Lysophospholipase from *Trichoderma reesei*

An application to amend the Australia New Zealand Food Standards Code with a Lysophospholipase preparation produced by a genetically modified strain of *Trichoderma reesei*

PROCESSING AID APPLICATION

Food Standards Australia New Zealand

Applicant: IFF AUSTRALIA PTY LTD (Trading as Danisco Australia Pty Ltd)

2nd March 2026

CONTENTS

3.1 General requirements for applications

A. Executive Summary

An Executive Summary is provided as a separate copy with this application.

B. Applicant details

(a) Applicant:
This application is made by Danisco Australia (IFF)

(b) Company:
Danisco Australia Pty Ltd

(c) Address:
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Chatswood NSW 2067
AUSTRALIA

(d) Contact Details:
[redacted]
Regulatory Affairs Manager Australia/NZ
Danisco NZ Ltd
Tel: [redacted]
Email: [redacted]

(e) Email address:
See above

(f) Nature of Applicants Business:
Danisco Australia Pty Ltd – A subsidiary of International Flavors and Fragrances Inc (IFF), manufacturer/marketer of specialty food ingredients, food additives and food processing aids. Danisco Australia is also an affiliate of Genencor International Ltd, the manufacturer of the product and another subsidiary of International Flavors and Fragrances Inc (IFF).

(g) Details of Other Individuals.:
No other individuals, companies or organisations are associated with this application.

C. Purpose of the application

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a genetically modified strain of *Trichoderma reesei* as a permitted source of lysophospholipase. The intended use of the processing aid is for use in carbohydrate processing to produce glucose syrups and other starch hydrolysates. The enzyme is designated as "Lysophospholipase" throughout the dossier, Appendix A1 (**Confidential Commercial Information**) outlines a summary of internal names that could be referenced in reports throughout this dossier.

This application is made solely on behalf of IFF Health & Biosciences (IFF), the manufacturer/marketer of the *Processing Aid*. When approved, the *Processing Aid* would be available for use by any food manufacturer in Australia and New Zealand.

D. Justification for the application

Currently no lysophospholipase from *Aspergillus niger* expressed in *Trichoderma reesei* is permitted as a Processing Aid, however lysophospholipase from *Aspergillus niger*, and from *Trichoderma reesei* containing the gene for lysophospholipase isolated from *Aspergillus niger* are listed in Schedule 18 section S18-4(5) as permitted enzymes. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed in Section 3.3.2, Part A.

No marketing or promotional activities have been undertaken for Lysophospholipase derived from *T. reesei* containing the gene for lysophospholipase from *A. niger* in the Australia/New Zealand market. Hence at this stage, no requests from food manufacturers are provided in support of this application. It is anticipated that support from the food processing industry will be submitted during the Public Notification period of this assessment.

D.1 Regulatory impact information

D.1.1 *Costs and benefits of the application*

The application and approval of the enzyme is not anticipated to impose regulatory restrictions or costs on industry or consumers. As outlined in Section 3.3.2, Part A, the approval of lysophospholipase from *T. reesei* for use in carbohydrate processing will deliver numerous production benefits to industry. In turn the government will only incur the burden of the required activities related to variation of Schedule 18.

More information on the benefit of this enzyme can be found in Section 3.3.2, Part A.

Enzyme preparations are widely used as processing aids in the manufacture of food products. Currently no lysophospholipase from *Aspergillus niger* expressed in *Trichoderma reesei* is permitted as a Processing Aid. Approval of this application would provide food processors with a new enzyme preparation offering the benefits and advantages as discussed previously

D.1.2 *Impact on international trade*

The inclusion of lysophospholipase from *A. niger* expressed in *T. reesei* in the Australia New Zealand Food Standards Code to Schedule 18 may promote international trade on products produced with this enzyme product and reduce technical barriers to trade.

E. Information to Support the application

Support for the application

No public health and safety issues related to the proposed change are foreseen. In accordance with the Food Standards Australia New Zealand (FSANZ) Application Handbook (FSANZ, 2024), in the following Section 3.3.2 Parts A through F, detailed data has been provided to support the quality, efficacy and safety of the Lysophospholipase under the proposed conditions of use. The data pertaining to Lysophospholipase derived from *T. reesei* presented in this application is representative of the commercial product for which approval is being sought.

The information is provided in this application to enable the objectives specified in Section 18 of the FSANZ Act to be addressed as follows:

a. The protection of public health and safety: Information to support objective (a) is provided in Section C of the application, in which the safety of Lysophospholipase derived from *T. reesei*, based on the available safety data, is discussed in detail.

b. The provision of adequate information relating to food to enable consumers to make informed choices: Data to support objective (b) are provided in Section F, in which the impact and purpose of Lysophospholipase are described in detail.

c. The prevention of misleading or deceptive conduct: Information supporting objective (c) is provided in Section F, in which the consumer awareness and potential behaviour in response to products manufactured using Lysophospholipase are described. This objective can also be further supported by human safety data contained in Section C.

F. Assessment procedure

This application seeks to modify Schedule 18 to Standard 1.3.3 Processing Aids to permit the use of a Processing aid that is currently not permitted. Based on guidance in the Application Handbook, IFF considers General Procedure Level 1 (up to 180 hours) to be the appropriate procedure for assessment of the application.

G. Confidential commercial information (CCI)

Certain (identified) technical and manufacturing information included in the Appendices A1-6, B1-8, C1-5, D1-5 and other information including amino acid sequences labelled with Confidential Commercial information is regarded by the applicant as "**Confidential Commercial Information**" and is provided in the application strictly on this basis. This information is the result of a significant research and development effort and investment by the applicant; it is not in the public domain and is considered as either proprietary or commercially sensitive. It would be disadvantageous to the applicant if this information were released into the public domain.

H. Other confidential information

Certain redactions throughout the dossier have also been made to avoid identification or disclosure of the proprietary strain, product or other details considered commercially sensitive. Both redacted and non-redacted versions of all documentation have been supplied to FSANZ.

The applicant requests that only redacted versions are provided for public consultation purposes.

I. Exclusive commercial capturable benefit (ECCB)

According to Section 8 of the FSANZ Act, this application is not expected to confer Exclusive Capturable Commercial Benefit (ECCB).

J. International and other national standards

J.1 International standards

Use of enzymes as processing aids in food is not restricted by any Codex Alimentarius Commission (Codex) Standards or any other known regulations. Additionally, Lysophospholipase from *A. niger* produced by *T. reesei* has not been reviewed by JECFA there is no specific Codex Standards relevant to this application.

