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

12 December 2007
[8-07]

DRAFT ASSESSMENT REPORT

APPLICATION A606

ASPARAGINASE AS A PROCESSING AID

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 6 February 2008
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

An Application has been received by Food Standards Australia New Zealand (FSANZ) on 30 April 2007 from Novozymes A/S Denmark (submitted by Novozymes Australia Pty Ltd) seeking the approval of a new enzyme, asparaginase, as a processing aid. Asparaginase is produced from a selected genetically modified strain of the host micro-organism *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene.

Application A606 seeks to amend Standard 1.3.3 – Processing Aids of the *Australia New Zealand Food Standards Code* (the Code) to approve an asparaginase enzyme preparation, (EC number 3.5.1.1), from *A. oryzae* containing a gene coding for asparaginase from *A. oryzae*, as a processing aid. The enzyme is proposed for use in food processing to convert the amino acid asparagine to aspartic acid to reduce acrylamide formation during processing of potato based products such as potato chips and French fries and wheat dough based products such as biscuits and crisp breads which are heated above 120°C. The enzyme is produced by a submerged batch-fed fermentation of an *A. oryzae* micro-organism expressing the *A. oryzae* asparaginase gene.

Acrylamide is formed as a reaction product between asparagine and reducing sugars contained in the food when heated above 120°C during baking or frying. Concerns about dietary exposure to acrylamide had arisen as a result of studies conducted in Sweden in 2002, which showed high levels of acrylamide were formed during the frying or baking of a variety of foods. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of acrylamide in 2005 and recommended that acrylamide be re-evaluated when results of ongoing carcinogenicity and long term neurotoxicity studies, which are being conducted around the world, become available and that appropriate efforts to reduce acrylamide concentrations in food should continue.

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 in the Code for this or any other asparaginase enzyme.

The enzyme preparation meets the international specifications for enzymes. The enzyme also has been self-affirmed GRAS (Generally Recognised As Safe in the US.

The Safety Assessment Report concluded that:

- the production organism has a history of safe use as a production strain for food-grade enzyme preparations and has been shown not to produce toxic metabolites;
- the recombinant DNA in the production organism is considered to be stable and unlikely to pose a safety concern;
- the enzyme preparation complies with international specifications;
- there was no evidence of toxicity in the 90-day toxicity study in rats; 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 this enzyme as a processing aid in food would not raise any public health and safety concerns. This is consistent with the findings of JECFA, which established an ADI of 'not specified' at their meeting in 2007, confirming that asparaginase is a substance of very low toxicity.

FSANZ reviewed the dietary exposure assessment for asparaginase provided by the Applicant and concluded that it was unnecessary to undertake more refined dietary exposure estimates.

Based on claimed levels of acrylamide reductions provided by the Applicant for foods that are major contributors to acrylamide dietary exposure, FSANZ concludes that it is likely the intended use of asparaginase as a processing aid will reduce total acrylamide dietary exposure. Such use of the enzyme is technologically justified to potentially reduce the formation of acrylamide in some potato based and wheat dough based products which are baked or fried.

The recommended option is to approve permission for the enzyme since it provides benefits to food manufacturers and consumers, by approving a treatment some food manufacturers can use to reduce the formation of acrylamide in processed food. There should not be any major costs or disadvantages to government regulators that outweigh the benefits.

Enzymes from microbial sources are permitted processing aids if they are listed in the Table to clause 17 of Standard 1.3.3. Therefore, if this enzyme is approved for use then a new entry needs to be made for asparaginase. Subclause 17(2) of Standard 1.3.3 allows that approved micro-organism sources of enzymes may contain additional copies of genes from the same organism. This is the situation for this asparaginase enzyme since the source micro-organism (*A. oryzae*) contains multiple copies of the asparaginase gene from the *A. oryzae* micro-organism. Therefore, the source micro-organism can be simply given as *Aspergillus oryzae* in the Table to clause 17 of Standard 1.3.3.

