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

1-04
18 February 2004

FINAL ASSESSMENT REPORT

APPLICATION A492

LYSOPHOSPHOLIPASE AS A PROCESSING AID (ENZYME)

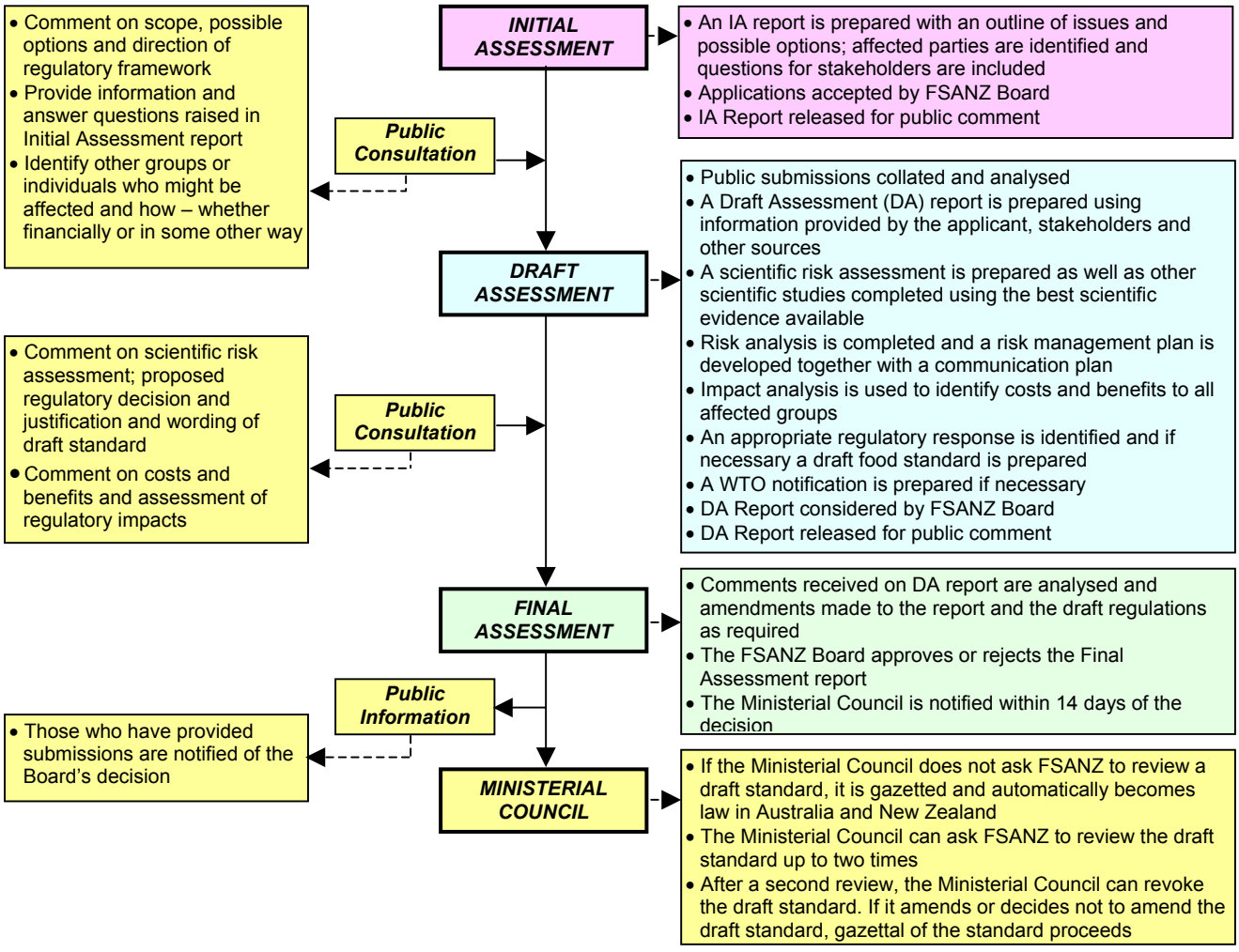
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



Final Assessment Stage

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

Further Information

Further information on this Application and the assessment process should be addressed to the FSANZ Standards Liaison Officer at one of the following addresses:

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Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from FSANZ's Information Officer at info@foodstandards.gov.au including other general enquiries and requests for information.

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Executive Summary and Statement of Reasons

FSANZ received an Application on 14 February 2003, from Genencor International to amend Standard 1.3.3 – Processing Aids - of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of a new enzyme, lysophospholipase (EC number 3.1.1.5). The Application is being progressed as a Group 3 (cost-recovered) application.

Lysophospholipase is sourced from *Aspergillus niger* which is the source organism for a number of approved enzymes within the Code. The enzyme is not sourced from a genetically modified organism.

The main function that lysophospholipase performs in food manufacturing, is as a processing aid to improve the filterability and therefore process efficiencies during the production of glucose syrups and maltodextrins from the hydrolysis of wheat starch. Lysophospholipase reduces the concentration of phospholipids during processing, which otherwise cause slow filtration.

Regulatory Problem

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. There is currently no approval for the use of lysophospholipase in the Code.

Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of lysophospholipase sourced from *Aspergillus niger*. Such an amendment needs to be consistent with the section 10 objectives of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act).

