FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment report – Application A1171 Endo-inulinase from *Aspergillus oryzae* as a processing aid (enzyme)

Executive summary

The purpose of the application is to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include the enzyme endo-inulinase (EC 3.2.1.7), from a genetically modified strain of *Aspergillus oryzae.* This production organism contains the endo-inulinase gene from *Aspergillus ficuum.* Endo-inulinase is proposed for use in hydrolysing inulin to produce fructo-oligosaccharides (FOS).

The evidence presented to support the proposed use of the enzyme provides adequate assurance that the enzyme, in its recommended form and amounts, is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme meets international purity specifications.

There are no public health and safety concerns associated with the use of endo-inulinase from *A. oryzae* as a food processing aid.

The production organism is not toxigenic nor pathogenic. *A. oryzae* has a long history of safe use as the production organism for a number of enzyme processing aids that are already permitted in the Code. Molecular characterisation of the production strain confirmed the sequence of the inserted DNA has not undergone any rearrangement, and the introduced DNA is stably inherited.

This endo-inulinase has been legally used in the EU, with no reports of adverse effects in consumers.

Results of genotoxicity assays were negative, and the enzyme shows no significant homology with known protein toxins. The No Observed Adverse Effect Level (NOAEL) in a 13-week repeat-dose oral gavage study in rats was 27500 UI/kg bw/day, equivalent in Total Organic Solids (TOS) to 189.65 mg/kg bw/day. The Theoretical Maximum Daily Intake (TMDI), expressed in TOS is 0.0069 mg/kg bw/day, and the Margin of Exposure (MoE) is therefore 27,486.

Bioinformatic analysis identified potential homology to minor allergens in tomato. Tomato is not considered by FSANZ to be a major allergen and is widely used as a food or ingredient of food.

Based on the reviewed toxicological data it is concluded that in the absence of any

identifiable hazard, an Acceptable Daily Intake (ADI) of ‘not specified’ is appropriate. A dietary exposure assessment was therefore not required.

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1 Introduction

Puratos NV applied to FSANZ for permission to use the enzyme endo-inulinase (EC 3.2.1.7) as a processing aid in hydrolysing inulin to produce fructo-oligosaccharides (FOS). This endo-inulinase is from a genetically modified (GM) strain of *Aspergillus oryzae,* containing the endo-inulinase gene from *Aspergillus ficuum.*

Currently, Schedule 18 of the Australia New Zealand Food Standards Code (the Code) permits the use of 18 different enzymes produced by *A. oryzae* (both GM and non-GM). However, the Code does not currently include a permission to use endo-inulinase produced by a GM strain of *A. oryzae* that contains the endo-inulinase gene from *A. ficuum.* Therefore, this enzyme needs a pre-market assessment before permission can be given for its use as a processing aid.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid

- 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 of the enzyme

The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using an appropriate enzyme nomenclature reference (IUBMB 2017).

Accepted IUBMB[1]/common name:	inulinase[2]
Systematic name:	1-β-D-fructan fructanohydrolase
IUBMB enzyme nomenclature:	EC 3.2.1.7
CAS[3] number:	9025-67-6
Other names:	**endo-inulinase**; inulase; indoinulinase;

[1] International Union of Biochemistry and Molecular Biology.
[2] As the accepted IUBMB name, with the EC number EC 3.2.1.7, 'inulinase' is the name used in the variation to the Code for this enzyme.
[3] Chemical Abstracts Service.

	exoinulinase; 2,1-β-D-fructan fructanohydrolase
Reaction:	Endohydrolysis of (2→1)-β-D-fructosidic linkages in inulin

2.1.2 Technological purpose of the enzyme

Inulin is a generic term used to describe polysaccharides of various lengths composed of fructose, typically with a single terminal glucose. It is naturally present in a range of plant foods including chicory root, Jerusalem artichoke, garlic and onion.

The technological purpose of endo-inulinase, as described by the applicant, is to catalyse the hydrolysis of (2→1)-β-D-fructosidic linkages in inulin to form FOS. This technological purpose is supported by the scientific literature (Zittan 1981).

FOS can be added to a variety of processed foods such as dairy products, cereal bars, meal replacement beverages, infant formula and infant foods as a sugar alternative, low caloric bulking agent, and for dietary fibre supplementation.

