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Supporting document 1

Risk and technical assessment – Application A1266

Endo-1,4-beta-xylanase from GM *Trichoderma reesei* (gene donor: *Fusarium verticillioides*) as a processing aid

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Executive summary

IFF Australia Pty Ltd (IFF) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme endo-1,4-β-xylanase (EC 3.2.1.8) as a processing aid in starch processing and the production of potable alcohol.[1] This enzyme is sourced from a genetically modified (GM) strain of *Trichoderma reesei* containing a protein-engineered variant of the endo-1,4-beta-xylanase gene from *Fusarium verticillioides.*

FSANZ has undertaken an assessment and concludes that the proposed use of endo-1,4-beta-xylanase as a processing aid in starch processing and the production of potable alcohol is consistent with its known technological function to hydrolyse glycosidic bonds in starch molecules.

Endo-1,4-beta-xylanase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

No public health and safety concerns were identified in the assessment of endo-1,4-beta-xylanase from GM *T. reesei* under the proposed use conditions. The *T. reesei* host was selected from a safe strain lineage and is neither pathogenic nor toxigenic. Analysis of the genetically modified production strain confirmed the presence and stability of the inserted DNA.

For the endo-1,4-beta-xylanase, a no observed adverse effect level (NOAEL) of 1000 total organic solids (TOS)/kg bw/day was identified in a 90-day oral toxicity study in rats. The theoretical maximum daily intake (TMDI) of the enzyme was calculated to be 0.02 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in a large margin of exposure

[1] The enzyme will be referred to in this report as endo-1,4-beta-xylanase.

of approximately 50,000. Based on the reviewed data it is concluded that in the absence of any identifiable hazard, an acceptable daily intake ‘not specified’ is appropriate. FSANZ concludes there are negligible health and safety concerns to consumers.

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1 Introduction

IFF Australia Pty Ltd (IFF) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme endo-1,4-β-xylanase (EC 3.2.1.8) as a processing aid in starch processing and the production of potable alcohol. The enzyme will be referred to in this report as endo-1,4-beta-xylanase. This enzyme is sourced from a genetically modified (GM) strain of *Trichoderma reesei* containing a protein-engineered variant of the endo-1,4-beta-xylanase gene from *Fusarium verticillioides.*

There are permissions for use as a processing aid of endo-1,4-beta-xylanase from both GM and non-GM microbial sources in the Code, including from *T. reesei*. However, the source requested is not specified as permitted. If a pre-market assessment leads to permission being granted, this endo-1,4-beta-xylanase will provide an additional option for starch processors and manufacturers of potable alcohol.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is a solely technological purpose (function) and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety concerns that may arise from the use of this enzyme, produced by a GM microorganism, as a processing aid, specifically by considering the:
  - history of use of the gene donor and production microorganisms
  - characterisation of the genetic modification(s), and
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity and properties of the enzyme

The production microorganism of the enzyme is a GM strain of *T. reesei.* The donor microorganism for the endo-1,4-beta-xylanase gene is *F. verticillioides* (further details contained in Section 3). The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclature database (IUBMB 2018). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	Endo-1,4-β-xylanase
Other names:	endo-(1→4)-β-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; β-1,4-xylanase; endo-1,4-xylanase; endo-β-1,4-xylanase; endo-1,4-β-D-xylanase; 1,4-β-xylan xylanohydrolase; β-xylanase; β-1,4-xylan xylanohydrolase; endo-1,4-β-xylanase; β-D-xylanase

IUBMB No.:	EC 3.2.1.8
CAS number:	9025-57-4
Reaction:	Catalyses the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans

IUBMB: International Union of Biochemistry and Molecular Biology; CAS: Chemical Abstracts Service

For a graphical representation of the hydrolysis reaction catalysed by endo-1,4-beta-xylanase, refer to its record in the enzyme database BRENDA (Chang, Jeske et al. 2020).