J.1.1 Supporting evaluations

No lysophospholipase has been approved by the Joint Expert Committee on Food Additives (JECFA).

Cellulase from *T. reesei* and Glucoamylase from *T. reesei* expressed in *T. reesei* have been reviewed by the Joint Expert Committee on Food Additives (JECFA) of FAO/WHO and an acceptable daily intake (ADI) "not specified" has been set.

J.2 Other national standards or regulations

Please find below the status of submission to other countries for the Lysophospholipase from *A.niger* expressed in *T. reesei*, as of February 2025.

United States of America

The enzyme
The subject of this application has been determined to be Generally Recognized as Safe (GRAS) in the United States as a food processing aid for use as an enzyme in carbohydrate processing (e.g., high fructose corn syrup) in the USA. US FDA has issued a no questions response to submission of this self-GRAS opinion GRN 964.

A similar lysophospholipase from *A. nishimurae,* produced in *T. reesei*, was issued a "no questions" letter for GRAS by the US FDA for use in starch processing (GRN 653).

[redacted]

Europe

This Lysophospholipase from a genetically modified strain of *T. reesei* has been approved by [redacted] (Appendix C1 and C2, **Confidential Commercial Information**).

In Europe, most enzyme preparations used in food processing are classified as processing aids, meaning that they have their technological function in the food-processing stage and not in the final food. They are excluded from the Food Additives Framework Directive. On 16 December 2008 the European Parliament and the Council adopted Regulation 1332/2008 EC on food enzymes which aims to harmonise authorisation and safety assessment procedures of enzymes used in food processing in the EU (EC, 2008), (Annex 9). Several years yet will be needed for

the new rules to become fully applicable across the EU. Until then, all national provisions on the use of food enzymes in individual EU Member States remain valid and applicable. Only France and Denmark have legislation covering all food-use enzymes. In Denmark and France, approval is needed prior to use. The information contained in the application dossier necessary for approval should follow the EFSA guidelines laid down in the 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 (EFSA, 2021). France has some additional national requirements specified in the Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires as amended. In the other EU countries, enzyme preparation should be proved to be safe for use in food before being sold in EU according to the General EU Food Law. It is the producer's responsibility how to meet this requirement. IFF uses the USA GRAS system as the backbone for this.

[redacted]

Other countries

Outside of the US and Europe, Lysophospholipase from genetically modified strain of *T. reesei* has been approved by the following countries:

- [redacted]
- [redacted]
- [redacted]

Refer to Appendices C3-5 (**Confidential Commercial Information**) for further information.

L. Checklist

General Requirements		
Check	**Page No.**	**Mandatory Requirements**
		A. Form of application
☒		Application in English
☒		Executive Summary (separated from main application electronically)
☒		Relevant sections of Part 3 clearly identified
☒		Pages sequentially numbered
☒		Electronic copy (searchable)
☒	28	All references provided
☒	3	B. Applicant Details
☒	4	C. Purpose of the application
☒	4	D. Justification for the application
☒		Regulatory impact information
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☒	5	E. Information to support the application
☒		Data requirements
☒	5	F. Assessment procedure
☒		General
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☐		Minor
☐		High level health claim variation
☒	5	G. Confidential commercial information
☒		CCI material separated from other application material
☒		Formal request including reasons
☒		Non-confidential summary provided
☒	5	H Other confidential information
		Confidential material separated from other application material
		Formal request including reasons
☒	6	I Exclusive Capturable Commercial Benefit
		Justification provided
☒	6	J International and other national standards
		International standards
		Other national standards
☒	8	K Statutory Declaration
☒	9	L Checklist/s provided with application
☒		3.1.1 Checklist
☒		All page number references from application included
☒		Any other relevant checklists for Chapters 3.2–3.7

Processing Aids		
Check	**Page No.**	**Mandatory Requirements**
		A. Technical information on the processing Aid
☒	11	A.1 Type of processing aid
☒	11	A.2 Identification information
☒	12	A.3 Chemical and physical properties
☒	15	A.4 Manufacturing process

☒	17	A.5 Specification information
☒	17	A.6 Analytical method for detection
N/A	17	B. Information related to the safety of a chemical processing aid
	18	C. Information related to the safety of an enzyme processing aid
☒	18	C.1 Information on enzyme use on other countries
☒	19	C.2 Toxicity information of enzyme
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☒	22	C.4. Overseas safety Assessment Reports
	22	D. Additional information related to the safety of an enzyme processing aid derived from a microorganism
☒	22	D.1 Information on source organism
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	23	E. Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism
☒	23	E.1 Nature of genetic modification of source organism
	24	F. Information related to the dietary exposure to the processing aid
☒	24	F.1 List of foods likely to contain the processing aid
☒	24	F.2 Anticipated residue levels in foods
☒	27	F.3 Information on likely level of consumption
☒	27	F.4 Percentage of food group to use processing aid
☒	27	F.5 Information on residues in foods in other countries (if available)
☒	27	F.6 Where consumption has changed, information on likely consumption

3.3.2 Processing Aids

A. Technical information on the processing aid

A.1. Information on the type of the processing aid

The Lysophospholipase enzyme is a liquid enzyme of microbial origin produced by submerged fermentation of *Trichoderma reesei*, carrying the lysophospholipase gene from *Aspergillus niger*.

This Processing Aid belongs to the category "Enzymes of microbial origin" from the Food Standard Code, Standard 1.3.3 Processing Aids, Section 1.3.3-6 Enzymes.

The Lysophospholipase enzyme preparation is to be used in the food industry as a processing aid during the processing of raw materials containing carbohydrates. Lysophospholipase catalyses the hydrolysis of 2-lysophosphatidylcholine to form glycerophosphocholine and carboxylate.

The Lysophospholipase enzyme preparation is used in the following food manufacturing processes:

- starch hydrolysis for the production of glucose syrup and other starch hydrolysates

The Lysophospholipase intended for use in carbohydrate processing in foods at a dose of maximum 100 g enzyme product/metric tonne dry material which is equivalent to 24.16 mg TOS/kg dry material in accordance with the principles of current Good Manufacturing Practice (GMP).

A.2. Information on the identity of the processing

A.2.1. Enzyme identity

The systematic name of the principal enzyme activity is lysophospholipase. Other 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.

- EC number: 3.1.1.5
- CAS number: 9001-85-8

Biological source:
The Lysophospholipase enzyme is an enzyme produced by submerged fermentation of *Trichoderma reesei*, carrying the lysophospholipase gene from *Aspergillus niger*. This lysophospholipase has not been protein engineered.