Purpose

The Application is seeking the approval of a new enzyme, asparaginase, which has a microbial source, being *A. oryzae* expressing the asparaginase gene from *A. oryzae*. The asparaginase enzyme is used to reduce acrylamide formation in some foods during processing.

Preferred Approach

FSANZ recommends the proposed draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids, to permit the use of the enzyme asparaginase sourced from *Aspergillus. oryzae* expressing the *A. oryzae* asparaginase gene.

Reasons for Preferred Approach

This Application has been assessed against the requirements for Draft Assessment in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). FSANZ recommends the proposed draft variation to Standard 1.3.3 for the following reasons.

- A detailed safety assessment has concluded that the use of the enzyme does not raise any public health and safety concerns.

- Use of the enzyme is technologically justified as a treatment to reduce the formation of acrylamide in some foods.

- No issues were raised in submissions to the Initial Assessment identifying any risks associated with the proposed approval of the enzyme.

- The impact analysis concluded that the benefits of permitting the use of the enzyme to reduce the formation of acrylamide in some treated foods outweigh any associated costs.

- The proposed variation is consistent with the FSANZ Act section 18 objectives.

Consultation

Public comment on the Initial Assessment Report was sought from 8 August 2007 to 19 September 2007. Five submissions were received, with three submitters supporting the Application and two reserving their position until after the Draft Assessment as they wished to review the assessment of the safety of the enzyme in food preparation. A small number of issues raised in the submissions have been addressed in this Report.

CONTENTS

INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variations to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the 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 FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as confidential commercial information. Section 114 of the FSANZ Act requires FSANZ 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 need to be received by FSANZ by 6pm (Canberra time) 6 February 2008.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

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 Management Officer at the above address or by emailing standards.management@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

INTRODUCTION

This Application was received from Novozymes A/S (submitted by Novozymes Australia Pty Limited) on 30 April 2007 seeking to vary the *Australia New Zealand Food Standards Code* (the Code). The proposed variation to Standard 1.3.3 – Processing Aids would permit the enzyme asparaginase (also called L-asparagine amidohydrolase) (EC 3.5.1.1), as a processing aid. Asparaginase is produced using recombinant DNA techniques, from a strain of the host micro-organism *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene.

The Applicant claims that the enzyme hydrolyses the amino acid asparagine to aspartic acid by hydrolyzing the amide in asparagine to the corresponding acid, aspartic acid. It is claimed that the enzyme is intended for use as a processing aid during food manufacture to convert asparagine to aspartic acid to reduce acrylamide formation in baked or fried wheat dough based products such as biscuits and crackers and cut vegetable products such as sliced potato chips and French fries.

Acrylamide is formed as a reaction product between the amino acid asparagine and reducing sugars contained in the food when heated above 120°C during baking or frying.

1. Background

1.1 Current Standard

Standard 1.3.3 regulates the use of processing aids in food manufacture, prohibiting their use unless there is a specific permission in the Standard. There are currently no permissions in Standard 1.3.3 for use of asparaginase as a processing aid in manufacturing food products. Processing aids not permitted in the Code may not be used for food manufacture until there has been a pre-market assessment of their use.

Clause 1 of Standard 1.3.3 defines a processing aid as:

> ***processing aid*** *means a substance listed in clauses 3 to 18, where –*
>
> *(a) the substance is 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; and*
>
> *(b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.*

The Applicant has requested that, if approved, the permission for use of the enzyme be included in the Table to clause 17 – Permitted enzymes of microbial origin as asparaginase EC 3.5.1.1 with the source being *A. oryzae* expressing the *A. oryzae* asparaginase gene. Under clause 17, the processing aids listed in the Table to this clause may be used as enzymes in the course of manufacture of any food provided the enzyme is derived from the corresponding source or sources specified in the Table.

