the enzyme as given in The EFSA Comprehensive European Food Consumption Database[ii]. According to this database the summarized intake of relevant food products, taken as the maximum average intake over 17 countries and all age groups, is 10.7 g "Grains and grain-based products"/kg bw/day.

It is supposed that the totality of the enzyme will end up in the final food. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted or removed in certain processing steps.

It is also assumed that all grains and grain-based products are produced with the enzyme, which is not the case.

The baking processes (mixing flour and water and other relevant ingredients followed by a heating step) on average results in 140 g of final baked product from 100 g of flour. Therefore, an intake per kg bw per day of 10.7 g of baked "Grains and grain-based products" corresponds to 10.7 x 100 / 140 = 7.64 g flour/kg bw/day.
The dosage for the enzyme in baking and other cereal based processes is up to 60 NXU per kg flour corresponding to 1.00 mg TOS.
Based on this, 7.64 g flour/kg bw/day will result in an exposure of 0.0076 mg TOS/kg bw/day.
Thereby, the Theoretical Maximum Daily Intake (TMDI) of the enzyme by consumers is 0.0076 mg TOS/kg bw/day.

Other information as necessary

10. ***Date on which data could be submitted to JECFA:***

November 2015

[i] Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26

[ii] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm. Used information: Chronic food consumption statistics - reported in g/kg bw/day (Published 2 March 2011) - Excel sheet L1 'Consumers Only' for "Grains and grain-based products" (Other children, Bulgaria).