Safety Assessment

The safety assessment concluded that:

- the source organism, *Aspergillus niger* has a long history of safe use in the production of food enzymes, is the source for many approved enzymes in the Code, and is regarded as non-pathogenic and non-toxigenic;
- the enzyme preparation complies with international specifications;
- the enzyme causes no mutagenic effects in *in vitro* studies and there are no acute toxicity effects in animal studies; and
- a sub-chronic study in rats produced an ADI for lysophospholipase of 3 mg/kg bw per day.

Lysophospholipase has been 'self-assessed' by the Applicant as Generally Recognized As Safe (GRAS) under the US FDA GRAS system for use in food in the USA. France has approved the use of lysophospholipase as a food enzyme.

Regulatory Options

The only regulatory options considered were to approve or not approve the use of lysophospholipase sourced from *Aspergillus niger* as a processing aid. Approval of the Application provides advantages to manufacturers of glucose syrups and maltodextrins by improving filtration rates so improving process efficiencies. There should be no added costs to government regulators or consumers.

Consultation

Public comment on the Initial Assessment Report had been sought from 19 March 2003 till 30 April 2003. Three submissions were received; two submissions supported the Application, while one deferred comments until after the Draft Assessment Report.

Public comment on the Draft Assessment Report had been sought from 16 July 2003 till 27 August 2003. Eight submissions were received. Seven submitters supported the Application while one submitter raised two minor issues which have been addressed (paragraph 6 above addresses one issue).

The Final Assessment Report concludes that approval of lysophospholipase sourced from *Aspergillus niger* as a processing aid is technologically justified and does not raise any public health and safety concerns.

Statement of Reasons

FSANZ has agreed to approve the draft variation to Standard 1.3.3 – Processing Aids for the use of lysophospholipase sourced from *Aspergillus niger* as a processing aid for the following reasons.

- Use of the enzyme does not raise any public health and safety concerns.

- Use of the enzyme is technologically justified since it has a role in improving filtration rates and hence efficiencies in the process of hydrolysing wheat starch to produce caloric sweeteners such as glucose syrups and maltodextrins.

- The source organism, *Aspergillus niger* has a long history of safe use in the production of food enzymes, is the source for many approved enzymes in the Code, and is regarded as non-pathogenic and non-toxigenic.

- The proposed draft variation of the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, FSANZ has addressed the protection of public health and safety by undertaking a safety assessment of the enzyme. The assessment is based on the best available scientific data.

- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.

1. Introduction

FSANZ received an Application on 14 February 2003, from Genencor International to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of a new enzyme, lysophospholipase (EC number 3.1.1.5). The Application is being progressed as a Group 3 (cost-recovered) application.

Lysophospholipase is sourced from *Aspergillus niger* which is the source organism for a number of approved enzymes within the Code. The enzyme is not sourced from a genetically modified organism.

The main function that lysophospholipase performs in food manufacturing is as a processing aid to improve the filterability and therefore process efficiencies during the production of glucose syrups and maltodextrins from the hydrolysis of wheat starch.

2. Regulatory Problem

Processing aids are required to undergo a pre-market safety assessment before approval for use in Australia and New Zealand. A processing aid is a substance used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food.

There is currently no approval for the use of lysophospholipase in the Code. Lysophospholipase is not listed in the Table to clause 17 of Standard 1.3.3, for permitted enzymes of microbial origin.

The source organism *Aspergillus niger* is listed as an approved source for a large number (22) of other permitted enzymes in the Table to clause 17 of Standard 1.3.3.

3. Objective

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

The objective of this assessment is to determine whether the Code should be amended to permit the use of lysophospholipase derived from *Aspergillus niger* as a processing aid.

FSANZ has addressed the protection of public health and safety by ensuring that there are no significant health risks associated with approval of the new enzyme. This report has used the best available scientific data for the purposes of conducting a risk assessment. Approval of this Application will encourage an efficient and internationally competitive food industry and will promote consistency with other international food standards.

4. Background

Prior to 1980, starch based sweeteners were produced almost exclusively from maize. With the introduction of microbial enzymes that facilitate the processing and hydrolysis of wheat starch to form such starch based sweeteners, wheat became the raw material of choice, especially in Australia where there is a ready supply.

The processing of wheat starch hydrolysates is limited by poor filtration. Use of lysophospholipase during processing of wheat starch hydrolysates improves the filterability and process efficiencies.

5. Relevant Issues

5.1 Nature of the enzyme

The common name of the enzyme is lysophospholipase. Other alternative names include lecithinase B, lysolecithinase and phospholipase B, while the systematic name is 2-lysophosphatidylcholine acylhydrolase.

The Enzyme Commission number is EC 3.1.1.5 and the CAS registry number is 9001-85-8. The molecular weight of the enzyme is approximately 65 kD.

The enzyme is characterised by its ability to catalyse the reaction:

2-lysophosphatidylcholine + H_2O = glycerophosphocholine + a carboxylate.

Lysophospholipase is produced by fermentation of a commonly used fungal microorganism, *Aspergillus niger*.

