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Te Mana Kounga Kai - Ahitereiria me Aotearoa

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Supporting document 1

Risk and technical assessment – Application A1350

Lysophospholipase from *Trichoderma reesei* (gene donor: *Aspergillus niger*) for use as a processing aid

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Executive summary

Danisco Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme lysophospholipase (EC 3.1.1.5) from *Trichoderma reesei* containing the lysophospholipase gene from *Aspergillus niger*. The enzyme is proposed to be used as a processing aid in starch processing for production of glucose syrups and other starch hydrolysates.

The available evidence provides adequate assurance the proposed use of lysophospholipase from *T. reesei* as a processing aid is technologically justified. Lysophospholipase performs its technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code.

The microbiological assessment undertaken by FSANZ did not identify any public health or safety concerns associated with the use of the production strain. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

No significant homology between lysophospholipase from *T. reesei* and known toxins or allergens was identified. The enzyme was not genotoxic *in vitro*, and no adverse effects were observed in a 90-day oral toxicity study in rats. The no observed adverse effect level (NOAEL) was 1000 mg total organic solids (TOS)/kg bw/day, the highest dose tested.

The theoretical maximum daily intake (TMDI) of this lysophospholipase was calculated to be 0.1 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of approximately 10,000.

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. FSANZ concludes there are no public health and safety concerns with the proposed use of lysophospholipase from *T. reesei*.

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1 Introduction

Danisco Australia Pty Ltd[1] has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of lysophospholipase (EC 3.1.1.5) as a processing aid in starch processing. The enzyme is produced by *Trichoderma reesei* containing the gene for lysophospholipase from *Aspergillus niger*.

The enzyme will be used in the production of glucose syrups and other starch hydrolysates. It will be utilised at the minimum level required to achieve the intended technological purpose, in accordance with Good Manufacturing Practice (GMP).[2]

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely a technological 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 as a processing aid by considering the:
  - safety and history of use of the production microorganism
  - safety of the enzyme preparation.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided information regarding the identity of the enzyme, which has been verified using the IUBMB[3] enzyme nomenclature reference database (McDonald et al. 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	lysophospholipase
Systematic name:	2-lysophosphatidylcholine acylhydrolase
Other names/common names:	lecithinase B; lysolecithinase; phospholipase B; lysophosphatidase; lecitholipase; phosphatidase B; lysophosphatidylcholine hydrolase; lysophospholipase A1; lysophopholipase L2; lysophospholipase-transacylase; neuropathy target esterase; NTE; NTE-LysoPLA; NTE-lysophospholipase
IUBMB enzyme nomenclature:	EC 3.1.1.5

[1] A subsidiary of International Flavors and Fragrances Inc.

[2] GMP is defined in section 1.1.2—2 of the Code as follows: ***GMP*** *or* ***Good Manufacturing Practice****, with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*

(a) limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and

(b) to the extent reasonably possible, reducing the amount of the substance or its derivatives that:

*(i) remains as a *component of the food as a result of its use in the manufacture, processing or packaging; and*

(ii) is not intended to accomplish any physical or other technical effect in the food itself;

(c) preparing and handling the substance in the same way as a food ingredient.

[3] International Union of Biochemistry and Molecular Biology.

CAS number:	9001-85-8
Reaction:	lysophospholipase catalyses the below chemical reaction (see Figure 1) 2-lysophosphatidylcholine + H_2O = glycerophosphocholine + a carboxylate

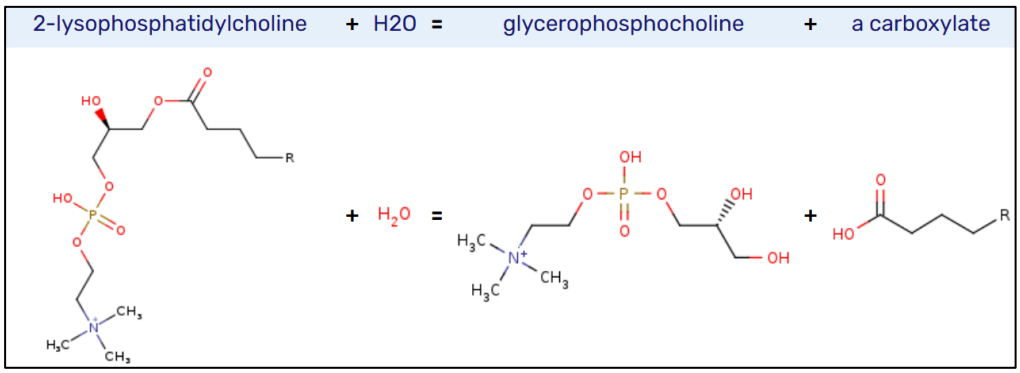

Figure 1: Reaction catalysed by lysophospholipase (source: BRENDA)

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps.