Schedule 18 of the Code currently permits the use of only one inulinase (EC 3.2.1.7) sourced from *A. niger.* However, the application notes that different inulinases can vary in their pH and temperature optima. Therefore, if permitted, this endo-inulinase will provide an additional option for producers of FOS. Which form of the enzyme a FOS manufacturer will use will depend on a range of factors, including performance under certain conditions and commercial considerations.

The physical and chemical properties of the enzyme preparation are presented in Table 1.

Table 1 *Endo-inulinase enzyme preparation physical/chemical properties*

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	2500 – 3000 IU/ml[4]
Appearance	Brown liquid
Temperature optimum	60 – 70°C
Thermal stability	Not thermally stable above 70°C
pH optimum	4 – 6
Storage stability	>12 months between 0°C and 7°C

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 technical data sheet provided by the applicant advises that the optimum use level should be assessed by individual manufacturers through trials that reflect their processes and the required degree of hydrolysis of inulin.

Endo-inulinase is denatured at >80°C after performing its technical function. As such, there is no active enzyme remaining in the FOS or the foods to which FOS is added.

[4] The method by which the enzyme activity is measured, including an explanation of the units has been provided as part of the applicant's CCI material.

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme is produced using submerged fed-batch fermentation, which is the commonly used and well understood production method to produce food enzymes. The fermentation process is completely closed to prevent contamination from any foreign microorganisms and other contaminants from the outside. Fermentation is ceased when the optimal level of biomass and enzymatic activity is obtained.

The recovery process involves separating the biomass from the enzyme-containing culture medium by a series of filtration steps, resulting in an enzyme-containing liquid that is concentrated and free from any microorganisms or other impurities. The applicant has provided information to demonstrate that the manufacture of the enzyme follows current Good Manufacturing Practice (cGMP) and the principles of HACCP.

The final enzyme product is sold under the commercial name Oligofruct'Ase 3000. FSANZ has assessed the formulation ingredients and can confirm that they are compatible with usage in food. The main steps of the manufacturing process are shown in Figure 2 below taken from the application.