5.2 Efficacy and technological justification

Lysophospholipase can be used to improve filtration rates in the process of hydrolysing wheat starch to produce caloric sweeteners. A major cause of poor filtration was found to be due to a monoacyl lipid compound (lysophospholipids), such as lysophosphatidylcholine. Lysophospholipids are water soluble and are efficient emulsifiers. Lysophospholipids, when concentrated, form micelles which reduce the filtration rate of the hydrolysate. Use of lysophospholipase removes the emulsifying properties of the phospholipid by cleaving a fatty acid producing separate water insoluble (fatty acid) and water soluble (glycerophosphatide) molecules.

The Applicant supplied a letter from the Manildra group in Australia, which expressed support for this Application. The Manildra group manufacture glucose syrups. The letter states that using lysophospholipase improves the filtration rate, which is often a rate limiting step in the glucose syrup manufacturing process.

It would appear there are no dietary implications with this Application since lysophospholipase is used as a processing aid during the filtration step in the manufacture of sweeteners. Heating steps during subsequent processing would inactivate the enzyme while other purification treatments such as carbon filtration and ion exchange refining would remove most of the inactivated enzyme, which would be present as protein, in the final sweeteners.

The Food Technology Report (**Attachment 4**) provides further information about the purpose and efficacy of the enzyme.

5.3 Safety assessment

Aspergillus niger is the source for the enzyme and has a long history of safe use in the production of food enzymes. *Aspergillus niger* is regarded as non-pathogenic and non-toxigenic.

Enzyme preparations used in food processing are generally considered to have low potential toxicity. The main toxicological consideration is in relation to possible contaminants arising from the host organism and the enzyme preparation production processes.

The production organism *Aspergillus niger* is considered non-toxic and non-pathogenic. The enzyme preparation complies with international standards for enzyme preparations and with the recommended purity specifications for food-grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[1].

Six toxicological studies were submitted in support of this Application. These consisted of one acute toxicity study, two irritation studies, one subchronic study, and two genotoxicity studies.

The safety assessment of lysophospholipase from *Aspergillus niger* concluded that:

- the source organism has a long history of safe use as a production strain for food-grade enzyme preparations;
- the enzyme preparation complies with international specifications;
- there was no evidence of toxic effects of lysophospholipase in the acute toxicity study in animals;
- in a sub-chronic study in rats, decreased ovaries weights and an increased incidence in centrilobular hepatocytic vacuolation in livers in males at 1000 mg lysophospholipase /kg bw per day was observed. The No Observed Adverse Effect Level (NOAEL) from the sub-chronic feeding study is 300 mg/kg bw per day. Using a safety factor of 100 for intra- and inter-species variation, the ADI for lysophospholipase is set at 3 mg/kg bw per day; and

1. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Add. 9, pp. 37-39.

- the enzyme preparation produced no mutagenic or cytogenic effects in *in vitro* assays;

From the available information, it is concluded that the use of lysophospholipase as a processing aid in food would raise no public health and safety concerns. The full toxicological evaluation is at **Attachment 3**.

5.4 Other international regulatory standards

Lysophospholipase preparations meet the current Food Chemical Codex (FCC, 4th Edition) and JECFA (footnote 1 on the previous page) specifications for food grade enzyme preparations.

Lysophospholipase has been 'self-assessed' by the Applicant as Generally Recognized As Safe (GRAS) for use in food in the USA (see section 5.5.2).

France has approved the use of lysophospholipase derived from *Aspergillus niger* as a food enzyme.

5.5 Issues addressed from submissions

There were two issues raised by one submitter (New Zealand Food Safety Authority, NZFSA) to the Draft Assessment Report which are both discussed in this section.

5.5.1 Dietary modelling of the denatured enzyme in the food supply

5.5.1.1 Modelling from the Application

The Safety Assessment Report reports a NOAEL of the enzyme of 300 mg/kg bw per day, with an ADI of 3 mg/kg bw per day (using a safety factor of 100). The NZFSA asked if the Applicant supplied any dietary modelling data in relation to the levels of the processing aid (in its denatured form) in the final foods, to justify that there are no safety concerns. The Applicant supplied worst case dietary modelling within the Application. The enzyme will be denatured by the heating steps of the starch hydrolysis process, while the various carbon and ion-exchange refining steps of the starch hydrolysis products would remove nearly all, if not all, of the enzyme preparation.

The Applicant has used ELISA tests for many other enzymes also used for starch hydrolysis and have found no detectable enzyme protein in starch hydrolysis products.

The Application provided some worst case scenarios for total human dietary exposure. The Applicant assumed that 90% of the enzyme preparation total organic solids (TOS) are removed during processing (being very conservative). The maximum recommended dosage of the enzyme is 500 U/kg starch d.s. (dry starch), which is equivalent of 2.3×10^{-2} g TOS/ kg of starch (on a dry basis). Including 90% removal that leaves a residual amount of 2.3×10^{-3} g TOS/kg starch (2.3 µg TOS/g starch product).

The Applicant also assumed that the enzyme is used to produce all caloric sweeteners. According to USDA reports (2001)[2] in 1997 the average per capita consumption of caloric sweeteners in the USA was 154.1 pounds per year (corresponds to 191.4 g/person/day). They used a typical male weighing 70 kg and typical female weighing 58 kg to model the intake as follows.