Enzyme Preparation:
The enzyme will be formulated into a liquid a commercial preparation. The commercial preparation may contain only the lysophospholipase enzyme, or it could be formulated with other permitted enzyme concentrates depending on the required end application.

The typical composition of an enzyme concentrate is:

Water	55-72 % w/w
Glycerol	25-35 % w/w
Sodium Chloride	5.0-6.5% w/w
Lysophospholipase	0-1 % w/w
Sodium Benzoate	0.3-0.4 % w/w
Potassium sorbate	0.15-0.25 % w/w

Other enzymes:
Downstream processing concentrates and purifies the enzyme product. The resulting enzyme preparation will not be totally pure, and traces of other enzyme activities (e.g. protease) might be found but their level will be very low.

A.2.2. Host Organism

The host (recipient) organism *Trichoderma reesei* strain [redacted] was obtained from Dr Bland S. Montenecourt. The derivation and characterisation of strain [redacted] have been published (Sheir-Neiss and Montenecourt, 1984).

A.2.3. Donor Organism

The donor strain used as a source for the Lysophospholipase sequence is *Aspergillus niger* [redacted], which has a long history of safe use and is widely used in biotechnology for the production of enzymes (Pel *et al.*, 2007).

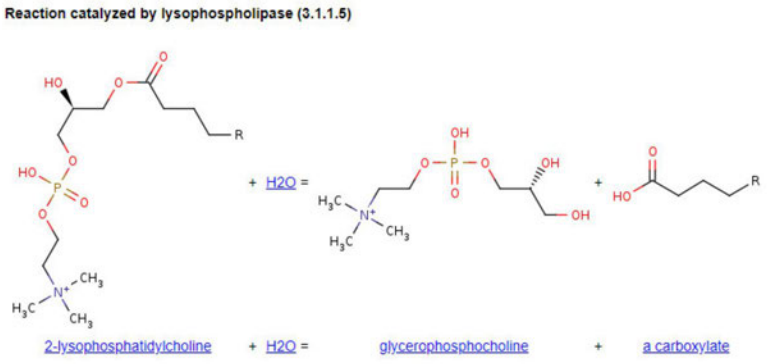
A.3. Information on the chemical and physical properties of the processing aid

Like any other enzyme, Lysophospholipase acts as a biocatalyst: with the help of the enzyme, a certain substrate is converted into a certain reaction product. As the catalytic activity is very specific, it is not to be expected that the food enzyme will act in another way than described below. After the conversion has taken place, the enzyme no longer performs a technological function in the final food.

A.3.1. Function of the food enzyme

The **substrate(s)** for Lysophospholipase is 2-lysophosphatidylcholine.

The **function** of Lysophospholipase is to catalyse the following reaction: -



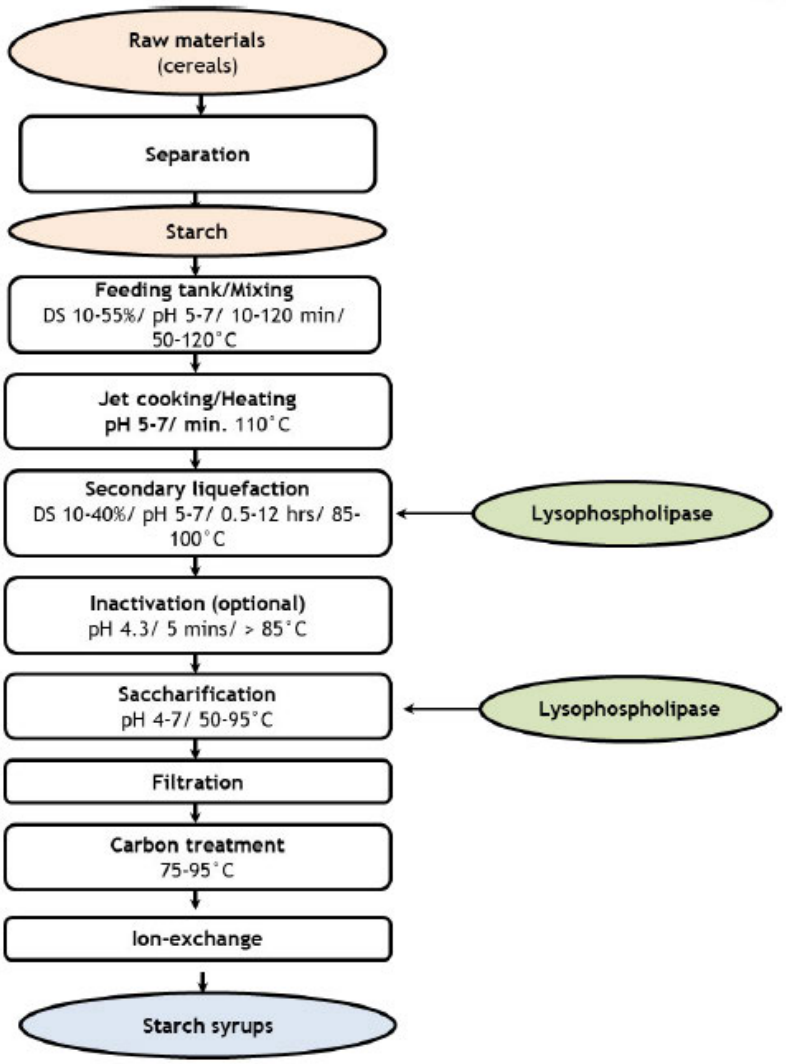
The substrate(s) for Lysophospholipase is 2-lysophosphatidylcholine. 2- lysophosphatidylcholine is a lysophospholipid which can be found in cereal starches, predominantly in wheat starch (Matser *et al*, 1998; Morrison W, 1988). Consequently, the substrate for lysophospholipase occurs naturally in foods.

The function of Lysophospholipase is to catalyse the hydrolyses 2-lysophosphatidylcholine resulting in the formation of glycerophosphocholine and free fatty acids. Like the substrate, the enzyme lysophospholipase is described to naturally occur also in other organisms, such as microorganisms (Kim *et al*, 2020). Consequently, also the enzyme occurs naturally in foods.

The reaction product(s) of the hydrolysis of 2-lysophosphatidylcholine with the help of Lysophospholipase are glycerophosphocholine and free fatty acids. Like the substrate and the enzyme, the reaction product(s) also naturally occur(s) in various organisms, including plants and animals. Consequently, also the reaction product(s) occur(s) naturally in foods.