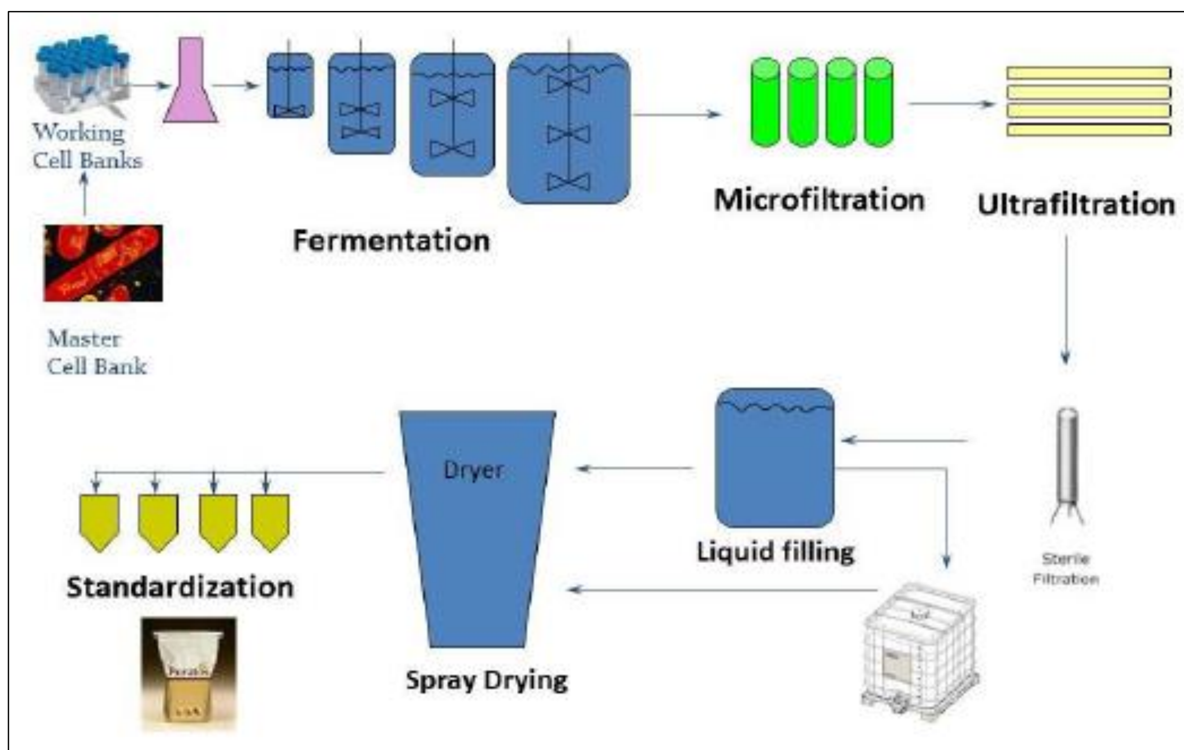

Figure 2 *Production process of endo-inulinase food enzyme from fermentation*

2.2.2 Allergen considerations

The fermentation medium contains a carbon source that may comprise, for example, sucrose, maltose, glucose, maltodextrins or starch. It also contains a nitrogen source chosen from, for example, peptones, protein hydrolysates, yeast extracts, glutamate or urea. The medium is also typically supplemented with various inorganic salts. The applicant states that the enzyme preparation does not contain any allergens that could originate from the

fermentation medium. Further, Appendix 7 indicates nil presence of allergens in the enzyme preparation (through both the ingredients or possible cross contamination).

2.2.3 Specifications

The JECFA Compendium of Food Additive Specifications (2017) and the Food Chemicals Codex 11th edition are international specifications for enzymes used in the production of food. These are primary sources of specifications listed in section S3—2 of Schedule 3 of the Code. Enzymes need to meet these specifications. Schedule 3 of the Code also includes specifications for heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

Table 2 provides a comparison of the analysis of different batches of the endo-inulinase product 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 preparation meets all relevant specifications.

Table 2 *Analysis of Puratos enzyme endo-inulinase compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes (3 batches)*

Analysis	**Puratos analysis**	**Specifications**		
		JECFA	*Food Chemicals Codex*	*Australia New Zealand Food Standards Code (section S3—4)*
Lead (mg/kg)	<0.10	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	<0.10	-	-	≤1
Cadmium (mg/kg)	<0.010	-	-	≤1
Mercury (mg/kg)	<0.010	-	-	≤1
Coliforms (cfu/g)	<10	≤30	≤30	-
Salmonella (in 25 g)	Absent	Absent	Negative	-
E. coli (in 25 g)	Absent	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-
Mycotoxins	<LOQ	No toxicologically significant levels		

2.3 Food technology conclusion

The use of this endo-inulinase in hydrolysing inulin to produce FOS is clearly described in the application. The evidence presented to support its proposed use provides adequate assurance that the enzyme, in the form and amounts used (which must be consistent with GMP), is technologically justified and effective in achieving its stated purpose. The enzyme meets international purity specifications.

3 Safety assessment

3.1 History of use

3.1.1 Host organism

Aspergillus oryzae (Ahlburg) *Cohn* var. *oryzae* is a biosafety level 1 organism not commonly associated with human disease. *A. oryzae* strains have been used for centuries for the production of fermented products such as sake, miso and soy sauce. Therefore, these bacterial strains have a long history of safe use in food (Abe and Gomi, 2008). The combination of its safe use in food with the ability to grow in large-scale cultures and to express heterologous proteins, means *A. oryzae* is a preferred strain for the industrial production of food enzymes (Braaksma and Punt, 2008).

The recipient strain of *A. oryzae* was purchased from the Belgian Co-ordinated Collections of Microorganisms (BCCM). Sequence analysis of the genetically modified production strain targeting the ITS and 18S genes confirmed the strain as *A. oryzae*.

3.1.2 Gene donor organisms

Aspergillus ficuum is classified as a biosafety level 1 organism. Although not commonly used for the production of food components, it has been used for the industrial production of enzymes used in animal feed such as phytase (Bogar et al, 2003), without adverse effects.

Aspergillus nidulans is classified as a biosafety level 1 organism, however some strains have been associated with opportunistic infections in immunocompromised individuals (Gabrielli et al, 2014; Sadarangani et al, 2015). As the gene sequence has been manipulated through standard DNA cloning methods subsequent to the original isolation from the donor organism, extraneous material from *A. nidulans* would not be carried across to the enzyme production organism.

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of DNA to be introduced and method of transformation

The endo-inulinase gene and terminator sequence was isolated from *A. ficuum* by targeted amplification of genomic DNA. The amplification primers used allowed modification of the existing signal peptide to a secretion signal peptide, at the amino-terminus. This signal peptide is removed during intracellular processing and is not present in the mature product secreted from the production strain. An expression plasmid was then constructed using a pBlueScriptSK vector, with the endo-inulinase gene and terminator sequence sitting behind a well characterised promoter sourced from *A. nidulans*.