The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020b).

Lysophospholipase is produced by submerged fermentation of a *T. reesei* strain carrying the gene for lysophospholipase from *A. niger*. The batch or fed-batch fermentation process includes inoculating pure culture into fermentation media, followed by seed and main fermentation stages. Once fermentation is complete, the enzyme-containing media is separated from biomass and cell remnants, concentrated, and filtered using polish and germ filtration. The resulting product is then formulated as a liquid enzyme preparation.

The typical composition of the liquid enzyme preparation is shown in Table 1.

Table 1: Typical composition of lysophospholipase preparation

Component	Approximate % w/w
Lysophospholipase enzyme	0-1
Water	55-72
Glycerol	25-35
Sodium chloride	5.0-6.5
Sodium benzoate	0.3-0.4
Potassium sorbate	0.15-0.25

The applicant has stated the enzyme is manufactured with appropriate GMP controls and processes to ensure compliance with relevant specifications and that the final product does not contain hazardous or toxic impurities. The resultant product meets the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Food Chemicals Codex (FCC) (see also section 2.2.2 of this report).

Information on the raw materials used in the production and recovery of the enzyme preparation has been evaluated by FSANZ but deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) and cannot be disclosed in this report.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by JECFA in its Compendium of Food Additive Specifications (FAO JECFA Monographs 34 (2025)), explicitly FAO/WHO (2006), and in FCC (14th edition, 2022), referenced in section S3—2 of Schedule 3 of the Code. Enzymes used as processing aids need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3.

Schedule 3 of the Code includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant provided a certificate of analysis for three independent batches of the lysophospholipase enzyme preparation (section A.5 and Annex 5 of the application). Table 2 provides a comparison of the summary results of those analyses with the international specifications established by JECFA and FCC, as well as those in the Code. Based on those results, the enzyme met all relevant specifications.

In addition, the specification for identity and purity of the enzyme product provided by the applicant indicates an absence of the production strain and several mycotoxins.

Table 2: Analysis of three representative batches of the enzyme preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Test parameters	Test results	Specifications		
		JECFA	Food Chemicals Codex	The Code – section S3—4
Lead (mg/kg)	<0.01, <0.01, <0.5	≤5	≤5	≤2
Arsenic (mg/kg)	<0.01, <0.01, <0.5	-	-	≤1
Cadmium (mg/kg)	<0.01, <0.01, <0.1	-	-	≤1
Mercury (mg/kg)	<0.01, <0.01, <0.01	-	-	≤1
Coliforms (cfu/g)	1, <1, <1	≤30	≤30	-
***Salmonella* (in 25 g)**	Negative	Absent	Negative	-
***Escherichia coli* (in 25 g)**	Negative	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-
Mycotoxins	Negative for several mycotoxins[a]	No toxicologically significant amounts	-	-

[a] Information about the identity of the mycotoxins tested was provided in a confidential appendix.
ND = not detected; LOD = limit of detection; cfu = colony forming units

2.3 Technological purpose

The lysophospholipase enzyme preparation is intended for use as a processing aid in starch processing to produce glucose syrups and other starch hydrolysates. The applicant requested use of the enzyme at GMP levels, with doses up to 100 g of enzyme per metric tonne of dry material.

Lysophospholipase catalyses the cleavage of a fatty acid chain from a lysophospholipid. For example, 2-lysophosphatidylcholine is hydrolysed into glycerophosphocholine and a free fatty acid by this enzyme (see section 2.1).

In the manufacture of glucose syrups and other starch hydrolysates, lysophospholipids that are present in cereal starches can aggregate and interfere with processing steps that involve filtration of the hydrolysate (Morrison 1988, Matser and Steeneken 1998). As stated in the application, the use of lysophospholipase to break down lysophospholipids during starch processing can improve filtration efficiency and enhance syrup clarity.

The technological purpose stated by the applicant is consistent with the typical function of lysophospholipase. This is supported by scientific literature, which indicates the enzyme is principally responsible for the hydrolysis of lysophospholipids and is utilised in starch processing to improve the filtration of hydrolysates (Słomińska and Niedbach 2009, Nazir and Sajjad 2025, Köhler et al. 2006).

