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

10/03
16 July 2003

DRAFT ASSESSMENT REPORT

APPLICATION A492

LYSOPHOSPHOLIPASE AS A PROCESSING AID (ENZYME)

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:
27 August 2003
(See 'Invitation for Public Submissions' for details)

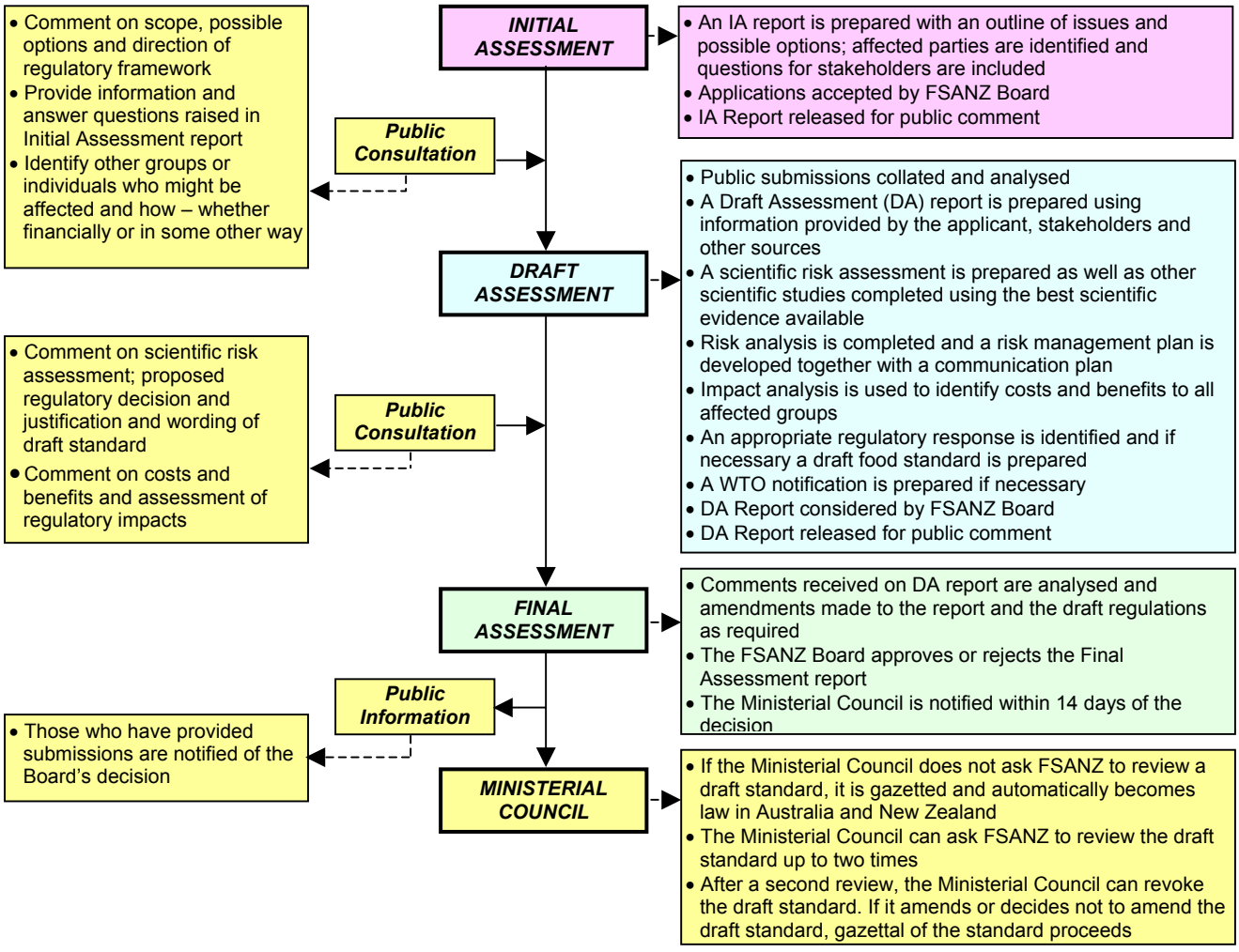
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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.