2.2 Manufacturing process

2.2.1 Production of the enzyme

IFF's endo-1,4-beta-xylanase is produced by submerged fed-batch fermentation of GM *T. reesei.* The main fermentation steps are inoculum, seed fermentation, and main fermentation. The fermentation processes are consistent with the scientific literature and references provided with the application (Aunstrup 1979). This is followed by the recovery stage which involves primary separation, concentration, and germ filtration to achieve the desired enzyme activity and/or to increase the ratio of enzyme activity to total organic solids (TOS) before formulation. The resulting product is a concentrated enzyme solution that the applicant states is free of the production strain. This is followed by formulation of the enzyme into an enzyme preparation.[2]

The application states that the enzyme is produced in accordance with Good Manufacturing Practices (GMP). Details of the manufacturing process, raw materials and ingredients used in the production of the endo-1,4-beta-xylanase enzyme preparation were provided in the application, some as Confidential Commercial Information (CCI).

The typical composition of the applicant's enzyme preparation is:

Water	54.4–58.4%
Glycerol	33.0%
Sodium chloride	7.0%
Endo-1,4-beta-xylanase	1–5%
Sodium benzoate	0.4%
Potassium sorbate	0.2%

2.2.2 Allergen considerations

Glucose syrup derived from wheat is used in the fermentation media for the manufacture of the enzyme. The applicant states that the wheat-derived glucose syrup is exempt from the declaration of wheat in accordance with S9—3, Schedule 9 of the Code. The applicant also provided the allergen declaration for the enzyme preparation. It states that the following allergens are not present, other cereals containing gluten, eggs, fish, peanuts, milk (including lactose), nuts (including but not limited to almond, hazelnuts, cashews, Brazilians,

[2] Enzymes are generally sold as enzyme preparations, which consist of the enzyme(s) and other ingredients, to facilitate their storage, sale, standardisation, dilution or dissolution.

macadamias, pecans, pistachios, pinolis, and chestnuts), celery, mustard, sesame seeds, sulphur dioxide and sulphites, lupine and products thereof, molluscs and products thereof, natural latex, soybeans and crustaceans. Section 3.3.4 confirms there were no matches with known allergens found for this enzyme.

2.2.3 Specifications

There are international specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2017) and the Food Chemicals Codex (FCC) (USPC, 2018). These specifications are included in the primary sources listed in section S3—2 of Schedule 3 of the Code and enzymes used as a processing aid must meet either of these specifications. Schedule 3 of the Code also includes specifications for arsenic and metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs[3]. In the case of endo-1,4-beta-xylanase, there is no individual monograph.

Table 1 provides a comparison of the analysis of three batches of the applicant's endo-1,4-beta-xylanase enzyme preparation with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, the enzyme met all relevant specifications in schedule 3 of the Code. Certificates of analysis have been provided which confirm the results below.

[3] http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

Table 1 *Analysis of enzyme endo-1,4-beta-xylanase compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes (three non-sequential batches)*

Analysis	Results from Applicant	JECFA	Food Chemicals Codex	Australia New Zealand Food Standards Code (section S3-4)
Lead (mg/kg)	<0.01	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	<0.01	-	-	≤1
Cadmium (mg/kg)	<0.00	< 0.5	-	≤1
Mercury (mg/kg)	<0.01	< 0.5	-	≤1
Coliforms (cfu/g)	<1	≤30	≤30	-
Salmonella (in 25 g)	Negative	Absent	Negative	-
E. coli (in 25 g)	Negative	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-

ND: Not detected; CFU: Colony-forming unit

Note: Analysis was performed on three batches of enzyme preparation.

While the manufacturing processes ensure the production microorganism is removed from the final enzyme preparation, the food enzyme is a biological isolate of variable composition, containing the enzyme protein, as well as organic and inorganic material derived from the microorganism and fermentation process. Refer to section 3.4 below for the total organic solids (TOS) value.