The **benefits** of using Lysophospholipase in starch processing for the enzymatic conversion of 2-lysophosphatidylcholine are both technical and economic including, improved filtration efficiency and enhanced syrup clarity.

The process flow of starch processing presented below shows the typical application of the Lysophospholipase activity and shows the conditions under which the Lysophospholipase activity is used.



A.3.2. Fate in food

Lysophospholipase performs its technological function during food processing. Like endogenous lysophospholipase in raw materials and ingredients, it does not perform any technological function in the final food.

The reasons why an enzyme does not exert any (unintentional) enzymatic activity in the final food can be due to a combination of various factors, depending on the application and the process conditions used by the individual food producer. These factors include depletion of the substrate, denaturation of the enzyme during processing, lack of water activity, wrong pH, etc. In some cases (e.g. after alcohol distillation, products resulting from starch processing), an enzyme may no longer be present in the final food.

In starch processing, the enzyme is added to cooked starch during the liquefaction and/or saccharification steps. The syrup is filtered and clarified by carbon treatment and ion exchange chromatography to remove any objectionable flavour or colour (Watson *et al*, 1987; Alexander, 1998; Corn Refiners Association, 2006).

The purified hydrolysate passes a check filter, and the clear hydrolysate is evaporated to reduce the amount of water. The various additional purification steps (filtration, carbon treatment, ion exchange) to which the sugar syrup is subjected effectively removes all of enzyme protein. Lysophospholipase is denatured by heat during a dedicated inactivation step or removed during subsequent carbon or ion exchange treatment. Negligible carryover of lysophospholipase is expected.

A.3.3. Activity

The activity of the Lysophospholipase is defined in U/g (units per gram). The substrate employed in the assay is L-α-Lysophosphatidylcholine.

Lysophospholipase preparations' enzyme activity will depend on the final product. An example product has the Lysophospholipase activity range of 3740-5060 U/g. The method, by which the enzyme activity is measured, including an explanation of the Units, is given in Appendix A3 (**Confidential Commercial Information**).

A.3.4. Information on the activity of the food enzyme under various reaction conditions

The activity of the food enzyme Lysophospholipase from *Trichoderma reesei* was measured under various pH and temperature conditions. The results and the methods are presented in Appendices 4-6.

It can be concluded from the results, the food enzyme lysophospholipase from *Trichoderma reesei* exhibits activity from pH 3.5 till pH 7, and from 30°C till 70°C. The optimum pH range lies between pH 3.5 and 5.5, whereas the optimum temperature range lies between 50 and 70°C. No enzyme activity is left at temperatures above 75°C after an incubation of 15 min at pH 4.5.

A.3.5. Storage

Food enzymes are not sold as such but formulated into various enzyme preparations in order to obtain standardised and stable products. The stability thus depends on the type of formulation, not on the food enzyme as such.

According to Standard 1.2.5 of Food Standards Code, the date of minimum durability or use-by-date is indicated on the label of the food enzyme preparation. If necessary, special conditions of storage and/or use will also be mentioned on the label.

A.4. Manufacturing process

The enzyme is produced by a submerged fermentation process using appropriate substrate and nutrients. The following sections describe the manufacturing process for the enzyme, which follows standard industry practice.

A manufacturing process flow is provided in Annex 1.

The production of Lysophospholipase is monitored and controlled by analytical and quality assurance procedures that ensure that the finished preparation complies with the specifications and is of the appropriate quality for use as a processing aid in food processing applications.

A.4.1. Raw materials

The raw materials used in the fermentation and recovery process for this Lysophospholipase concentrate are standard ingredients used in the enzyme industry (Kroschwitz, 1994; Aunstrup, 1979 and Aunstrup *et al.*, 1979). All the raw materials conform to the specifications of the Food Chemicals Codex, 13th edition ("FCC"), except for those raw materials that do not appear in the FCC. For those not appearing in the FCC, internal requirements have been made in line with FCC requirements and acceptability of use for food enzyme production. Danisco US Inc. uses a supplier quality program to qualify and approve suppliers. Raw materials are purchased only from approved suppliers and are verified upon receipt.

Regarding potential major food allergens, glucose (which may be derived from wheat) will be used in the fermentation process and is consumed by the microorganism as nutrients. No other major allergen substances will be used in the fermentation, recovery processes, or formulation of this product.

Materials used in the fermentation process (inoculum, seed and main fermentation) are:

- Potable water
- A carbon source
- A nitrogen source
- Vitamins (typically part of one of the used complex fermentation materials)
- Salts and minerals
- An inducer (that encourages the microorganism to produce the intended enzyme protein)
- pH adjustment agents
- Foam control agents

A list of raw materials used for the production of the food enzyme is provided in Appendix D5 (**Confidential Commercial Information**).

A.4.2. Fermentation process

The production of food enzymes from microbial sources follows the process involving fermentation as described below. Fermentation is a well-known process that occurs in food and has been used for the production of food enzymes for decades. This section describes the different fermentation process steps.

A.4.2.1. Inoculum

A suspension of a pure culture of the production strain is aseptically transferred to an inoculum flask containing fermentation medium.

The culture is grown in the flask until a sufficient amount of biomass is obtained, which can subsequently be used as inoculum for the seed fermentation.

A.4.2.2. Seed fermentation

The inoculum is aseptically transferred to the seed fermenter containing fermentation medium. The seed fermentation is run under the specifically defined and controlled process conditions

The fermentation is run under controlled agitation and controlled air supply. When a sufficient amount of biomass has developed, the content of the seed fermenter is used for inoculation of the main fermentation.

A.4.2.3. Main fermentation

Biosynthesis of the enzyme protein by the production organism occurs during the main fermentation.

The fermentation in the main fermenter is operated as a batch or fed-batch fermentation. In both cases, the content of the seed fermenter is aseptically transferred to the main fermenter containing fermentation medium.

In the case of a fed-batch process, additional fermentation medium is added during the fermentation. In order to control the growth of the production organism and the enzyme production, the feed-rate of this medium is based upon a predetermined profile or on deviation from defined set points for pH or dissolved oxygen concentration.

During growth, the fermentation broth can be drawn from the bottom of the main fermenter to storage tanks if the production exceeds the capacity of the main fermenter. The main fermentation is run under the following defined process conditions.

The fermentation process is continued for a predetermined time or until laboratory test data show that the desired enzyme production has been obtained or that the rate of enzyme production has decreased below a predetermined production rate. When these conditions are met, and depending on the batch size, the fermentation is completed.