1.2 Basis of Application

The Applicant proposes introducing asparaginase as a processing aid to be added to food products during processing to convert L-asparagine to L-aspartate and ammonia to reduce the quantity of acrylamide formed during production of products such as potato chips and French fries and wheat dough based products such as biscuits and crisp breads. Both asparagine and reducing sugars are commonly found in the ingredients of many food products. The Applicant claims that by using asparaginase, the asparagine content will be reduced, resulting in reduced acrylamide formation and consequently a reduced acrylamide content in the final product benefiting consumers by decreasing acrylamide intake through consumption of processed food products.

1.3 Acrylamide in Food

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook an evaluation of acrylamide at its sixty-fourth meeting, at the request of the Codex Committee on Food Additives and Contaminants (JECFA 2005)[1]. The Committee had not previously evaluated acrylamide. Concerns about dietary exposure to acrylamide had arisen as a result of studies conducted in Sweden in 2002, which showed high levels of acrylamide were formed during the frying or baking of a variety of foods. JECFA recommended that acrylamide be re-evaluated when results of ongoing carcinogenicity and long term neurotoxicity studies become available and that appropriate efforts to reduce acrylamide concentrations in food should continue.

The Confederation of the Food and Drink Industries of the EU (CIAA, Confédération des Industries Agro-Alimentaires de l'UE) produced an Acrylamide 'Toolbox' in September 2006 (revision 9) to assist the food industry to utilise methods to minimise the formation of acrylamide in their processed food[2]. It specifically mentions using asparaginase in food processing, understanding that regulatory approval is first required.

In April 2007, the Codex Committee on Contaminants in Food (CCCF) commenced work on a draft Code of Practice for the Reduction of Acrylamide in Food[3]. This document flags the potential use of the enzyme asparaginase to reduce asparagine and hence acrylamide formation in food, specifically potato products made from potato doughs and cereal-based products.

1.4 Nature of the Enzyme and Source Organism

The systematic name of the enzyme is L-asparagine amidohydrolase, and the accepted name is asparaginase[4] which is the name used in this report. The commercial name of the Novozymes asparaginase preparation is Acrylaway® L.

[1] Joint FAO/WHO Expert Committee on Food Additives (JECFA) *Report on 64th meeting* (Rome, 8-17 February 2005), Acrylamide, pp7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf, accessed on 19 September 2007.
[2] The CIAA Acrylamide 'Toolbox', Rev 9, 29 September 2006, http://www.ciaa.be/documents/brochures/ciaa_acrylamide_toolbox_Oct2006.pdf, accessed 19 September 2007.
[3] Codex Committee on Contaminants in Foods (Beijing 16-20 April 2007) Proposed Draft Code of Practice for the Reduction of Acrylamide in Food, at ftp://ftp.fao.org/codex/cccf1/cf01_15e.pdf, assessed on 19 September 2007.
[4] International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/5/1/1.html, accessed on 19 September 2007.

The enzyme has the Enzyme Commission (EC) number of 3.5.1.1 and a Chemical Abstracts Service (CAS) number of 9015-68-3.

The enzyme preparation is a clear to pale brown water soluble liquid. The enzyme is stable between pH 5.0 to 9.0. The enzyme activity range occurs between pH 5.0 to 9.0, with its optimum activity at pH 7.0. The optimum temperature of use is 60ºC. The molecular weight of the enzyme was determined to be 36 kDa.

Asparaginase catalyses the hydrolysis of the amino acids L-asparagine and L-glutamine to yield L-aspartate and L-glutamate. Probably owing to steric hindrance it has no activity on asparagine or glutamine residues in peptides or proteins.

The Application indicates that the source micro-organism is a genetically modified selected strain of *A. oryzae* which contains extra copies of the asparaginase gene obtained from *A. oryzae*. The extra copies of the asparaginase gene inserted into the source micro-organism improves the yield of the enzyme during the fermentation. The Applicant indicated that modification also removed unwanted side activities and metabolites.