For males:
191.4 g sweeteners/day/70 kg = 2.7 g sweetener/kg/day x 2.3 µg TOS/g
= 6.2 µg TOS/kg body weight/day

For females the value is
191.4 g sweeteners/day/58 kg = 3.3 g sweetener/kg/day x 2.3 µg TOS/g
= 7.6 µg TOS/kg body weight/day

This is equivalent to
43.78 µg enzyme/kg/day for males
and
52.96 µg enzyme/kg/day for females.

Both these numbers are well below the ADI of 3 mg/kg bw/day (by a factor of 56 for males and 68 for females).

5.5.1.2 FSANZ's modelling for Australia and New Zealand

The estimated intakes supplied in the Application were compared to an estimated worst case intake based on Australian sugar consumption (natural and added) data. The 2 to 3 years age group in Australia was identified as having the highest consumption of sugar on a per kilogram of body weight basis in Australia and New Zealand (1998[3], 1999[4]). Therefore, this population group is considered the most vulnerable to lysophospholipase dietary exposure in Australia and New Zealand. The 90th percentile intake of sugar for this age group was 9.9 µg/kg body weight/day. The average body weight for this age group is 16 kg. Using these figures, combined with the residual amount of 2.3 µg TOS/kg starch product supplied in the Application, the estimated intake of TOS for the 2-3 years age group is:

9.9 g sugar/kg/day x 2.3 µg TOS/g
= 22.8 µg TOS/kg body weight/day.

Based on the ratio of TOS to enzyme (calculated from the data presented in the Application) this is equivalent to 162.8 µg enzyme/kg body weight/day). This is higher than the estimated intakes supplied by the Applicant using the US average per capita caloric sweetener consumption, however it is a worst case scenario and is still well below the ADI of 3 mg/kg body weight/day.

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[2] United States Department of Agriculture (USDA). Food consumption, prices, and expenditures. 1970-97. http://www.ers.usda.gov/publications/sb965; 2001

[3] National Nutrition Survey: Nutrient Intakes and Physical Measurements Australia 1995 (4805.0), Australian Bureau of Statistics, Canberra, 1998.

[4] NZ Food: NZ People – Key results of the 1997 National Nutrition Survey, Ministry of Health, Wellington, 1999.

5.5.3 GRAS (Generally Recognised As Safe) status

The GRAS status of the enzyme in the USA was questioned. The NZFSA sought confirmation of the GRAS status of the enzyme, as there appeared to be no listing for the enzyme in the Food Chemicals News Guide or in the US FDA (Food and Drug Administration) GRAS lists which can be searched on the FDA web sites.

The FDA has a notification system that allows manufacturers to 'self-affirm' GRAS status, which puts the onus on the manufacturer to ensure that their products are safe. The Applicant has confirmed that it has not filed a GRAS notice with the FDA but has 'self-affirmed' the GRAS status. Part of its justification for this judgement is based on the earlier FDA letter of no objection for GRAS Notice No. 111 for the enzyme lipase, sourced from the same organism as for this enzyme, *Aspergillus niger*. The US FDA no longer routinely provides no objection letters.

6. Regulatory Options

The Regulatory Options available for this Application are:

Option 1. Maintain the status quo and not approve the use of lysophospholipase sourced from *Aspergillus niger* as a food processing aid.

Option 2. Approve the use of lysophospholipase sourced from *Aspergillus niger* as a food processing aid.

7. Impact Analysis

Parties likely to be affected by the Regulatory Options are:

1. those sectors of the food industry wishing to produce and market food products produced using lysophospholipase as a processing aid;

2. consumers; and

3. Commonwealth, State, Territory and New Zealand governments

7.1 Option 1 - Maintain the status quo and not approve the use of lysophospholipase sourced from *Aspergillus niger* as a food processing aid

There are no perceived benefits to industry, government or consumers if this option is taken.

There are disadvantages to those food industries that wish to use the lysophospholipase enzyme.

7.2 Option 2 - Approve the use of lysophospholipase sourced from *Aspergillus niger* as a food processing aid

There are advantages to food manufacturers to be able to use lysophospholipase. It can be used to improve the filterability and therefore process efficiencies during the production of glucose syrups and maltodextrins from the hydrolysis of wheat starch.

There should be no added costs to government or consumers.

Option 2, which supports the approval of lysophospholipase as a food processing aid is the preferred option, since it has advantages for the food industry and consumers but has no significant cost for government regulators, consumers or manufacturers.

8. Consultation

8.1 Public consultation

Public comment on the Initial Assessment Report was sought from 19 March till 30 April 2003. Three submissions were received, with two accepting the Application and one deferring comment until the Draft Assessment Report.

Public comment on the Draft Assessment Report was sought from 16 July 2003 till 27 August 2003. Eight submissions were received. Seven of the 8 submissions supported the Application while the eighth (New Zealand Food Safety Authority) did not make an explicit statement but raised two issues which they asked to be addressed in the Final Assessment Report. These two issues are discussed in section 5.5 of this report.

Attachment 2 summarises the submissions received during the two rounds of public comment.

8.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Amending the Code to approve lysophospholipase as a processing aid is unlikely to have a significant effect on trade. The enzyme preparations are also consistent with the international specifications for food enzymes of Food Chemicals Codex (4th Edition, 1996) and JECFA so the WTO was not notified.

9. Conclusion and Approval

The Final Assessment Report concludes that approval of the use of lysophospholipase as a processing aid is technologically justified and does not pose a risk to public health and safety.