A.4.3. Recovery process

The purpose of the recovery process is:

- to separate the fermentation broth into biomass and fermentation medium containing the desired enzyme protein,
- to concentrate the desired enzyme protein and to improve the ratio enzyme activity/TOS

During fermentation, the enzyme protein is excreted by the producing microorganism into the fermentation medium. During recovery, the enzyme-containing fermentation medium is separated from the biomass.

During recovery, the enzyme-containing fermentation medium is separated from the cell remnants.

A.4.4. Formulation and standardisation process

Subsequently, the food enzyme is formulated. The resulting product is defined as a 'food enzyme preparation'.

Food enzymes can be sold as dry or liquid preparations, depending on the final application where the enzyme is intended to be used. For all kinds of food enzyme preparations, the food enzyme is standardised and preserved with food ingredients or food additives regulated according to Annex III of the EU Food Additive Regulation No 1333/2008 and which fulfil specifications for food additives listed in Commission Regulation No 231/2012.

For the manufacture of liquid food enzyme preparations, the food enzyme is standardised with food grade ingredients and adjusted to the desired activity. The preparation is in certain cases stabilised with preservatives.

A.5. Specification for identity and purity

The specification for impurities and microbial limits for the Lysophospholipase product can be found in Annex 2. Certificates of Analysis for three lots of product are provided in Annex 5.

The Lysophospholipase meets or exceeds the specifications for enzyme preparations established by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA") in 2006 and FCC, 13th edition. These specifications are provided in Annexes 3 and 4.

Impurity profile:
Appropriate GMP controls and processes are used in the manufacture of Lysophospholipase to ensure that the finished preparation does not contain any impurities of a hazardous or toxic nature. The specification for impurities and microbial limits is as follows:

Metals:	
Lead	less than 5 mg/kg
Arsenic	less than 1 mg/kg
Cadmium	less than 1 mg/kg
Mercury	less than 1 mg/kg
Microbial:	
Total Viable Count	less than 50,000 CFU/ml
Total coliforms	less than 30 CFU/ml
E.coli	absent in 25ml
Salmonella	absent in 25ml
Antibiotic activity	Negative by test
Production strain	Negative by test
Physical properties	
Appearance	Off white powder

A.6. Analytical method for detection

According to the FSANZ Application Handbook, July 2024, this is not required for enzymes used as processing aids.

B. Information related to the safety of a chemical processing aid

This Section is not applicable to chemical, but not enzymatic processing aids.

C. Information related to the safety of an enzyme processing aid

C.1 General information on the use of the enzyme as a food processing aid in other countries

Enzyme products are developed for a specific function, i.e. to catalyse a specific chemical reaction. That reaction determines the IUBMB classification. Enzyme variants may be selected to have a better performance of that function under the specific conditions of the application (e.g. temperature or pH). Enzymes of a certain IUBMB classification share conserved structural elements, called domains, which are needed for their specific function. As such the enzymes of our approval procedures do resemble those already permitted by FSANZ both in function and in structure.

Figure 1 below shows an example of natural variation of alpha-amylases. The same holds for any other enzyme type. While significant differences in sequence amongst the various species exist, they all catalyse the same reaction and therefore fit under the same IUBMB entry. There will also be natural variation within one species. All this also applies to the enzymes under the current approval procedures by FSANZ:

% amino acid sequence identity	*B. amyloliquefaciens*	*B. licheniformis*	*G. stearothermophilus*	*A. niger*	*A. oryzae*	*Z. mays*	*O. sativa*	*H. vulgare*	*P. vulgaris*	*H. sapiens*
Bacillus amyloliquefaciens	100									
Bacillus licheniformis	80	100								
Geobacillus stearothermophilus	65	65	100							
Aspergillus niger	21	21	22	100						
Aspergillus oryzae	23	24	24	66	100					
Zea mays (corn)	24	26	25	28	27	100				
Oryza sativa (rice)	25	27	25	27	26	89	100			
Hordeum vulgare (barley)	25	23	24	25	28	70	69	100		
Phaseolus vulgaris (bean)	26	27	25	24	27	67	65	64	100	
Homo sapiens (human)	25	33	29	22	28	23	22	23	24	100

α-amylases in nature have divergent

amino acid sequences but have the same catalytic activity and IUBMB number

Figure 1. Variation of enzymes in nature.

The expressed mature enzyme amino acid sequence of lysophospholipase shows a clear conserves PLA2_B superfamily domain, characteristic for lysophospholipases (EC 3.1.1.5).

Lysophospholipase enzyme, the subject of this dossier, is one of the permitted processing aids on Schedule 18 of the ANZ Food Standards Code i.e. from *Aspergillus niger*. In our case the enzyme protein is expressed from *Trichoderma reesei* carrying the lysophospholipase gene from *Aspergillus niger*

The lysophospholipase enzyme derived from *Trichoderma reesei* carrying the lysophospholipase gene from *Aspergillus niger* has been determined to be GRAS in the United States and has been approved by several authorities, [redacted] There have not been any adverse events reported since this aminopeptidase has been in commercial use in these countries.

Please refer to Section 3.3.1, J and Appendix C (**Confidential Commercial Information**) of different authorisations and evaluations.

C.2 Information on the potential toxicity of the enzyme

Toxin homology study
A bioinformatics (BLAST) search for similarity of the Lysophospholipase to known toxins was performed. The mature *Aspergillus niger* lysophospholipase sequence is given in Appendix D4 (**Confidential Commercial Information**).

The UniProt annotated Protein Knowledge database1 (http://www.uniprot.org), release 2025_04 of October 15, 2025, contains 573,661 reviewed proteins, of which 7,061 sequences are manually annotated as toxins and 7,445 as venom proteins (http://www.uniprot.org/biocuration_project/Toxins/statistics). These toxin and venom sequences are grouped in the animal toxin database subset (http://www.uniprot.org/program/Toxins).

A BLAST search for homology of the Lysophospholipase sequence against the complete Uniprot database was performed, with a threshold E-value of 0.1. The majority of the 50 database matches were phospholipases and lysophospholipases, with none being annotated as either toxin or venom. Please refer to Appendix B1 for analysis results (**Confidential Commercial Information**).

In addition, a specific BLAST search for homology of the mature Lysophospholipase sequence was performed against the Uniprot animal toxin database. This yielded no matches. Refer to Annex 7 for the output result from this search.

Therefore, the Lysophospholipase sequence does not share homology with a known toxin or venom sequence.