The recommended use levels in starch processing provided by the applicant are 1.71–4.28 mg TOS/kg of raw material for wheat and 1.07–4.28 mg/kg of raw material for starch.

2.3 Technological purpose and justification

Xylanases belong to the hydrolase enzyme class, which are enzymes that break or hydrolyse chemical bonds. They are further categorised as glycosidases as they act on glycosidic bonds. Endo-1,4-beta-xylanase is a glycosidase that breaks down xylan, a non-starchy hemicellulosic polysaccharide found in plant cell walls. Specifically, it hydrolyses bonds in the middle of the xylan molecule, hence the ‘endo’ designation. IFF’s endo-1,4-beta-xylanase is intended to be used in starch processing and the production of potable alcohol.

Many carbohydrate sources contain both starchy and non-starchy polysaccharides. Non-starchy polysaccharides are highly viscous when mixed with water. This slows the rate of hydrolysis and decreases the amount of smaller oligosaccharides produced. Xylanase reduces the viscosity of the mixture, allowing for more efficient starch hydrolysis (Buchilina et al 2021). This increases the amount of oligosaccharides produced and reduces the energy

and water required for the process. Reduced viscosity also supports more efficient fermentation for the production of potable alcohol, improving yield and purity (Sørensen, Pedersen et al. 2006). Carbohydrate sources also include both soluble and insoluble polysaccharides. Xylanase converts insoluble arabinoxylan in wheat flour to soluble arabinoxylan, which supports the separation of gluten and starch. This improves both the quality and the amount of gluten extracted (Wang, Vliet et al. 2004, Ramalingam 2010).

Use of commercial enzyme preparations should follow Good Manufacturing Practice (GMP), where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction. The conditions of the proposed use of the enzyme in food processing will depend on a number of factors including the nature of the application and the individual food manufacturers' production processes. The optimum use level should be assessed and adjusted using trials that reflect their particular processes.

The applicant provided a description of the colorimetric assay method used for determining enzyme activity.

Physical and chemical properties of commercial enzyme preparation	
Enzyme activity*	8000–12,000 NGXU/g
Appearance	Off-white powder
Optimum temperature range for maximum activity	42–69°C
Thermostability	No activity above 79°C (when holding for 30 minutes)
pH optimum	4.8–7.0

** As determined by NGX xylanase Konelab activity assay*

2.5 Food technology conclusion

FSANZ concludes that the proposed use of endo-1,4-beta-xylanase from GM *T. reesei* as a processing aid in starch processing and the production of potable alcohol is technologically justified. This is because it is consistent with its known technological function of hydrolysis of xylan which increases efficiency of starch processing and supports increased yield and purity of fermentation in potable alcohol production.

Analysis of the evidence provides adequate assurance that the proposed use of this enzyme, at a level not higher than necessary to achieve the desired enzyme reaction under GMP usage levels, is technologically justified.

Endo-1,4-beta-xylanase performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

3 Safety assessment

The objective of this safety assessment is to evaluate any potential public health and safety concerns that may arise from the use of this enzyme, produced by this microorganism, as a processing aid.

Some information relevant to this section is CCI, so full details cannot be provided in this public report.

3.1 History of use

3.1.1 Host organism

T. reesei (teleomorph: *Hypocrea jecorina*) is a species under the genus of *Trichoderma* (Family:*Hypocreaceae*; Order: *Hypocreales*; Class: *Sordariomycetes*; Phylum: *Ascomycota*; Kingdom:Fungi. *T. reesei* grows on decomposing plant material in nature (Schmoll, 2022).

T. reesei is frequently used to produce enzymes to catalyse the conversion of complex carbohydrates to simple sugars in food, feed and alcohol production, and has a long history of safe use (Nevalainen et al., 1994). FSANZ has approved 17 enzymes produced by *T. reesei* for use as processing aids, including: arabinofuranosidase, serine endopeptidase, alpha-glucosidase, and glucoamylase. Endo-1,4-beta-xylanases produced by *T. reesei* with the xylanase gene sourced from *Talaromyces leycettanus*, *Aspergillus niger*, and *Thermopolyspora flexuosa* respectively, are among the processing aids already approved by FSANZ.