The draft variation to Standard 1.3.3 – Processing Aids of the Code, approving the use of lysophospholipase sourced from *Aspergillus niger* as a processing aid is recommended for the following reasons.

- Use of the enzyme does not raise any public health and safety concerns.

- Use of the enzyme is technologically justified since it has a role in improving filtration rates and hence efficiencies in the process of hydrolysing wheat starch to produce caloric sweeteners such as glucose syrups and maltodextrins.

- The source organism, *Aspergillus niger* has a long history of safe use in the production of food enzymes, is the source for many approved enzymes in the Code, and is regarded as non-pathogenic and non-toxigenic.

- The proposed draft variation of the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, FSANZ has addressed the protection of public health and safety by undertaking a safety assessment of the enzyme. The assessment is based on the best available scientific data.

- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Summary of Public Submissions
3. Safety Assessment Report
4. Food Technology Report

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

To commence on gazettal

[1] ***Standard 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 17 –

Lysophospholipase EC [3.1.1.5]	*Aspergillus niger*

Attachment 2

Summary of Public Submissions

Round One

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	Tony Downer
2	Food Technology Association of Victoria	David Gill
3	Agriculture, Fisheries and Forestry – Australia, Department of (Australian Quarantine and Inspection Service section)	Peter Maple

Submitter	Position	Comments
Australian Food and Grocery Council	Supports	The Council supports the Application. It considers that FSANZ will find the use of the enzyme technologically justified and on further examination (safety assessment and technological function at Draft Assessment) will approve the enzyme as a processing aid.
Food Technology Association of Victoria	Supports	The Technical Sub Committee agrees to accept option 2 - to approve the use of the enzyme as a processing aid.
Agriculture, Fisheries and Forestry – Australia, Department of (Australian Quarantine and Inspection Service section)	Defer comment until the Draft Assessment	It will defer comment until the Draft Assessment Report.

Round Two

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	Tony Downer
2	Food Technology Association of Victoria	David Gill
3	Agriculture, Fisheries and Forestry – Australia, Department of (Australian Quarantine and Inspection Service section)	Trent Brady, Kerrie Boulton
4	New Zealand Food Safety Authority	Carole Inkster
5	Environmental Health Unit, Queensland Health	Gary Bielby
6	Ayesha Khatun, student, Department of Food Science, University of Auckland	
7	Amy Choi, student, Department of Food Science, University of Auckland	
8	Sajith Kanchana Wimalaratne, student, Department of Food Science, University of Auckland	

Submitter	Position	Comments
Australian Food and Grocery Council	Supports	The Council supports the Application, reiterating its earlier support. Specifically now having seen that the safety assessment (contained in the Draft Assessment Report) concluded use of the enzyme would pose no risk to public health and safety. It supports the technological justification – in particular the specificity and economic use. It also believes the benefits outweigh any costs.
Food Technology Association of Victoria	Supports	It supports option 2, to approve the Application.

Department of Agriculture, Fisheries and Forestry (Australian Quarantine and Inspection Service)	Supports	It believes the proposed amendment will have no regulatory impact for imported foods (under the *Imported Food Control Act 1992*).
New Zealand Food Safety Authority	Not explicitly stated	It had two comments to make. • It wished to see data showing the levels of the enzyme (denatured) in the final product, and whether there should be modelling studies to confirm there are no safety concerns with dietary intake since there is an ADI determined. (The Application contained worst case dietary modelling which has been added into this Final Assessment Report, contained in section 5.5.1). • It would like the US GRAS listing confirmed since they could not find any evidence of this. (FSANZ communicated with the Applicant and they provided a letter stating that they have not filed a GRAS notice with the FDA (so not able to be searched) but have 'self-affirmed' the GRAS status of the enzyme preparation. Part of this justification is from the GRAS FDA letter for the GRAS notice 111 for lipase also sourced from *Aspergillus niger*. Discussed briefly in section 5.5.2)
Environmental Health Unit, Queensland Health	Support	It accepts that the use of the enzyme is technologically justified and poses no risk to public health and safety. It also agrees that the source organism has a history of safe use, is the source organism for 22 permitted enzymes and is regarded as non-pathogenic and non-toxigenic.
Ayesha Khatun	Support	She supported the Application and provided a report justifying this assessment including some references.
Amy Choi	Support	She supported the Application and provided a detailed report justifying this assessment including a number of references.
Sajith Kanchana Wimalaratne	Support	He supported the Application and provided a report justifying this assessment including some references.

Attachment 3

Safety Assessment Report

A492 – LYSOPHOSPHOLIPASE DERIVED FROM *ASPERGILLUS NIGER*

1. Introduction

Application A492 seeks approval for the use of lysophospholipase from a non-genetically modified *Aspergillus niger* as a processing aid.

The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

2. The source (production) organism – *Aspergillus niger*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as a processing aid. *Aspergillus niger* is the source organism for a number of approved enzymes within the Code. *Aspergillus niger* is considered to be non-pathogenic and nontoxic, and has a long history of safe use as a production strain for food-grade enzyme preparations[1].

3. Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic. The detailed specifications to which the preparation was found to conform are shown in Table 1.