INVITATION FOR PUBLIC SUBMISSIONS
The Authority has prepared a Draft Assessment Report of Application A492 and prepared a draft variation to the *Australia New Zealand Food Standards Code.*

The Authority invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the *Australia New Zealand Food Standards Code* for the purpose of preparing an amendment to the *Australia New Zealand Food Standards Code* for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist the Authority in preparing the Final Assessment for this application. Submissions should, where possible, address the objectives of the Authority as set out in section 10 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Information providing details of potential costs and benefits of the proposed change to the *Australia New Zealand Food Standards Code* from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires the Authority to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions should be received by the Authority **by 27 August 2003**. Submissions received after this date may not be considered, unless the Project Manager has given prior agreement for an extension. While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Liaison Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website or alternatively paper copies of reports can be requested from the Authority's Information Officer at either of the above addresses or by emailing 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.

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. 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*.

The main function that lysophospholipase has 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.

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 is listed as Generally Recognized As Safe (GRAS) for use in food in the USA. France has approved the use of lysophospholipase as a food enzyme.

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.

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.

The Draft 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

The draft variation to Standard 1.3.3 – Processing Aids of the Code, thereby giving approval for 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 process 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 to the Code is consistent with the section 10 objectives of the FSANZ Act. FSANZ has addressed the protection of public health and safety by undertaking a safety assessment of the enzyme and using 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 has 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 listed in the Table to clause 17 of Standard 1.3.3.

3. Objective

The objective of this assessment is to determine whether the Code should be amended to permit the use of lysophospholipase derived from *Aspergillus niger*. The assessment will include consideration of the section 10 objectives of the FSANZ Act.

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 *Food Standards Australia New Zealand Act 1991*. 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.

FSANZ will address 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

The applicant claims 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 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 supporting this application from the Manildra group in Australia. 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 more 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.

From the available data, the production organism *Aspergillus niger* is 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 NOEL 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
- the enzyme preparation produced no mutagenic or cytogenic effects in *in vitro* assays;

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.

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) and JECFA compendium of specifications for food grade enzyme preparations.

Lysophospholipase is listed as Generally Recognized As Safe (GRAS) for use in food in the USA.

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

6. Regulatory Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and governments in Australia and New Zealand. The benefits and costs associated with the proposed amendment to the Code will be analysed using regulatory impact principles.

The following two regulatory options are available for this application:

Option 1. 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

The affected parties to this application include:

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 government enforcement agencies that enforce food regulations.

7.1 Option 1

There are no perceived benefits to industry, government regulators 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

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 regulators 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 for this application 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. Attachment 2 summarises the submissions received during the first round of public comment.

FSANZ is seeking further public comment on this Draft Assessment Report to assist in assessing this application at Final Assessment.

Comments on the following topics would be useful:

- technological justification;
- safety considerations;
- other scientific aspects; and
- costs and benefits.

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 JEFCA so there does not appear to be a need to notify the WTO.

9. Conclusion and Recommendation

The Draft 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, thereby giving approval for 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 process 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.
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- 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

Submitters

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	
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	Comments
Australian Food and Grocery Council	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	The Technical Sub Committee agree 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)	It will defer comment until the Draft Assessment Report.

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 NOEL 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.

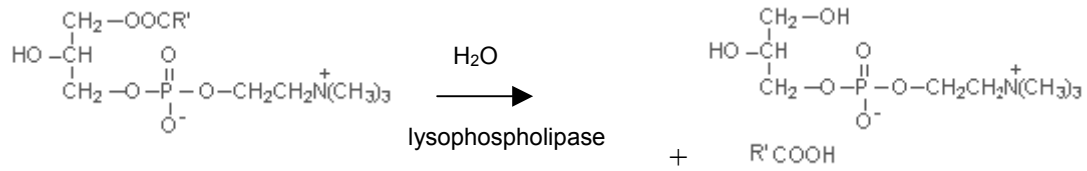
Lysophospholipase

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