A second plasmid was generated containing the acetamidase gene (*amdS)* from *A. nidulans* (Kelly and Hynes, 1985). The *amdS* gene allows for selection of transformants on acetamide-containing media and has been widely used as a selection marker in fungal transformations. The inclusion of this selection marker is standard in filamentous fungi systems producing a variety of recombinant gene products (Gryshyna *et al.,* 2016) and there are no safety concerns.

Both plasmids contain an ampicillin resistance gene to allow for selection of transformants during passaging in *Escherichia coli*. Expression of the antibiotic resistance gene is driven by a bacterial promoter that is not functional in fungal species.

The two plasmids were introduced into the filamentous fungal host using a polyethylene glycol-mediated protoplasts transformation method.

3.2.2 Characterisation of the inserted DNA

Southern blotting, using a probe targeting the entire coding region of endo-inulinase gene, was performed on genomic DNA extracted from the production strain (MUCL 44346) and parental host strain. The results showed that a single copy of the enzyme plasmid is integrated into the genome of the production strain. Hybridisation with a probe targeting the ampicillin resistance gene also confirmed the existence of single copies of each plasmid.

3.2.3 Stability of the production organisms and inheritance of the introduced DNA

Polymerase chain reaction (PCR) targeting the entire coding region of the endo-inulinase gene was performed on genomic DNA extracted from the production strain (MUCL 44346), comparing an early isolate of the production strain with three separate cultures run for 10 days. The parental host was used as a negative control and the expression plasmid was used as a positive control for the PCR. The results confirmed presence of the gene across several generations, indicating the inserted DNA is genetically stable and inherited.

3.2.4 Presence of recombinant DNA in the enzyme product

Three different batches of the enzyme product, prior to formulation, were tested for the presence of recombinant DNA from MUCL 44346. Based on the verified limits of detection, no recombinant DNA was detected.

3.3 Safety of endo-inulinase

3.3.1 History of safe use

The enzyme that is the subject of this application has been legally used in the European Union (EU), with no reports of adverse effects. It has been submitted for evaluation to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA).

FSANZ has previously approved an inulinase produced by another fungus in the same genus as the production and donor organisms, *A. niger*, and permits 16 other enzymes produced by *A. oryzae* for use as processing aids.

3.3.2 Bioinformatic assessment of enzyme toxicity

The applicant provided results of an *in silico* analysis using the NCBI protein database[5] comparing endo-inulinase to proteins associated with the keyword “toxin”. A BLAST search with a threshold E-value of 0.1 did not identify any similarity to known protein toxins.

3.3.3 Toxicology studies in animals

Reports of two oral gavage studies in rats were provided by the applicant. The first study, a two-week repeat-dose study, served as a dose-rangefinder for the second study, a 13-week repeat-dose study. The test article for both studies was the enzyme that is the subject of this application.

Two-week repeat-dose oral gavage study in Sprague Dawley rats (CIT 2012). Regulatory

[5] https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins

status: Not GLP

Sprague Dawley rats were acclimated to standard laboratory environmental conditions for 5 days prior to commencement of dosing. Rats were group-housed, 3/cage, in polycarbonate cages, and had ad libitum access to standard rat food and to water. Rats were assigned to groups, 3/sex/group, by a computerized stratification procedure. Rats were 6 weeks old at the start of dosing. The negative control article was water. Rats were dosed with 0, 2750, 11000 or 27500 UI/kg/day endo-inulinase by oral gavage, daily for 14 days, at a daily dose volume up to 10.58 mL. Rats were subject to twice-daily moribundity/mortality checks, a daily cageside clinical observation and a weekly detailed clinical observation. Bodyweights were recorded prior to Day 1, on Day 1 of dosing, and at least once weekly during the in-life phase. Food consumption was measured once weekly. At the end of the dosing period, rats were fasted overnight, weighed, anesthetized with intraperitoneal sodium pentobarbital and exsanguinated. Fresh organ weights of brain, heart, kidneys, liver, lungs, spleen, thymus, gonads and, in the case of males, epididymides were recorded and all those organs, and also adrenal glands, were preserved, although no histopathology was performed.

All rats survived to scheduled termination and no abnormal clinical signs were observed. Treatment had no effect on group mean values for food consumption, bodyweight, bodyweight change, or absolute or relative organ weight. There were no gross findings at necropsy in any rat.

13-week repeat-dose oral gavage study in Sprague Dawley rats (CIT 2014). Regulatory status: GLP; based on OECD Guideline 408.