The US Food and Drug Administration accepted the GRAS status of a range of enzymes produced by *T. reesei* in food production. For example, endo-1,4-β-xylanase produced by *T. reesei* carrying a gene that expresses endo-1,4-β-xylanase from *T. flexuosa* was accepted with a GRAS claim by FDA in 2015 (GRN 000628).

The production strain of *T. reesei* referred to in this application is *T. reesei* LVS-ETD-FVEXYN4-CL8-D3#15.2.3. This GM strain of *T- reesei* was derived from the wild type strain QM6a, registered as ATCC 13631 in the American Type Culture Collection.

T. reesei strains derived from QM6a are considered non-pathogenic and non-toxigenic and there is a long history of safe use in the production of industrial enzymes (Nevalainen et al., 1994; Blumenthal CZ 2004, Schmoll, 2022). A range of enzymes produced from the *T. reesei* QM6a safe strain lineage have been accepted as food processing aids by the U.S. Food and Drug Administration and Danish Veterinary and Food Administration. *T. reesei* LVS-ETD-FVEXYN4-CL8-D3#15.2.3 was selected as the production strain from within this safe strain lineage.

Consistent with enzyme preparations applied as processing aids, endo-1,4-beta-xylanase preparation referred to in this application does not contain the production strain. FSANZ is satisfied with the testing data provided by the applicant that demonstrated that the endo-1,4-beta-xylanse preparation does not contain any antimicrobial activity.

3.1.2 Gene donor organism

F. verticillioides is a pathogenic fungus associated with plants, particularly maize, and is able to produce fumonisins. Fumonisin-contaminated food has been linked to oesophageal

cancer in humans (Pitt, 2014; Choi and Shim, 2008). Fumonisins or other extraneous factors from *F. verticillioides* would not be carried across to the production organism because standard DNA methods were used to introduce the endo-1,4-beta-xylanase gene into *T. reesei* (see Section 3.2.1).

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of the DNA to be introduced and method of transformation

An expression cassette containing the endo-1,4-beta-xylanase gene was introduced into the genome of the host *T. reesei* strain by non-homologous recombination. The endo-1,4-beta-xylanase gene is an engineered variant from *F. verticillioides* and placed under the control of the *T. reesei cbh1* gene promotor and terminator. The expression cassette also contained an endogenous *T. reesei* orotate phosphoribosyl transferase (pyr2) selectable marker gene.

Data provided by IFF and analysed by FSANZ confirmed the identity of the endo-1,4-beta-xylanase enzyme. The enzyme has been protein engineered.

3.2.2 Characterisation of inserted DNA

FSANZ has analysed data provided by IFF and confirms the presence of the inserted DNA in the production strain.

3.2.3 Genetic stability of the inserted gene

The assessment confirmed the inserted gene is stably integrated into the genome of the production strain and does not have the ability to replicate autonomously.

To provide further evidence that the inserted gene is stable, the applicant provided genome sequencing results of the production strain at the beginning and end of industrial-scale fermentation. This analysis confirmed that the gene encoding the endo-1,4-beta-xylanase enzyme is stably maintained over multiple generations.

3.3 Safety of endo-1,4-beta-xylanase

3.3.1 History of safe use of the enzyme

Multiple endo-1,4-beta-xylanase enzymes are currently permitted as processing aids in Schedule 18 of the Code. However, *F. verticillioides* endo-1,4-beta-xylanase is not permitted and does not have a history of safe use in Australia or New Zealand. The applicant stated that the enzyme has been used for potable alcohol production and starch processing in Denmark since 2018.

There are no known reports of adverse effects arising from the consumption of xylanase, or specifically in relation to *F. verticillioides* xylanase, when used as a processing aid.