Safe Strain Lineage concept
The Safe Strain Lineage concept has been discussed by Pariza and Johnson (2001) in their publication on the safety of food enzymes and is commonly used by enzyme companies in the determination of safety for their products and their specific uses, as appropriate.

The primary issue in evaluating the safety of a production strain is its toxigenic potential, specifically the possible synthesis by the production strain of toxins that are active via the oral route. The toxigenic potential of the production organism is confined to the Total Organic Solid (TOS) originating from the fermentation.

As the toxicological evaluation is based on the TOS originating from fermentation of the production organism, studies conducted on strains from the Safe Strain Lineage can support other production strains pertaining to this same Safe Strain Lineage.

Although *T. reesei* is scientifically determined by IFF as a Safe Strain Lineage, the food enzyme object of the current dossier is supported by toxicological studies on the specific food enzyme object of this dossier. The toxicological studies on *T. reesei* LPL are thus one of the pillars supporting the IFF *T. reesei* Safe Strain Lineage. The position of the food enzyme in the IFF *Trichoderma reesei* Safe Strain Lineage is presented in Appendix B2 (**Confidential Commercial Information**).

Due to the consistency of the findings supporting the safety of enzyme preparations derived from different *T. reesei* strains, it is reasonable to expect that most enzyme preparation produced from *T. reesei* strains would have a similar toxicological profile.

Toxicological testing
To assess the safety of Lysophospholipase, different endpoints of toxicity were investigated and are evaluated and assessed in this document:

- Ames test: no mutagenic activity under the given test conditions
- Chromosomal aberrations: no clastogenic activity under the given test conditions
- 90-day oral toxicity on rats: the NOAEL (no observed adverse effect level) is established at the highest dose tested, 1000 mg TOS/kg bw/day,

Toxicological text summaries are provided in Annex 8, and full study reports included with this submission in Annexes 8a-c.

In addition, safety was further assessed according to the decision tree in the Pariza-Johnson guidelines (2001) for assuring the safety of a new enzyme preparation (Refer to Annex 10).

- Overall safety assessment

In the 90-day oral (gavage) study of Trehalase, a NOAEL was established at 1000 mg Total Organic Solids (TOS) /kg bw/day. The study was designed based on OECD guideline No. 408 and conducted in compliance with both the FDA Good Laboratory Practice Regulations and the OECD Good Laboratory Practice. Since human exposure to *Aspergillus niger* Lysophospholipase is through oral ingestion, selection of this NOAEL is thus appropriate.

NOAEL = 1000 mg TOS/kg bw/day.

Determination of margin of safety
It is prudent to ascertain that the most suitable available NOAEL is sufficiently high to accommodate the intended uses. The margin of safety was calculated by dividing the NOAEL obtained from the 90-day oral (gavage) study in rats by the human exposure (worst case scenario) assessed in Section F. As the margin of safety was determined to be greater than 100 (i.e.), it suggests that the available toxicology data support the proposed uses and application rates.

$$\text{Margin of safety} = \frac{\text{No observed adverse effect level}}{\text{Maximum daily exposure}}$$

$$\text{Margin of safety} = \frac{1000 \text{ mg TOS/kg bw/day}}{0.087 \text{ mg TOS/kg bw/day}}$$

Margin of Safety = 11494

C.3 Information on the potential allergenicity of the enzyme processing aid

The most current allergenicity assessment guidelines developed by the Codex Commission (Codex, 2009) and Ladics *et al*. (2011); (Ladics & Silvanovich, 2026) recommend the use of FASTA or BLASTP search for matches of 35% identity or more over 80 amino acids of a subject protein and a known allergen. Ladics *et al*. (2011); (Ladics & Silvanovich, 2026) further discussed the use of the "E-score or E-value in BLAST algorithm that reflects the measure of relatedness among protein sequences and can help separate the potential random occurrence of aligned sequences from those alignments that may share structurally relevant similarities." High E-scores are indicative that any alignments do not represent biologically relevant similarity, whereas low Escores (<10-7) may suggest a biologically relevant similarity (i.e., in the context of allergy, potential cross reactivity). They suggest that the E-score may be used in addition to percent identity (such as > 35% over 80 amino acids) to improve the selection of biologically relevant matches. The past practice of

conducting an analysis to identify short, six contiguous identical amino acid matches is associated with false positive results and is no longer considered a scientifically defensible practice.

The Codex Commission states:

"A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens."

The mature *A. niger* Lysophospholipase sequence is given in Appendix D4 as "**Confidential Commercial Information**").

A full-length sequence alignment against known allergens in the Allergy Research and Resource Program (FARRP) AllergenOnline database, January 30, 2025 V23, containing 2334 peer-reviewed allergen sequences listed in the database (using *E*-value <0.1) yielded no sequence matches (see Appendix B4, **Confidential Commercial Information**).

The FASTA full-length (conventional) sequence analysis was found to produce less false positive results when compared to the sliding 80-amino acid window analysis and was determined to be the more appropriate analysis when evaluating for potential allergenic cross-reactivity of proteins (Ladics *et al.*, 2007; Cressman & Ladics, 2009). Nevertheless, a search for 80-amino acid stretches within the sequence matching to known allergens was performed using the same FARRP AllergenOnline database and yielded no sequence matches (see Appendix B5, **Confidential Commercial Information**).

As cautioned in Codex (2009), researched by Herman *et al.* (2009) and further elaborated by Ladics *et al.* (2011); (Ladics & Silvanovich, 2026) and on AllergenOnline.org, there is no evidence that a short identical contiguous amino acid match will identify a protein that is likely to be cross-reactive and could be missed by the conservative 80 amino acid match (>35%). The FARRP AllergenOnline database allows isolated identity matches of eight contiguous identical amino acids to satisfy demands by some regulatory authorities for this precautionary search. Performing the eight-contiguous identical amino acid search produced no matches (see Appendix B6, **Confidential Commercial Information**).

Additionally, microbial derived enzymes acting as environmental allergens have not been demonstrated to be active via the oral route. This concept was evaluated by Bindslev-Jensen *et al.* (2006) in which 19 microbial derived enzymes used in the food industry were tested for allergenicity in a double-blind placebo-controlled food challenge study in subjects with positive skin prick tests to inhalation allergens, food allergens, and bee and wasp allergens. None of the 19 microbial derived food enzymes were found to be food allergens and the authors concluded that ingestion of microbial derived food enzymes, in general, are not considered to be a concern regarding food allergy. Moreover, enzymes are processing aids and per definition, they are not present or active in the final food. Consequently, there is no exposure of the enzyme to the consumer.