Sprague Dawley rats were acclimatized to standard laboratory environmental conditions for 8 days prior to study start. Rats were pair-housed in polycarbonate cages and had *ad libitum* access to standard rat maintenance diet and to filtered tap water. Rats were assigned to treatment groups, 10/sex/group, using a computerized stratification procedure. Target dose levels were 0, 2750, 11000 and 27500 UI/kg bw/day endo-inulinase, in a dose volume up to 10.58 mL/kg bw/day, using water as the negative control article. However from Day 60, dose levels were 0, 3134, 12508 and 31285 UI/kg bw/day, because the test article was restocked from a more concentrated batch of enzyme. Rats were administered the control article or test article by oral gavage, once daily for 91 days.

Rats were subject to moribundity/mortality checks twice daily, and a cageside observation once daily, throughout the treatment period. Detailed clinical observations were conducted prestudy and once weekly during the in-life phase. Body weights were recorded prestudy, on Day 1 of dosing and once weekly during the in-life phase, and food consumption was recorded weekly. An ophthalmological examination was conducted prestudy on all rats, and on the control and 27500 UI/kg bw/day rats during the final week of the in-life phase. During week 11, all rats were subject to a detailed examination in the home cage, in-hand and in a standard arena. This included recording forelimb grip strength, landing foot splay, rectal temperature and a number of reflexes and responses, and measurement of motor activity over a 60-minute period. At the end of the in-life phase, rats were fasted overnight with access to water, prior to blood collection for haematology, prothrombin time and clinical chemistry. Rats were then anesthetized with sodium pentobarbital and killed by exsanguination. Each rat was subject to a complete gross necropsy which included recording fresh weights of adrenals, brain, heart, kidneys, liver, spleen, thymus and gonads, as well as epididymides of males and uterus of females. A comprehensive list of tissues was fixed from each rat. All tissues from the control and 27500 UI/kg bw/day rats were examined microscopically, and histopathological examination was also conducted on ovaries, uterus and vagina of females in the other treatment groups, due to findings in the high-dose group.

All rats survived to scheduled termination and there were no treatment-related clinical

observations. Motor activity and performance on the Functional Observational Battery, both assessed in Week 11, showed no treatment-related effects. Treatment did not affect group mean values for body weight, body weight change, or food consumption, and no treatment-related effects on haematology, prothrombin time, clinical chemistry or ophthalmology were discovered.

There were no treatment-related gross lesions on necropsy. A statistically significant increase in mean absolute and relative liver weight was observed in 27500 UI/kg bw/day males, but not in females. There were no microscopic correlates and this finding was considered to be of doubtful toxicological relevance. Incidence and severity of mucification of the vaginal epithelium was increased, relative to that of controls, in female rats treated with 27500 UI/kg bw/day. This was not considered to be an adverse effect. It was therefore concluded that the NOAEL was 27500 UI/kg bw/day, equivalent to 189.65 mg/kg bw/day Total Organic Solids (TOS).

3.3.4 Genotoxicity assays

Reports of two genotoxicity studies, a bacterial reverse mutation assay (Ames test), and a chromosomal aberration study in mouse lymphocytes, were provided by the applicant.

Bacterial reverse mutation assay (SafePharm 2003). Regulatory status: GLP; in compliance with OECD Guideline 471, Method B13/14 of Commission Directive 2000/32/EC ad the US-EPA (TSCA) OPPTS harmonised guidelines.

Test systems for this assay were *Salmonella typhimurium* strains TA1535, TA102, TA100, TA1537 and TA98. The test article was the enzyme that is the subject of the current application. The solvent and negative control article was sterile distilled water. For assays conducted in the absence of S9 mix for metabolic activation, positive control articles were N-ethyl-N'-nitro-N-nitrosoguanidine for TA100 and TA1535, 9-aminoacridine for TA1537, Mitomycin C for TA102 and 4-nitroquinoline-1-oxide for TA98. For assays conducted in the presence of S9 mix, the positive control articles were benzo(a)pyrene for TA98, 1,8-dihydroxyanthraquinone for TA102, and 2-aminoanthracene for TA100, TA1535 and TA1537. All assays were conducted in triplicate using the plate incorporation method.

Concentrations tested in the preliminary toxicity test, which was conducted using TA100 were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. For each plate, bacterial culture, top agar, test or control article, and S9 mix or phosphate buffer were mixed and overlaid on sterile plates containing agar. Plates were incubated for approximately 48 hours at 37°C and then assessed for numbers of revertant colonies and examined for effects on the growth of the bacterial lawn. There was no evidence of toxicity to TA100.