3.3.2 Bioinformatics concerning potential for toxicity

A BLAST search was performed (February 2022) by the applicant comparing the endo-1,4-beta-xylanase protein sequence against all entries in the UniProt[4] database using an E-value[5] threshold of 0.1. No matches to known toxins were found in the top 250 matches.

Another BLAST search was performed against the UniProt animal toxin database only. No sequence matches were found.

3.3.3 Toxicology data

The *F. verticillioides* endo-1,4-beta-xylanase test item used in the following toxicity studies was produced using GM *T. reesei* and represented the commercial enzyme product.

3.3.3.1 Animal studies

90-day repeated dose oral toxicity study in rats (MPI Research, Inc. 2015). Regulatory Status: GLP; conducted according to OECD Test Guideline (TG) 408.

The endo-1,4-beta-xylanase test item was administered to Sprague-Dawley rats Crl:CD(SD) (10/sex /group) at doses of 0, 250, 500 and 1000 mg TOS/kg bw/day by oral gavage for 13 weeks. The vehicle control was water.

Animals were observed daily for signs of toxicity. Body weight, food consumption and detailed clinical examinations for signs of toxicity were recorded weekly. Functional performance and locomotor activity testing, as well as ophthalmological examination, was conducted on all animals prior to treatment and at study termination. Gross pathology, histopathological examination and measurement of organ weights was conducted on all test animals at study termination.

No mortality occurred during the study. No treatment related effects were observed on feed consumption, body weights, haematology, clinical chemistry, ophthalmology, or functional observations or motor activity parameters. No remarkable macroscopic or histopathological changes were observed at necropsy.

The no observed adverse effect level (NOAEL) was 1000 mg TOS/kg bw/day enzyme, which was the highest dose tested.

3.3.3.2 Genotoxicity studies

Bacterial reverse mutation test (BioReliance 2015). Regulatory Status: GLP; conducted according to OECD TG 471.

The potential mutagenicity of endo-1,4-beta-xylanase was evaluated in *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA1535 and TA1537, and in the *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation using rat liver homogenate (S9). Mutation tests were conducted twice independently using the treat and plate method, over a

[4] UniProt database: https://www.uniprot.org

[5] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance.

dose range of 50–5000 µg protein/plate (51-5153 µg TOS/plate). The concentration range of the test item was based on the findings of a preliminary dose range finding study.

Positive controls in the absence metabolic activation were 2-nitrofluorene (TA98), *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (TA100, TA1535, WP2 *uvrA*) and ICR-191 (TA1537). The positive control in the presence of metabolic activation was 2-aminoanthracene (TA1535) for all strains. Sterile water was used as the vehicle control.

No concentration-related increases in revertant colonies were observed in cultures treated with the test item, with or without metabolic activation. All positive control treatments showed the anticipated increases in mutagenic activity demonstrating the validity of the assay. It was concluded that the endo-1,4-beta-xylanase test item was not mutagenic under the conditions of this test.

In vitro mammalian chromosomal aberration test (BioReliance 2015). Regulatory status: GLP; conducted according to OECD TG 473

The potential of endo-1,4-beta-xylanase to cause chromosomal aberrations in mammalian cells was tested using human lymphocytes isolated from peripheral blood, collected from a healthy female volunteer. Treatment with the endo-1,4-beta-xylanase test item was either a 4 hour pulse exposure with or without S9, followed by a 16 hour recovery; or 20 hours of continuous exposure without S9. Positive control assays were conducted in parallel using mitomycin C in the absence of S9 and cyclophosphamide in the short-term treatment with S9. The vehicle control was sterile water. The experiment was carried out once in duplicate.

Based on dose selection experiments, a dose range of 1000–5000 µg protein/mL of endo-1,4-beta-xylanase (1031–5153 µg TOS/mL) was examined for all test conditions.