Table 1. Complete specification of lysophospholipase preparation

Criteria	Specification
Phospholipase activity (U/g)	Between 1000 and 1163
Total viable count (cfu/g)	Not more than $5x10^4$
Anaerobic Bacteria, Sulfite Red (cfu/g)	Not more than 30
Total coliforms (cfu/g)	Not more than 30
E. Coli	Negative by test
Salmonella	Negative by test
Staphylococcus aureus	Negative by test
Moulds (cfu/g)	Not more than 100
Yeasts (cfu/g)	Not more than 100
Production strain	Negative by test
Antibacterial activity	Negative by test
Heavy Metals as Pb	Not more than 30 ppm
Arsenic	Not more than 3 ppm
Cadmium	Not more than 0.50 ppm

[1] Pariza, M.W. and E.A. Johnson, Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Reg. Toxicol. Pharmacol*. **33**, 173-186 (2001).

Mercury	Not more than 0.50 ppm
Lead	Not more than 5 ppm
Mycotoxins	Negative by test
Potassium Sorbate (% w/w)	Between 0.10 and 0.25
Sodium Benzoate (% w/w)	Between 1.3 and 1.7

Lysophospholipase from the source organism, *Aspergillus niger* complies with the recommended purity specifications for food-grade enzymes[2,3].

4. Evaluation of the submitted studies

Six toxicological studies were submitted in support of this Application. These were: a) acute oral toxicity study in rats, b) acute dermal irritation study in the rabbit, c) acute eye irritation study in the rabbit, d) a 90-day sub-chronic oral toxicity study in rats, e) a bacterial mutagenicity assay, and f) a human lymphocyte cytogenetic assay. The dermal and eye irritation studies were not evaluated, since they are not relevant for the safety assessment of lysophospholipase for public health safety in relation to food use.

4.1. Acute studies

Acute oral toxicity in the rat. (Acute toxic class method). Study Director: C. Longobardi, Research Toxicology Centre, Roma. Report no. 7396/T/264/99. 31 March 2000.

Test material	Lysophospholipase, batch number 991192B, 1114 U/g
Vehicle material	0.5% carboxymethylcellulose in water
Test Species	3 female and male Hsd: Sprague Dawley rats; administration by gavage
Dose	2000 mg/kg bw
GLP/guidelines	OECD guideline No. 423

A single group of 3 male and 3 female rats received single doses of lysophospholipase administered orally by gavage and were observed for mortality, morbidity, and clinical signs for 14 days post-dose. Body weights were measured prior to dosing, at day 8 and 15. At day 15 the animals were sacrificed and necropsy was performed. No clinical signs and mortality was observed. Body weights and necropsy revealed no treatment related effects.

[2] Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Add. 9, pp. 37-39.

[3] National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex. 1996. *Food Chemical Codex*, 4th edition, National Academy Press, Washington DC.

4.2. Sub-chronic toxicity

A 13-week oral toxicity study in rats. Study Director: C. Longobardi, Research Toxicology Centre, Roma. Report no. 7402/T/187/2000. 8 November 2000.

Test material	Lysophospholipase 1114 U/g
Control and vehicle material	Sterile water
Test Species	Sprague-Dawley CD rats 10 males and females per test dose; administration by gavage
Dose	0, 100, 300 and 1000 mg lysophospholipase /kg bw per day
GLP/guidelines	OECD guideline No. 408

Study conduct

Four groups of rats (10/sex/group) were treated with lysophospholipase by gavage at 0, 100, 300 or 1000 mg/kg bw per day for 90-days.

Clinical observations were recorded daily and more detailed clinical examination, including neurotoxicity was assessed once a week. In week 12 sensory reactivity and grip strength was assessed. Motor activity assessment was performed in 5 rats/sex/dose during week 12. Bodyweight and food consumption were recorded weekly; haematology and clinical chemistry before the end of the treatment period; and ophthalmology performed on all animals before the start of the study and near termination. At the end of the study, all animals were sacrificed and a complete necroscopy performed (gross examination, organ weights and histo-pathology on selected organs).

Results

One animal from the high-dose group died at day 22 post-treatment. The death was not considered treatment related, because no microscopic or macroscopic changes were observed which could be ascribed to a toxicological effect of the test substance. There were no treatment related clinical signs observed. There were no observed changes in bodyweights, food consumption, haematological, or ophthalmoscopical parameters during the treatment period. Potassium levels were significantly increased in males at the highest dose and in females at 300 mg/kg bw per day. The effects were not dose related and small, therefore not considered to be toxicologically relevant. In females there was a statistically significant dose-related decrease at all treatment levels in both absolute and relative ovaries weight (absolute ovaries weight: 0.095, 0.083, 0.083, 0.070 mg, for 0, 100, 300, 1000 mg/kg bw per day respectively; relative ovaries weight: 0.035, 0.031, 0.031, 0.029%, for 0, 100, 300, 1000 mg/kg bw per day respectively). The study authors considered the decrease in ovaries weight of no toxicological importance, because the control group values were abnormally high values compared to historical controls. These historical controls were not given. However, since a dose related decrease was observed both in absolute and relative ovaries weight, the effect was considered to be biologically significant at the highest dose (at the highest dose 26% decrease compared to controls in absolute weight). In 6/9 treated males at the highest dose, centrilobular hepatocytic vacuolation in the liver was observed, while no such effects were observed in any other group. The study author considered the effect related to the carbon dioxide used for necropsy. However, these effects were only observed at the highest dose and not in any other treatment group, therefore the increase in centrilobular hepatocytic vacuolation in the liver is considered to be treatment related.