Lysophospholipase performs its technological function during food manufacturing and has no function in the final food. The applicant stated that the enzyme is inactivated by processes that use high temperature or else removed during steps that purify the syrup or hydrolysate (e.g. filtration, carbon treatment and ion exchange). Thus, the enzyme meets the definition of a processing aid and does not function in the final food. Information on the physical and chemical properties of the enzyme, as provided by the applicant, are summarised in Table 3.

Table 3: Lysophospholipase enzyme preparation physical and chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	3740-5060 U/g[a]
Appearance	Liquid, light yellow colour
Temperature optimum	Optimum activity within range 50–70°C
Thermostability	Enzyme has no activity when incubated for 15 min at temperatures above 75°C (pH 4.5)
pH optimum	Optimum activity within pH range 3.5–5.5

[a] U = enzyme activity units, where 1 unit (U) is the amount of enzyme that will form 1 micromole of fatty acid per minute from L-α-lysophosphatidylcholine under specified conditions (temperature, pH, substrate type). The enzyme activity range provided is for an example product and may differ based on the final product.

2.4 Allergen considerations

The applicant provided an allergen statement for the enzyme preparation that is supplied to customers. Liquid glucose derived from wheat is used as a fermentation ingredient.

2.5 Food technology conclusion

The use of lysophospholipase as a processing aid in starch processing to produce glucose syrups and other starch hydrolysates is consistent with its typical function of lysophospholipid hydrolysis. The reported advantages when used in starch processing include improved filtration efficiency and enhanced syrup clarity. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified and effective in achieving its stated purpose.

Lysophospholipase performs its technological purpose during starch processing, after which it is inactivated and/or removed during processing steps, and does not perform a technological purpose in the final food. It is therefore functioning as a processing aid for the purposes of 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

This safety assessment aims to evaluate potential public health and safety concerns arising from using the lysophospholipase enzyme associated with a genetically modified strain of *T. reesei* as a processing aid.

Some information relevant to this section is CCI, so full details cannot be disclosed under the FSANZ Act.

3.1 Source microorganism

FSANZ has previously assessed the safety of *T. reesei* as the source organism for more than 18 processing aids as listed in Schedule 18. Several enzymes produced by *T. reesei* QM6a have Generally Recognized as Safe (GRAS) status with the Food and Drug Administration (FDA) or FDA had no questions about the GRAS conclusions contained in

GRAS submissions to FDA (US EPA 2012).

Trichoderma reesei is a biosafety level 1, common, hypercellulolytic, soil fungus that was initially isolated from deteriorating canvas made from cellulosic material. Strain QM6a is the original wild-type strain for *T. reesei* and has been registered with the American Type Culture Collection as ATCC13631 (Olempska-Beer et al. 2006). Strain QM6a is the parental strain of the majority of *T. reesei* industrial production strains (Nevalainen et al. 1994).

T. reesei has a history of safe use in industrial-scale enzyme production (Nevalainen et al. 1994, Blumenthal 2004, Nevalainen and Peterson 2014, Paloheimo et al. 2016, Frisvad et al. 2018). Food enzymes derived from *T. reesei* strains (including recombinant *T. reesei* strains) have been evaluated by JECFA and many countries which regulate the use of food enzymes, such as Australia and New Zealand, USA, France, Denmark and Canada.

T. reesei QM6a derived strains are non-pathogenic, not known to possess any virulence factors associated with either colonisation or disease, and do not present any human toxicity concerns (US EPA 2012). Although some *Trichoderma* species can produce various mycotoxins and antifungal metabolites, several review papers support the safety of *T. reesei* QM6a strains concluding that there was no production of known mycotoxins or antibiotics under conditions used for enzyme production (Nevalainen et al. 1994, Kubicek et al. 2007, Peterson and Nevalainen 2012, Frisvad et al. 2018). *T. reesei* QM6a strains are known to produce the peptaibol antibiotic paracelsin, but industry-standard submerged fermentation conditions are not linked to the production of paracelsin (US EPA 2012).

The applicant provided data that adequately demonstrates the production strain's identity as a derivative of *T. reesei* QM6a. Microbiological data was provided to FSANZ confirming the absence of the production organism and mycotoxins in the final enzyme preparation.

The microbiological assessment undertaken by FSANZ did not identify any public health or safety concerns related to the use of the applicant's *T. reesei* production strain as a production organism for lysophospholipase.

3.2 Characterisation of the genetic modification to the production strain

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

The gene encoding the lysophospholipase enzyme is a synthetic gene encoding the wild-type *A. niger* lysophospholipase. Data provided by the applicant and analysed by FSANZ confirmed the identity of the lysophospholipase gene.