1.5 International Permissions

The Applicant submitted a self-affirmed GRAS (generally recognised as safe) notification (GRAS Notice No. GRN 000201) for this same enzyme to the US Food and Drug Administration (FDA) for which it received the 'no questions letter' on November 2006. This information is contained in the Application, while the FDA letter can be obtained from the FDA website[5].

The Applicant states that the enzyme can already be legally sold in Germany, Great Britain, Italy, Ireland and the USA. An application seeking approval for the enzyme has been submitted to JECFA and Denmark and will be submitted in France also in 2007.

There is no Codex standard for the enzyme, since there are no specific Codex standards for enzymes. However, the Applicant states that the enzyme complies with the specifications for enzymes of both JECFA and the Food Chemicals Codex.

JECFA also examined the same enzyme from the same source micro-organism (and the same information as contained in the Application) at their sixty-eight meeting, 19-28 June 2007 in Geneva with the summary report available. This summary report indicated that the enzyme had an *ADI of 'not specified' when used in the applications specified and in accordance with good manufacturing practice*[6].

Separately, FSANZ is aware that another form of the asparaginase enzyme sourced from a genetically modified micro-organism (*A. niger* expressing the asparaginase gene from *A. niger*) produced from DSM Food Specialties has recently also been developed.

[5] US FDA Agency Response letter GRAS Notice No. GRN 000201, 24 November 2006. http://www.cfsan.fda.gov/~rdb/opa-g201.html assessed on 19 September 2007.

[6] Joint FAO/WHO Expert Committee on Food Additives, Sixty-eight meeting, Summary and Conclusions, 19-28 June 2007, Geneva, http://www.who.int/ipcs/food/jecfa/summaries/summary68.pdf accessed on 19 September 2007.

The DSM asparaginase has recently (March 2007) been considered GRAS (self-affirmed), with GRN 000214[7].

It is important to understand that if this current Application is successful it will not provide permission for the DSM asparaginase enzyme, since it is derived from a different source micro-organism, so a separate pre-market assessment would be required for such an enzyme before a separate permission could be granted. The Table to clause 17 of Standard 1.3.3 provides individual permissions to enzymes derived from specific source micro-organisms, so the permission is quite specific.

2. The Issue / Problem

Processing aids (which includes enzymes) are required to undergo a pre-market assessment before they are approved for use in food manufacture.

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There is currently no permission for the enzyme asparaginase, from any source in this Table. Therefore an assessment (which includes a safety assessment) of the use of the enzyme is required before it can be approved or used.

3. Objectives

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of asparaginase from the source, *A. oryzae* expressing the *A. oryzae* asparaginase gene.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 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

[7] US FDA Agency response Letter GRAS Notice No. GRN 000214, http://www.cfsan.fda.gov/~rdb/opa-g214.html, accessed on 19 September 2007.

- any written policy guidelines formulated by the Ministerial Council.

4. Key Assessment Questions

The key question which FSANZ needs to consider as part of this assessment is:

- Are there any public health and safety issues with approving the asparaginase enzyme sourced from *A. oryzae* expressing the *A. oryzae* asparaginase gene?

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Safety Assessment

A safety assessment was conducted as part of this Application. The full Safety Assessment Report is at **Attachment 2**. The safety assessment concluded that:

- the production organism has a history of safe use as a production strain for food-grade enzyme preparations and has been shown not to produce toxic metabolites;
- the recombinant DNA in the production organism is considered to be stable and unlikely to pose a safety concern;
- the enzyme preparation complies with international specifications;
- there was no evidence of toxicity in the 90-day toxicity study in rats;
- in the 90-day study in rats, the NOEL was 880 mg total organic substances (TOS) /kg bw per day. This is equivalent to 10 ml liquid enzyme concentrate (or approximately 46576 Asparaginase Units (ASNU)/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 this enzyme as a processing aid in food would not raise any public health and safety concerns. This is consistent with the findings of JECFA, which established an ADI of 'not specified' at its meeting in 2007, confirming that asparaginase is a substance of very low toxicity.