The definitive mutation test, using all the bacterial test strains, was conducted twice using the same method, with test article concentrations of 50, 150, 500, 1500 and 5000 µg/plate. There was no evidence of cytotoxicity and no significant increase in frequency of revertant colonies, compared to negative control plates, for any of the strains of *Salmonella* at any dose level of the test article, with or without metabolic activation. The positive control articles all induced the expected significant increases in numbers of revertant colonies, confirming the validity of the assay. It was concluded that the test article is not mutagenic under the conditions of this assay.

In vitro *micronucleus test in L5178Y $TK^{+/-}$ mouse lymphoma cells (CIT 2013). Regulatory status: GLP; in compliance with OECD guideline 487.*

The test article was the enzyme that is the subject of this application. The vehicle and negative control article was sterile water for injection. For both short (3 hour) and long (24

hour) treatment assays in the absence of S9 mix, the positive controls were Mitomycin C as a clastogen and colchicine as an aneugen. For short treatment with S9 mix, the positive control article was cyclophosphamide as a clastogen. A preliminary toxicity test was conducted with exposure for 3 hours with and without S9 mix, and for 24 hours without S9 mix. Test article concentrations were 10, 100, 500, 1000, 2500 and 5000 µg/mL. No precipitation was observed in the culture medium at any dose level. There was no clear evidence of dose-related cytotoxicity. Accordingly, test article concentrations for two mutagenicity experiments, with or without S9 mix, were 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL for the first experiment and 625 2150, 2500 and 5000 µg for the second experiment. Each treatment was conducted in duplicate in each experiment. In the first experiment, cells were exposed to the test article, with or without S9 mix, for 3 hours, followed by 24 hours recovery. In the second experiment, cells were either exposed to the test article without S9 mix for 24 hours with 20 hours recovery, or with S9 mix for 3 hours with 24 hours recovery. Incubation for all exposure and recovery periods was 37°C. Cells were examined for evidence of cytotoxicity, and for the presence of micronuclei in 1000 mononucleated cells/culture (2000 mononucleated cells per dose).

No noteworthy toxicity was observed in any culture, and there was no significant increase in the frequency of micronucleated cells in any culture. All positive control articles induced significant increases in micronucleated cells, confirming the validity of the assay.

It was concluded that the test article did not induce any chromosome damage or damage to the cell division apparatus in L5178Y TK$^{+/-}$ mouse lymphoma cells, with or without the presence of a metabolizing system.

3.3.5 Potential for allergenicity

Bioinformatics searches of the AllergenOnline database (http://www.allergenonline.org) were conducted by the applicant and verified by FSANZ, using the amino acid sequence of the enzyme without the signal peptide as the query. Using the Full Fasta and the 80 amino acid sliding window search, there were two hits, both putative minor allergens of the tomato (*Solanum lycopersicum*, also known as *Lycopersicon esculentum*). No matches were found using a search for 8 contiguous amino acids. Tomato is widely used in the diet and is not recognized as a significant source of allergic reactions.

The applicant states that the enzyme preparation does not contain any allergens that could originate from the fermentation medium.

3.3.6 Approvals by other regulatory agencies

No approvals by other regulatory agencies are available.

4 Discussion

There are no public health and safety concerns associated with the use of endo-inulinase from *A. oryzae* as a food processing aid.

The production organism is not toxigenic nor pathogenic. *A. oryzae* has a long history of safe use as the production organism for a number of enzyme processing aids that are already permitted in the Code. Molecular characterisation of the production strain confirmed the sequence of the inserted DNA has not undergone any rearrangement, and the introduced DNA is stably inherited.

This endo-inulinase has been legally used in the EU, with no reports of adverse effects in consumers.

Results of genotoxicity assays were negative, and the enzyme shows no significant homology with known protein toxins. The NOAEL in a 13-week repeat-dose oral gavage study in rats was 27500 UI/kg bw/day, equivalent in TOS to 189.65 mg/kg bw/day. The Theoretical Maximum Daily Intake (TMDI), expressed in TOS is 0.0069 mg/kg bw/day, and the Margin of Exposure (MoE) is therefore 27,486.

Bioinformatic analysis identified potential homology to minor allergens in tomato. Tomato is not considered to be a major allergen and is widely used in food.

5 Conclusion

Based on the reviewed data it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate. A dietary exposure assessment was therefore not required.

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