In the preputial gland, abscesses were found at a rate of 0, 0, 2, 3 in males at increasing doses and in 1 female of the highest dose. These lesions were considered to be evidence of spontaneous pathology normally seen in this species under the experimental conditions.

The NOAEL was 300 mg/kg bw per day, based on decreased ovaries weight in females and centrilobular hepatocytic vacuolation in the liver in males at the highest dose.

4.3. Genotoxicity studies

G-Zyme G999 reverse mutation in *Salmonella typhimurium* (treat and plate method) Study Director: S. Cinelli. Research Toxicology Centre, Roma, Report No. 7399-M-00700. 28 June 2000.

Test article

The test article, G-zyme G999, labelled as lysophospholipase, batch 991192B was used. The activity was 1114 U/g.

Study design

Lysophospholipase was examined for mutagenic activity in five strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537). Experiments were performed with or without metabolic activation using liver S9 fraction from chemically pre-treated rats. The study design is in accordance with OECD guideline 471 (adopted 1997).

A preliminary toxicity test was performed to select the concentrations of the test article to be used in the main assays. The study comprised of negative and positive controls with or without S9 metabolising system. Experiments for survival determination and estimation of mutant numbers were carried out in triplicates at each test point. Five doses of test substance were applied with 5 mg/plate as the highest dose level. The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (sodium azide, 9-aminoacridine, 2-nitrofluorene, 2-aminoanthracene, cumene hydroperoxide and dimethylsulphoxide).

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	Lysophospholipase	First test: 0, 313, 625, 1250, 2500, 5000 µg/plate, second test: 0, 648, 1080, 1800, 3000, 5000 µg/plate with and without S9 mix	*S. typhimurium* TA98, TA100, TA 102, TA1535, TA1537.	-ve

Results and conclusion

In the first experiment a two-fold increase of mutation frequency was observed at the highest dose in the TA1535 strain without metabolic activation. This effect was not observed in the repeat experiment and therefore is not considered to be relevant. With metabolic activation a dose related increase in mutation frequency was observed in the TA98 strain in the first series. The mutation frequency was 3.5, 5.7, and 6.8 for 0, 2.5 and 5.0 mg/plate, respectively. In the repeat experiment no dose related increase was observed in the TA98 strain. Therefore, the increased mutation frequency is not considered to be relevant.

No other dose-related increases in mutation frequency were observed. It was concluded that lysophospholipase did not exhibit mutagenic activity under the conditions of the test.

G-Zyme G999 Chromosome aberrations in human lymphocytes cultured in vitro Study Director: S. Cinelli. Research Toxicology Centre, Roma, Report No. 7400-M-01400. 28 June 2000.

Test article

The test article, G-zyme G999, labelled as lysophospholipase, batch 991192B was used. The activity was 1114 U/g.

Study design

The potential of lysophospholipase to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using duplicate human lymphocyte cultures from a healthy male donor. Tests were carried out in the presence and absence of S9 metabolic activation, over a broad range of doses. In the first experiment, both in absence and presence of S9, the cells were treated for three hours and the harvest time was 24 hours, corresponding to approximately 1.5 cell cycle, was used. Since in toxicity experiments effects on the mitotic index were absent, the treatment levels in the main studies were 1250, 2500 and 5000 μg/ml both in the absence and presence of metabolic activation. As negative results were obtained, a second experiment in the absence of S9 was performed using a continuous treatment until harvest at 24 hours.

Results and conclusion

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S9 metabolic activation. Positive controls, mitomycin-C (-S9) and cyclophosphamide (+S9), gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

5. Conclusion

The safety assessment of lysophospholipase from *Aspergillus Niger* concluded that:

- the source organism has a long history of safe use as a production strain for food-grade enzyme preparations;
- the enzyme preparation complies with international specifications;
- there was no evidence of toxic effects of lysophospholipase in the acute toxicity study in animals;

- in a sub-chronic study in rats, decreased ovaries weights in females, and an increased incidence in centrilobular hepatocytic vacuolation in livers in males at 1000 mg lysophospholipase/kg bw per day was observed. The NOAEL from the sub-chronic feeding study is 300 mg/kg bw per day. Using a safety factor of 100 for intra- and inter-species variation, the ADI of lysophospholipase is 3 mg/kg bw per day; and

- the enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;

From the available information, it is concluded that the use of lysophospholipase as a processing aid in food would pose no public health and safety risk.

Attachment 4

Food Technology Report

A492 – LYSOPHOSPHOLIPASE AS A PROCESSING AID (ENZYME)

Introduction

FSANZ received an Application from Genencor International to amend the *Australia New Zealand Food Standards Code* to approve the use of the enzyme lysophospholipase sourced from *Aspergillus niger* as a processing aid.