5.2 Dietary Exposure Considerations of Asparaginase and Acrylamide

FSANZ has reviewed the dietary exposure assessment for the enzyme asparaginase which was provided by the Applicant. The Dietary Exposure Assessment Report is at **Attachment 4**. The Applicant's estimate of maximum dietary exposure for asparaginase was 0.35 mg (TOS)/kg/day, assuming asparaginase contains 4% TOS. FSANZ considers this to be a conservative assessment (i.e. an overestimate) as it is based on the Budget Method, an internationally accepted methodology to screen food additives for safety concerns using very conservative assumptions. Based on claimed levels of acrylamide reductions provided by the Applicant for foods that are major contributors to acrylamide dietary exposure, FSANZ concludes that it is likely the intended use of asparaginase as a processing aid will reduce total acrylamide dietary exposure.

Given the Applicant is seeking the approval of the enzyme asparaginase, FSANZ considers a dietary exposure assessment for acrylamide is not necessary.

An Australian survey of acrylamide in carbohydrate-based foods found major contributors to total dietary exposure to include hot potato chips, potato crisps, white toast, sweet biscuits, breads and breakfast cereals (wheat biscuit style)[8]. The 2005 JECFA evaluation of acrylamide found similar contributors to total dietary exposure assessment as the Australian survey and indicated that coffee was an additional major contributor to total acrylamide dietary exposure [9].

5.3 Risk Characterisation

In animal studies, the highest dose of asparaginase tested was 880 mg TOS /kg bw per day. This is equivalent to 10 mL liquid enzyme concentrate (or approximately 46576 ASNU)/kg bw per day. No adverse effects were noted at this level of exposure, indicating asparaginase is a substance of low toxicity. This was confirmed by JECFA, which has allocated an ADI of 'not specified' indicating asparaginase can be used safely within the bounds of Good Manufacturing Practice (GMP).

Dietary exposure calculations by the Applicant estimate the maximum dietary exposure for asparaginase was 0.35 mg TOS/kg/day. Given that the estimate of maximum dietary exposure to asparaginase was derived from the Budget method, the conservative nature of which is recognised in the literature[10], it is likely that actual exposure will be lower than this.

Human exposure levels are therefore anticipated to be greater than 2500 times lower than the highest levels used in animal experiments, which were found to cause no adverse effects. Therefore, FSANZ did not consider it necessary to undertake more refined dietary exposure estimates. FSANZ concludes that there is unlikely to be any appreciable risk to public health and safety should the permission to use asparaginase for its intended use as a processing aid be granted.

5.4 Technological Justification

The Novozymes asparaginase enzyme preparation is produced from a submerged batch-fed fermentation of a selected genetically modified strain of *A. oryzae* containing a gene coding for asparaginase from *A. oryzae*. The commercial asparaginase preparation complies with internationally recognised specifications for the production of enzymes, being the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[11] and the Food Chemicals Codex[12].

The asparaginase enzyme preparation is proposed to be used to treat wheat dough based products such as biscuits and crackers and processed products based on potato such as French fries and potato chips which are heated above 120°C by baking or frying, to reduce the formation of acrylamide.

[8] Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin*. **21**(8):721-736

[9] Joint FAO/WHO Expert Committee on Food Additives, Report on Sixty-fourth meeting, Rome, 8-17 February 2005, Acrylamide, pages 7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

[10] International Life Sciences Institute (1997) An evaluation of the Budget Method for screening food additive intake.

[11] 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, Addendum 9, pp 37-39.

[12] Food Chemicals Codex (2004), National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex, 5th Edition, National Academy press, Washington DC, pp146-152.

Results reported in the Application of trials undertaken by Novozymes provided in a Technology Sheet (but not assessed independently or in a peer-reviewed scientific article) indicate reductions of acrylamide in potato and wheat dough based heat processed foods (which are high contributors to acrylamide exposure in the diet) range from 40% up to above 90%.