In conclusion, bioinformatic analyses based on sequence homology determined that the expressed *Aspergillus niger* Lysophospholipase is unlikely to pose a risk of food allergenicity.

As for all enzyme products, an MSDS for the Lysophospholipase product would include a precautionary statement that inhalation of enzyme mist/dust may cause allergic respiratory reactions, including asthma, in susceptible individuals on repeated exposure.

An allergen statement for the enzyme preparation is given in Annex 6. Liquid glucose derived from wheat is used as a fermentation ingredient, however, according to Clause S9-3 to Standard 1.2.3, this substance would be exempt from mandatory allergen declaration.

C.4 Safety assessment reports prepared by international agencies or other national government agencies, if available

Documentation on the approval of Lysophospholipase by other agencies or jurisdictions is provided in Section 3.1, Part J.2, and Appendix C2-5 CCI as **Confidential Commercial Information.**

D. Additional information related to the safety of an enzyme processing aid derived from a microorganism

D.1 Information on the source microorganism

The source organism was obtained by genetic engineering of the *T. reesei* [redacted] a known unmodified, safe host strain. The purpose of the modification is to produce the Lysophospholipase enzyme of the donor strain *Aspergillus niger*.

D.1.1 The production organism

The production organism is a strain of *Trichoderma reesei*, that has been genetically engineered by Danisco US Inc. to overexpress a Lysophospholipase gene from *Aspergillus niger*. The production organism is a strain of *Trichoderma reesei* designated *Trichoderma reesei* LPL for the purposes of this dossier, IFF internal strain naming details are provided in Appendix A1 as **Confidential Commercial Information**.

The production strain contains the *A. niger* lysophospholipase gene regulated under the expression signals of the endogenous *Trichoderma reesei cbh1* gene, and a copy of the expression cassette were integrated into the recipient chromosome using the *T. reesei pyr2* gene (orotate phosphoribosyl transferase) as selectable marker.

The Whole Genome Sequencing analysis for production strain *T. reesei* LPL is provided in Appendix B8 as **Confidential Commercial Information**.

D.1.2 The host

The host organism *T. reesei* strain [redacted] was obtained from Dr. Bland S. Montenecourt. The derivation and characterisation of strain [redacted] have been published (Sheir-Neiss and Montenecourt, 1984). Strain [redacted] is a cellulase over-producing strain that was obtained through several classical mutagenesis steps from the wild-type *T. reesei* strain (QM6a). Strain QM6a is present in several public culture collections, such as the American Type Culture Collection as ATCC 13631. *T. reesei* has more recently been identified as a clonal derivative or anamorph of *Hypocrea jecorina* (Khuls *et al*., 1996 and Dugan, 1998). *T. reesei* has a long history of safe use in industrial scale enzyme production. Taxonomic determination of host strain *T. reesei* [redacted] is provided in Appendix B7 as **Confidential Commercial Information**.

D.1.3 The vector

The genetic modification of the *T. reesei* host involved recombinant DNA techniques to introduce a synthetic gene ([redacted]) encoding the wild type *A. niger* lysophospholipase into the *T. reesei* host.

The *T. reesei* production strain LPL was created by transformation of a linear DNA expression cassette containing the [redacted] gene and the *pyr2* selectable marker. Insertion of the fragment into the *T. reesei* genome was by non-homologous recombination.

The inserted DNA was integrated into the recipient chromosome. All these modifications were performed in such a way that no bacterial vector DNA remains present in the strain. No antibiotic resistance markers were inserted into the new microorganism. A detailed description is provided in Appendix D2 as **Confidential Commercial Information**.

D.2 Information on the pathogenicity and toxicity of the source organism

T. reesei a mesophilic deuteromycete, has a long history of safe use in industrial scale enzyme production. The safety of this species as an industrial enzyme producer has been reviewed by Nevalainen *et al*. (1994), Blumenthal (2004), Olemska-Beer *et al*. (2006), and Frisvad *et al*. (2018). The organism is considered non-pathogenic for humans and does not produce fungal toxins or antibiotics under conditions used for enzyme production. It is generally recognised as a safe production organism and is the source organism of a range of enzyme products that are used as processing aids in the international food and feed industries. *T. reesei* is classified as a Biosafety Level 1 (BSL1) microorganism by the American Type Culture Collection (ATCC) based on assessment of the potential risk using U. S. Department of Public Health guidelines with assistance provided by ATCC scientific advisory committees, and also considered as suitable for Good Industrial Large Scale Practice (GILSP) worldwide and meets the criteria for a safe production microorganism as described by Pariza and Johnson (2001).

D.3 Information on the stability of the source organism

The parental strain of the production strain *Trichoderma reesei* QM6a and its derivatives have been used for industry scale enzyme manufacturing for decades by IFF and its parental companies and has demonstrated stable enzyme expression even at large scale fermentation. Please also refer to Appendix B3 for list of example enzyme preparations produced using QM6a and its derivatives. Furthermore, the production strain has demonstrated to be 100% stable as confirmed by genome sequencing. A report on the stability of the inserted gene is provided in Appendix D3, (**Confidential Commercial Information**). Refer also section E.1.

E. Additional information related to the safety of an enzyme processing aid derived from a genetically modified org

E.1 Information on the methods used in the genetic modification of the source organism.

The *T. reesei* strain LPL was constructed for Lysophospholipase production. The construction of the production strain consisted of:

- Expression cassette containing the Lysophospholipase gene from *A. niger* under the control of the *T. reesei cbh1* promoter and *T. reesei pyr2* marker gene
- Introduction of the expression cassette into the genome of *T. reesei* [redacted] recipient strain.
- The gene donated to produce Lysophospholipase from *Trichoderma reesei* strain LPL is not protein engineered.

Full details of the genetic modifications and stability of the inserted genes are provided in Appendix D1-D3 as **Confidential Commercial Information**.

F. Information relating to the dietary exposure to the processing aid

F.1 A list of foods or food groups likely to contain the processing aid or its metabolites processing.

According to the food group classification system used in Standard 1.3.1-Food Additives Schedule 15 (15-5), Lysophospholipase will be used to produce:

11.0 Sugars, honey and related products

These products will further be used as ingredients to produce beverage, bakery, dairy and other processed food applications.

F.2 The levels of residues of the processing aid or its metabolites for each food or food group.