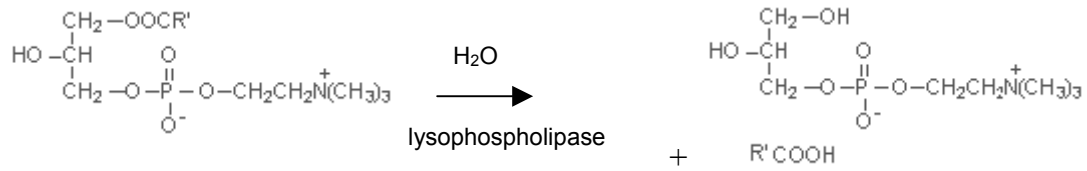
Lysophospholipase

The common name of the enzyme is lysophospholipase. Other alternative names include lecithinase B, lysolecithinase and phospholipase B, while the systematic name is 2-lysophosphatidylcholine acylhydrolase.

The Enzyme Commission number is EC 3.1.1.5 and the CAS registry number is 9001-85-8.

The enzyme is characterised by its ability to catalyse the reaction:

2-lysophosphatidylcholine + H_2O = glycerophosphocholine + a carboxylate.



Schematic of enzyme reaction

Lysophospholipase is produced by fermentation of a commonly used fungal microorganism, *Aspergillus niger*.

Technological Justification

Commercial food production enzymes are proteins that are able to catalyse chemical reactions more economically than traditional chemical or thermal processes. They are very important for many food manufacturing processes. Enzymes are able to be quite specific in the reactions they catalyse. Enzymes are able to catalyse chemical reactions with one or more of the following improved properties; reduction in time and temperature required for the reaction and greater specificity over reactions performed and products formed. Enzymes need to be stable for the conditions for their use.

The enzyme, lysophospholipase, meets the requirements of a food production enzyme. It is a protein which catalyses desired reactions during food preparation.

Advantages of using lysophospholipase

Phospholipids (commonly called 'lecithin') are found in all living cells; in animals and plants. Phospholipids in general are diacylglycerol molecules with the third carbon attached to a phosphate molecule. Phospholipids are commonly used as food emulsifiers due to their properties in having both water soluble and water insoluble functional groups in the molecules. Lysophospholipids are compounds where the second acyl group is missing from the middle carbon (carbon 2) of the glycerol backbone. They are also common phospholipids found in nature. Lysophospholipids are the predominant phospholipid found in wheat starch.

Lysophospholipase can be used to improve filtration rates in the process of hydrolysing wheat starch to produce caloric sweeteners. A major cause of the poor filtration is due to the presence of lysophospholipids, such as lysophosphatidylcholine. Lysophospholipids are water soluble and are efficient emulsifiers. This is because these compounds have both an ionic (hydrophilic, water soluble,) and long chain non-ionic carbohydrate (hydrophobic, water insoluble, R'COO-) group. Lysophospholipids, when concentrated, form micelles which reduce the filtration rate of the hydrolysate. Use of lysophospholipase removes the emulsifying properties of the phospholipid by cleaving a fatty acid producing separate water insoluble (long chain fatty acid) and water soluble (glycerophosphatide) molecules and therefore improves filtration rates.

Production of the enzyme

The enzyme preparation is produced using standard technologies employed for producing food grade enzymes. It is produced using a submerged fed-batch fermentation of the organism *Aspergillus niger*. Once the fermentation is complete the cells are removed and the preparation filtered, concentrated and stabilised with appropriate preservatives. Good Manufacturing Practice (GMP) is used throughout the production process meeting the requirements and specifications for food enzymes within Food Chemicals Codex (4th Edition, 1996) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the Compendium of Food Additives Specifications, Vol 1, Annex 1, Addendum 9 (2001) (and earlier relevant Addenda).

Specifications of the enzyme

The specifications for the lysophospholipase enzyme preparation meet the JECFA specifications mentioned above and listed in Table 1.

Table 1. Complete specification of lysophospholipase preparation

Criteria	Applicant Specification	JECFA Specification[1]
Heavy Metals as Pb	not more than 30 ppm	not more than 40 ppm
Potassium sorbate	0.10-0.25 % w/w	
Sodium benzoate	1.3- 1.7 % w/w	
Arsenic	not more than 3 ppm	not more than 3 ppm
Lead	not more than 5 ppm	not more than 5 ppm
Cadmium	not more than 0.50 ppm	

[1] Volume 1, Annex 1 of the Compendium of Food Additives Specifications, Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1992 and Addendum 9 (2001).

Total viable count (cfu/g)	not more than $5x10^4$	not more than $5x10^4$
Total coliforms (cfu/g)	not more than 30	not more than 30
Production organism (/g)	negative by test	
Mycotoxins	negative by test	negative by test
Antibacterial activity	negative by test	negative by test
pH	5.0 - 5.3	
Salmonella (/25 g)	negative by test	negative by test
Escherichia coli (/25 g)	negative by test	negative by test

Conclusion

The use of the enzyme lysophospholipase sourced from *Aspergillus niger* as a processing aid is technologically justified to improve filtration rates in the process of hydrolysing wheat starch to produce caloric sweeteners.

References

IUBMB (International Union of Biochemistry and Molecular Biology) Enzyme Nomenclature internet site, http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/1.html.

Phospholipids section in Encyclopaedia of Food Science Food Technology and Nutrition, Academic press, London, 1993, Edited by R. Macrae, R.K. Robinson and M.J. Sadler

Matser, A. M. and Steeneken, P.A.M., Origins of the poor filtration characteristics of wheat starch hydrolysates, *Cereal Chem*., 1998, 75 (3), 289-293.