The use of the asparaginase enzyme sourced from *A. oryzae* as a processing aid is technologically justified to treat some potato based and wheat dough based products which are baked or fried to reduce the formation of acrylamide in the final products.

A Food Technological Report at **Attachment 3**, contains some more detail about the nature and use of the enzyme in food preparation.

RISK MANAGEMENT

6. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sections of the community, especially relevant stakeholders who may be affected by this Application. The benefits and costs associated with the proposed amendment to the Code have been analysed using regulatory impact principles.

Enzymes (being processing aids in the Code) used in Australia and New Zealand are required to be listed in Standard 1.3.3, and it is not appropriate to consider non-regulatory options.

Two regulatory options have been identified for this Application:

Option 1 Not permit the use of asparaginase sourced from *A. oryzae* as a processing aid.

Option 2 Permit the use of asparaginase sourced from *A. oryzae* as a processing aid.

7. Impact Analysis

The impact analysis represents likely impacts on all stakeholders and affected parties by the Application, if successful. The impact analysis is designed to assist in the process of identifying the affected parties and the likely or potential impacts the regulatory provisions will have on each affected party.

7.1 Affected Parties

The affected parties to this Application include the following:

1. those sectors of the food industry, including importers of food, wishing to produce and market food products manufactured using this enzyme;

2. consumers; and

3. The Governments of Australia (State and Territory) and New Zealand.

7.2 Benefit Cost Analysis

In developing food regulatory measures for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the relevant food industries and governments. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits arising from the regulation and its health, economic and social impacts. At Final Assessment FSANZ will use the Office of Best practice Regulation Business Cost Calculator to calculate the compliance costs of regulatory options where medium to significant competitive impacts or compliance costs are likely.

7.2.1 Option 1 – Not permit the use of asparaginase sourced from A. oryzae *as a processing aid*

There are no perceived benefits to the food industry, consumers or government agencies if this option is progressed.

Not approving the use of this asparaginase enzyme would disadvantage consumers, some food industries and could leave government agencies open to criticism that not all viable treatments to reduce the formation of acrylamide in food have been investigated and supported. Consumers and relevant food industries where the enzyme could reduce the formation of acrylamide in their products would be disadvantaged since not all methods available to reduce the formation of acrylamide in food would be available.

7.2.2 Option 2 – Permit the use of asparaginase sourced from A. oryzae *as a processing aid*

This option does provide benefits to consumers, the food industry and indirectly to government agencies. The reason for this is that the asparaginase enzyme has been developed and assessed to reduce the formation of acrylamide in some processed foods so assisting in reducing the levels of this compound in the food supply of consumers. It also provides some food industries a viable commercial method to reduce the formation of acrylamide without compromising the quality, flavour or characteristics of their processed food. While government agencies are able to indicate to international agencies (specifically JECFA) that they are assisting the food industry in developing procedures to reduce the formation of acrylamide in the food supply.

There should not be any significant compliance costs for government enforcement agencies since they would not need to analyse for the presence of the enzyme, nor would it be expected that they would need to analyse for acrylamide due to this Application. It may well be that acrylamide analyses in food will be required as part of survey work in the future but that should not be due to this Application.

7.3 Comparison of Options

Option 2 is favoured since there is no benefit derived for any affected party for option 1, while consumers, relevant food industries and government agencies all would be advantaged by option 2. This is since the outcome of approving the use of this asparaginase enzyme as a processing aid can reduce the formation of acrylamide in some processed food products.

7.4 Drafting name for microbial source organism

To give effect to option 2, giving permission for the enzyme, required an assessment of how to incorporate the enzyme and the source micro-organism into the Code. Approved enzymes from microbial sources are listed in the Table to clause 17 of Standard 1.3.3, so an entry for the enzyme in this Table is required.