F.2.1 Level of use and residues in food

Estimated Food Intake

Commercial food enzyme preparations are generally used following the Quantum Satis (QS) principle, i.e. at a level not higher than the necessary dosage to achieve the desired enzymatic reaction – according to Good Manufacturing Practice. The amount of enzyme activity added to the raw material by the individual food manufacturer has to be determined case by case, based on the desired effect and process conditions. Therefore, the enzyme manufacturer can only issue a recommended enzyme dosage range. Such a dosage range is the starting point for the individual food producer to fine-tune their process and determine the amount of enzyme that will provide the desired effect and nothing more. Consequently, from a technological point of view, there are no 'normal or maximal use levels' and Lysophospholipase is used according to the QS principle. A food producer who would add much higher doses than needed would experience untenable costs as well as negative technological consequences.

The dosage of a food enzyme depends on the activity of the enzyme protein (in this case Lysophospholipase) present in the final food enzyme preparation (i.e. the formulated food enzyme). However, the activity Units as such do not give an indication of the amount of food enzyme actually added. Microbial food enzymes contain – apart from the enzyme protein in question – also some substances derived from the producing microorganism and the fermentation medium. The presence of all organic materials is expressed as Total Organic Solids (TOS, FAO/WHO, 2006). Whereas the dosage of a food enzyme depends on the enzyme activity present in the final food enzyme preparation, the dosage on basis of TOS is more relevant from a safety point of view. Therefore, the use levels are expressed in TOS.

Lysophospholipase is intended for use in carbohydrate processing (starch hydrolysis) for the enzymatic conversion of 2-lysophosphatidylcholine found in cereal starches, resulting in the formation of glycerophosphocholine and free fatty acids. The Lysophospholipase enzyme preparation is used at the minimum level required to achieve the desired effect, in accordance with the principles of current Good Manufacturing Practice (GMP).

The use level for starch processing ranges from 0.01-24.16 mg TOS/ kg raw material. The maximum use level is 24.16 mg TOS/kg raw material. The Table below shows the range of recommended use levels for each application where the food enzyme may be used.

Application	Raw material (RM)	Recommended use levels (mg TOS/kg RM)	Maximal recommended use levels (mg TOS/kg RM)
Starch processing for glucose syrups production and other starch hydrolysates	Cereal (starch)	0.01-24.16	24.16

As outlined above, Lysophospholipase from *Trichoderma reesei* may be used in the manufacture of glucose syrups and starch hydrolysates.

The exposure to Lysophospholipase via carbohydrate processing is outlined below via the Budget Method (Hansen, 1966; Douglass *et al.*, 1997). This method has been used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001). The method enables to calculate a 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.

The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake. The Budget Method is based on the following assumed consumption of targeted important foodstuffs and beverages (for less important foodstuffs, e.g., snacks, lower consumption levels are assumed). The assumption is for Processed food (50% of total solid food) and for soft drinks (25% of total beverages).

Average consumption over the course of a lifetime/kg body weight/day	Total solid food (kg)	Total non-milk beverages (l)	Processed food (50% of total solid food) (kg)	Soft drinks (25% of total beverages) (l)
	0.025	0.1	0.0125	0.025

The recommended use level of Lysophospholipase is given, based on the raw materials used in the food process. The calculation considers how much solid or liquid food is obtained per kg raw material, and it is assumed that all the TOS will end up in the final product. Therefore, the concentration of TOS from lysophospholipase in the applications can be calculated/summarised as in the table below:

Application	Liquid Food	Solid Food
	Sugar syrups	Modified starch Sugar syrups
Raw material (RM)	Starch	Starch
Final Food (FF)	Sweetened beverages	Bakery, Dairy
Dose (mg TOS/kg RM	24.16	24.16
Yield (RM /FF)	0.12	0.05
Concentration (TOS mg/L, final food)	2.90	1.21

Dietary Exposure assessment

In this assessment, the Budget method is used. This method was previously used by JECFA (FAO/WHO, 2001) and contains the following assumptions:

1) Level of consumption of foods and beverages:

For solid foods, the daily intake is set at 25 g/kg bw based on a maximum lifetime energy intake of 50 Kcal/kg bw/day. For non-milk beverages, a daily consumption of 100 ml/kg bw is used corresponding to 6 litres per day for a 60-kg adult.

2) Concentration of enzymes in foods and beverages:

The concentration of enzyme in foods and beverages is the maximum application rate.

3) Proportion of foods and beverages that contain the enzymes:
    a. A default of 50% of all solid foods is used to represent processed foods (i.e., 12.5 g/kg bw/day).
    b. A default of 25% is used to represent non-milk beverages that may contain the enzyme (i.e., 25 ml/kg bw/day).
4) Estimation of the theoretical maximum daily intake (TMDI)

To represent a worst-case scenario, TMDI for solid foods will be combined with the TMDI for beverages in the risk assessment.

Estimation of the TMDI for Liquid Foods:

Since exposure of carbohydrate processing is the sole liquid food application represented and the max dosage used in this application represents the worst-case scenario, in which we assume that 25% of all consumed beverages are manufactured from raw materials treated with Lysophospholipase.

Beverage (non-milk) intake)	100	ml/kg BW/day
Processed Beverage intake (25%)	25	ml/kg BW/day
Enzyme TOS in beverage via carbohydrate processing as worst case	2.90	mg TOS/L beverage
TMDI liquid food	0.072	mg TOS/kg BW day

Estimation of the TMDI for Solid Foods:

Carbohydrate processing is the sole solid food application represented, and the max dosage used in this application represents the worst-case scenario.

Solid food intake	25	g/kg BW/day
Processed food treated with enzyme (50%)	12.5	g/kg BW/day
Enzyme TOS in solid food as worst case	1.21	mg TOS/kg final food
TMDI solid food	0.015	mg TOS/kg BW day

The Theoretical Maximum Daily Intake (TMDI)- total:

TMDI solid food	0.072	mg TOS/kg BW day
TMDI liquid food	0.015	mg TOS/kg BW day
TMDI all food	0.087	mg TOS/kg BW day

F.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption.

Not applicable. Lysophospholipase is not expected to be used in production of any foods or food groups that are currently not listed in NNSs. If such usage arises, an application would be made to inform FSANZ.

F.4 The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid

The enzyme would be used as a processing aid in about % of the tonnage of starch-based carbohydrates sold in Australia and New Zealand.

F.5 Information relating to levels of residues in foods in other countries

Applications and levels of use of the Lysophospholipase preparation in other countries is the same as presented in section F2.

F.6 For foods where consumption has changed in recent years, information on likely current consumption.

Not applicable. Consumption of processed foods produced with Lysophospholipase is not expected to have a significant change.

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