There were no treatment related increases in chromosomal aberrations observed in peripheral blood lymphocytes following exposure to the endo-1,4-beta-xylanase test item, relative to the vehicle controls, under any of the conditions tested. The positive controls demonstrated a statistically significant increase in chromosomal aberration, validating the sensitivity of the experimental methodology. It was concluded that endo-1,4-beta-xylanase did not cause chromosome aberrations in peripheral blood lymphocytes, under the conditions of the study.

3.3.4 Potential for allergenicity

A FASTA search was performed using the amino acid sequence of endo-1,4-beta-xylanase using the AllergenOnline[6] database (queried February 2022) using two sequence alignments: an 80 mer sliding window (>35% homology) and the full-length protein (E-value less than 0.1). No matches with known allergens were found.

3.3.5 Assessments by other regulatory agencies

A letter of approval for the enzyme from the Ministry of Environment and Food in Denmark was provided by the applicant.

[6] AllergenOnline: http://www.allergenonline.org/

The applicant provided a generally recognised as safe (GRAS) expert opinion, which is not an assessment by the FDA and not accepted by FSANZ as an assessment by another agency.

3.4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worst-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the endo-1,4-beta-xylanase preparation remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass *et al* 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive. The TMDI can then be compared to an acceptable daily intake or a NOAEL to estimate a margin of exposure (MOE) for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020). The method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

In their budget method calculation, the applicant made the following assumptions:

- the maximum physiological requirement for solid food (including milk) is 25 g/kg bw/day;
- 50% of solid food is processed;
- all solid foods contain a ratio of raw material (wheat or starch) to the final food of 0.12 (1:8.3);
- the maximum physiological requirement for liquid is 100 mL/kg bw/day (the standard level used in a budget method calculation for non-milk beverages);
- 25% of non-milk beverages are processed;
- non-milk beverages contain a ratio of raw material (wheat or starch) to the final food of 0.05 (1:20);
- all solid foods and non-milk beverages contain the highest use level of 4.28 mg TOS/kg in the raw material (wheat or starch);
- all of the TOS from the enzyme preparation remains in the final food;
- all producers of alcoholic beverages and sweeteners (sugars and sugar syrups) would use this endo-1,4-beta-xylanase preparation;
- the final foods containing the theoretical amount of the endo-1,4-beta-xylanase preparation would be consumed daily over the course of a lifetime.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.011 mg TOS/kg bw/day.

As assumptions made by the applicant differ from those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI using the following assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

- The maximum physiological requirement for solid food (including milk) is 50 g/kg bw/day (the standard level used in a budget method calculation where there is potential for the enzyme preparation to be in baby foods or general purpose foods that would be consumed by infants).
- FSANZ would generally assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst-case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations for solid food is and non-milk beverages is 0.02 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes the assumption that all of the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that the enzyme is likely to either be inactivated or removed during processing. If any inactivated enzyme remained after processing, it would be present in insignificant quantities and perform no function in the final food to which the ingredient is added.

4 Discussion and Conclusion

No public health and safety concerns were identified in the assessment of endo-1,4-beta-xylanase from GM *T. reesei* under the proposed use conditions. *T. reesei* has a long history of safe use as a source of enzyme processing aids, including several that are already permitted in the Code. The *T. reesei* host is neither pathogenic or toxigenic. Analysis of the genetically modified production strain confirmed the presence and stability of the inserted DNA.

The enzyme did not show significant homology with known toxins, or with known food allergens. Results of genotoxicity assays were negative. For the endo-1,4-beta-xylanase, a NOAEL of 1000 mg TOS/kg bw/day was identified in a 90-day oral toxicity study in rats. The TMDI was calculated by the applicant and by FSANZ and is estimated to be 0.02 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in a large MOE of approximately 50,000.

Based on the reviewed data, it is concluded that in the absence of any identifiable hazard, an acceptable daily intake 'not specified' is appropriate.

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