The gene was inserted into the genome of the host *T. reesei* through non-homologous recombination and placed under the control of a *T. reesei cbh1* promotor. The inserted DNA also contained the *T. reesei pyr2* gene, which was used as a selectable marker.

3.2.2 Characterisation of the inserted DNA

High-coverage PacBio whole-genome sequencing data was provided by the applicant. This sequencing approach enabled near-complete (>99.9%) genome assemblies, ensuring sufficient resolution to detect the location of the inserted expression cassette and any rearrangements. The analysis demonstrated that the inserted DNA remained present at the expected genomic locus, with no additional integration sites and no changes in flanking genomic sequences. The data also confirmed the absence of bacterial vector DNA in the final production strain.

3.2.3 Stability of the introduced DNA

Genetic stability of the production strain was assessed by whole-genome sequencing of DNA obtained from a seed culture and three independent end-of-fermentation batches. These data confirmed the gene is expressed stably over multiple generations.

3.3 Safety of the enzyme

3.3.1 History of safe use

Several lysophospholipase enzymes from other sources are already permitted for use as processing aids in the Code. Lysophospholipase from *A. niger* is listed in S18—4 (Permitted enzymes). Lysophospholipase sourced from *T. reesei* containing the gene for lysophospholipase isolated from *Aspergillus nishimurae* is listed in S18—9 (Permitted processing aids—various technological purposes) for use in starch processing, including the production of syrups, at GMP.

The applicant's lysophospholipase enzyme has been determined to be GRAS in the USA and the US FDA has issued a 'FDA has no questions' response to the applicant's submission (US FDA 2021). The enzyme has also been approved by several overseas authorities, however their identities are claimed as CCI. No adverse events have been reported since the enzyme has been in commercial use in these countries.

3.3.2 Bioinformatic assessment of homology with known toxins

The applicant conducted two BLAST searches for similarity of the lysophospholipase amino acid sequence with known toxins using the UniProt annotated Protein Knowledge database[4]. The first search was for homology of the lysophospholipase sequence against the complete UniProt database, using a threshold E-value[5] of 0.1. The majority of the database matches were phospholipases and lysophospholipases, and no matches were annotated as being a toxin or venom. In addition, a specific BLAST search for homology with sequences in the UniProt animal toxin database was performed. No matches were identified. Based on these assessments the lysophospholipase sequence does not share homology with a known toxin or venom.

3.3.3 Toxicology data

The applicant submitted reports of two genotoxicity studies of the lysophospholipase enzyme concentrate as well as a 90-day oral toxicity study in rats. The enzyme concentrate tested was produced using the manufacturing method reviewed in section 2, and the total organic solids (TOS) content was 17.83%.

Short-term toxicity

90-day oral toxicity study in rats (Jai Research Foundation 2023a) Regulatory status: GLP; conducted in accordance with OECD Test Guideline 408 (2018)

Lysophospholipase (Batch/Lot no. 22RA082) was administered by oral gavage to Wistar (RccHan: WIST) rats (10/sex/group) at doses of 0, 250, 500 or 1000 mg TOS/kg bw/day for 90 days. The vehicle/control was water. Clinical signs were monitored throughout the study and body weight, body weight change and food consumption were recorded weekly. An

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

[5] In BLAST (Basic Local Alignment Search Tool), the E-value or *Expect value* is a key metric used to assess the statistical significance of an alignment between the query sequence and sequences in a database. Lower E-values indicate that the match is less likely to have occurred by chance.

ophthalmological examination was performed on all rats before the start of treatment and before study termination. Neurobehavioural observations were performed weekly and during week 12 of the study functional observational battery (FOB) parameters were evaluated. Vaginal smears were collected on the day of necropsy from all female rats to determine the stage of the oestrus cycle. At the end of treatment blood and urine samples were collected from all rats for haematology, coagulation, thyroid hormone assays, clinical chemistry, and urinalysis. Rats were subjected to a full gross necropsy at the end of the study. Organ and tissue weights were recorded and histopathological examination was performed on organs and tissues from the control and high dose groups, as well as any tissues with gross lesions from the low and mid dose groups.

No mortality, morbidity or clinical signs of toxicity were observed during the study. There were no treatment-related changes in body weight, body weight gain, food consumption, ophthalmological findings, neurobehavioural observations, FOB, oestrous cyclicity, haematology, coagulation, thyroid hormones, clinical chemistry or urinalysis. No treatment-related effects were observed on organ weights or upon gross and microscopic examination.