Subclause 17(2) of Standard 1.3.3 states that:

> *The sources listed in the Table to this clause may contain additional copies of genes from the same organism.*

This is the situation for asparaginase derived from *A. oryzae*. Therefore, the source micro-organism can be simply given as *Aspergillus oryzae*. The draft variation is provided in **Attachment 1**.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

It is considered that this Application is a routine matter. Therefore, FSANZ has applied a basic communication strategy. This will involve advertising the availability of assessment reports for public comment in the national press and making reports available on the FSANZ website.

The Applicant and individuals and organisations who make submissions on this Application will be notified at each stage of the assessment of the Application. If approval is recommended, once the FSANZ Board has approved the Final Assessment Report, FSANZ will notify the Ministerial Council. The Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website.

FSANZ provides an advisory service to the jurisdictions on changes to the Code.

9. Consultation

Public comment on the Initial Assessment Report for this Application was sought from 8 August 2007 until 19 September 2007. Five submissions were received of which three submissions supported approving the enzyme and two reserved their position until the Draft Assessment. **Attachment 5** summarises the submissions received during this first round of public comment.

Issues raised in these submissions and FSANZ's response to these are discussed in section 9.1 below.

FSANZ is seeking further public comment on this Application to assist in finalising the assessment.

9.1 Issues raised in submissions

Three issues were raised in one submission to the Initial Assessment Report, which were asked to be addressed at Draft Assessment. They have been dealt with already in the body of the report and in the attachments but will be summarised here as well.

9.1.1 Safety of residues in food

One submission asked that two issues be considered as part of the Draft Assessment. They both relate to residues in the final processed food.

The first issue was whether any residual asparaginase would be destroyed during the cooking process.

The second concern was whether there are any residues of the genetically modified micro-organism present in the enzyme preparation and then subsequently incorporated into the final food.

9.1.1.1 FSANZ evaluation

For the first point: any residual asparaginase enzyme is inactivated during the heating process (frying or baking to 120°C) and the subsequent inactivated enzyme is considered standard protein.

For the second point: the specifications for enzymes produced from micro-organisms, which the Applicant states their enzyme met and have provided data in the Application supporting this, require that no source organism be present in the final enzyme preparation. Therefore, no genetically modified micro-organism are present in the enzyme preparation and so none would be in contact with any treated food or remain as residues on the food.

9.1.2 Cost analysis issues relating to enforcement

One jurisdiction submission made mention that their analytical services laboratory does not currently have suitable analytical methodology that may be required for this Application if the asparaginase enzyme is approved. That is, their laboratory could not analyse for acrylamide in food, measure asparaginase activity or detect the genetically modified *A. oryzae.*

9.1.2.1 FSANZ evaluation

If this Application is successful and the Novozymes asparaginase enzyme is approved it would not be expected that any particular enforcement work would be required for day to day activities. There should not be any asparaginase activity in the final produced foods since the enzyme is inactivated during the heating step (frying or baking to 120°C). Also, there should be no presence of the production micro-organism (*A. oryzae*) in the treated food since the Novozymes specification requires no presence of the production organism in the enzyme preparation.

Analyses for acrylamide in the final food should not be required as part of this Application, though there may well be further acrylamide survey and analyses work required as part of some broader work relating specifically to acrylamide. It would be expected that such analytical work measuring acrylamide levels in food would be undertaken by a commercial laboratory such as National Measurement Institute (NMI, formerly the Australia Government Analytical Laboratories) who undertook the earlier acrylamide analyses for the survey of acrylamide levels in Australian food undertaken in late 2002 and who have the analytical methods available for such measurements[13]. If the jurisdiction wished to or was required to perform some acrylamide analytical work they could maybe engage the NMI to perform this work for them.

9.2 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.

There are not any relevant international standards for processing aids or specifically enzymes and amending the Code to allow permission to use asparaginase sourced from *A. oryzae* containing additional copies of the *A. oryzae* gene encoding asparaginase is unlikely to have a significant effect on international trade. The enzyme preparation is consistent with the international specifications for food enzymes of JECFA and the Food Chemicals Codex so there does not appear to be a need to notify the WTO. For these reasons FSANZ decided not to notify the WTO under the either the Technical Barriers to Trade (TBT) or Sanitary and Phytosanitary Measures (SPS) Agreements.