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

Genotoxicity

Genotoxicity studies with the lysophospholipase enzyme concentrate comprised a bacterial reverse mutation test and an *in vitro* mammalian micronucleus test. The test item had the same Batch/Lot no. as that used in the 90-day oral toxicity study. These studies were GLP compliant and conducted according to OECD Test Guidelines. The positive controls in these studies produced the expected responses.

As summarised in Table 4, lysophospholipase from *T. reesei* showed no evidence of mutagenicity, clastogenicity or aneugenicity in these studies.

Table 4: Genotoxicity studies with lysophospholipase from *T. reesei*

Test	Test object	Concentration	Results	Reference
Bacterial reverse mutation test (OECD TG 471); preincubation method (treat and plate)	*Salmonella typhimurium* strains TA1535, TA100, TA1537 & TA98; *Escherichia coli* WP2uvrA (pKM101)	Test I: 1.5 – 5000 µg/plate Test II: 156.25 – 5000 µg/plate Test III: 156.25 – 5000 µg TOS/plate	Negative ± S9[a]	Jai Research Foundation 2023b
In vitro mammalian micronucleus test (OECD TG 487)	Human peripheral blood lymphocytes	1250 – 5000 µg TOS/mL	Negative ± S9[b]	Jai Research Foundation 2023c

[a] Test I with metabolic activation conducted using 5% v/v S9 mix; Tests II and III with metabolic activation conducted using 10% v/v S9 mix.
[b] 4-hour exposures conducted in the presence and absence of metabolic activation; 24-hour exposure conducted in the absence of metabolic activation.

3.3.4 Potential for allergenicity

The potential allergenicity of the applicant's lysophospholipase was assessed by comparing its amino acid sequence with those of known allergens. The following searches were conducted by the applicant using the AllergenOnline[6] database v23 (30 January 2025):

- Full length alignments using an E-value of < 0.1
- > 35% identity over 80 amino acids
- Exact matches over 8 contiguous amino acids

No matches with known allergens were found. A literature search also found no reports of food allergy to lysophospholipase enzymes.

Based on the available information, lysophospholipase from *T. reesei* is unlikely to pose an allergenicity concern.

3.3.5 Assessments by other regulatory agencies

As noted in section 3.3.1, lysophospholipase from *T. reesei* has been approved by several overseas authorities, however safety assessments from these agencies are not available to FSANZ.

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 all of the TOS from the lysophospholipase enzyme 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 (ADI) 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 2020a). The method is used by overseas regulatory bodies and 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 body weight/day
- 50% of solid food is processed
- all solid foods contain the highest use level of 2.9 mg TOS/kg in the final food
- 25% of non-milk beverages is processed

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

- the daily consumption for non-milk beverages is 100 mL/kg body weight/day (the standard level used in a budget method calculation for non-milk beverages)

- all non-milk beverages contain the highest use level of 1.21 mg TOS/kg in the final food

- sweetened beverages contain 0.12 kg starch per litre of final product, and bakery and dairy products contain 0.05 kg starch per kg of final food

- all the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.087 mg TOS/kg body weight/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 body weight/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 and non-milk beverages is 0.1 mg TOS/kg bw/day.

Both FSANZ and the applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed 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 not present or active in the final food.

5 Discussion

The use of lysophospholipase from *T. reesei* containing the lysophospholipase gene from *A. niger* as a processing aid in starch processing for the manufacture of glucose syrups and other starch hydrolysates is consistent with the typical function of the enzyme.

The enzyme is intended to be used to hydrolyse lysophospholipids in starch, where it may confer benefits including improved filtration efficiency and enhanced syrup clarity.

Lysophospholipase is functioning as a processing aid for the purposes of the Code and does not perform a technological purpose in the food for sale. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

There are relevant identity and purity specifications for the enzyme in the Code, and the

applicant has provided evidence that the enzyme meets these specifications.

The microbiological assessment undertaken by FSANZ did not identify any public health or safety concerns associated with the use of the production strain. Analysis of the production strain confirmed the presence and stability of the inserted DNA.

No significant homology between lysophospholipase from *T. reesei* and known toxins or allergens was identified. The enzyme was not genotoxic *in vitro*, and no adverse effects were observed in a 90-day oral toxicity study in rats. The NOAEL was 1000 mg TOS/kg bw/day, the highest dose tested.

The TMDI was calculated by FSANZ to be 0.1 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a MOE of approximately 10,000.

In the absence of any identifiable hazard an ADI ‘not specified’ is appropriate for lysophospholipase.

FSANZ concludes there are no public health and safety concerns with the proposed use of lysophospholipase from *T. reesei*.

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