CONCLUSION

10. Conclusion and Preferred Approach

This Application has been assessed against the requirements for Draft Assessment in the FSANZ Act. FSANZ recommends the proposed draft variation to Standard 1.3.3 as at **Attachment 1**.

Preferred Approach

FSANZ recommends the proposed draft variation to the Table to clause 17 of Standard 1.3.3 – Processing Aids to permit the use of the enzyme asparaginase sourced from *Aspergillus. oryzae* expressing the *A. oryzae* asparaginase gene.

10.1 Reasons for Preferred Approach

FSANZ recommends the proposed draft variation to Standard 1.3.3 for the following reasons.

- A detailed safety assessment has concluded that the use of the enzyme does not raise any public health and safety concerns.

[13] Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin.* **21**(8):721-736

- Use of the enzyme is technologically justified as a treatment to reduce the formation of acrylamide in some foods.

- No issues were raised in submissions to the Initial Assessment identifying any risks associated with the proposed approval of the enzyme.

- The impact analysis concluded that the benefits of permitting the use of the enzyme to reduce the formation of acrylamide in some treated foods outweigh any associated costs.

- The proposed variation is consistent with the FSANZ Act section 18 objectives.

11. Implementation and Review

If this Application is successful the variation to the Code will take effect on gazettal and would be subject to existing compliance arrangements.

ATTACHMENTS

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

Attachment 1

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

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

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–

Asparaginase EC [3.5.1.1]	*Aspergillus oryzae*

Attachment 2

Safety Assessment Report

A606 – ASPARAGINASE AS A PROCESSING AID

Summary and Conclusion

Application A606 seeks approval for the use of asparaginase from *Aspergillus oryzae* as a processing aid. This strain of *A. oryzae* contains multiple copies of a gene encoding asparaginase from *A. oryzae*.

The enzyme is used as a processing aid only, and any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The safety assessment concluded that:

- the production organism has a history of safe use as a production strain for food-grade enzyme preparations and has been shown not to produce toxic metabolites;
- the recombinant DNA in the production organism is considered to be stable and unlikely to pose a safety concern;
- the enzyme preparation complies with international specifications;
- there was no evidence of toxicity in the 90-day toxicity study in rats;
- in the 90-day study in rats, the NOEL was 880 mg TOS /kg bw per day. This is equivalent to 10 ml liquid enzyme concentrate (or approximately 46576 ASNU)/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 this enzyme as a processing aid in food would not raise any public health and safety concerns. This is consistent with the findings of JECFA, which established an ADI of 'not specified' at their meeting in 2007, confirming that asparaginase is a substance of very low toxicity.

1. Introduction

Application A606 seeks approval for the use of the enzyme asparaginase from *A. oryzae* as a processing aid. The production organism, *A. oryzae*, contains additional copies of an endogenous asparaginase gene.

The enzyme is also known as L-asparagine amidohydrolase (EC 3.5.1.1, CAS No. 9015-68-3), and hydrolyses the amino acid asparagine to aspartic acid. The asparaginase enzyme preparation produced by the Applicant is called Acrylaway®L and has a typical activity of 3500 ASNU/g. One ASNU is the amount of enzyme that produces one µmole ammonia per minute under specific reaction conditions. The products of this reaction, aspartic acid and ammonia are normal constituents of food.

The applicant's intent is for the enzyme preparation to be used as a processing aid in wheat dough-based products such as cookies and crackers, as well as other processed foods such as potato chips and French fries. Asparaginase will be inactivated during the cooking of these foods.

2. 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. Asparaginase from the production organism, *A. oryzae* complies with the purity criteria recommended for enzyme preparations in the Fifth Edition of Food Chemicals Codex, 2004 (NAS FNB, 2004), and the JECFA specification for asparaginase(JECFA, 2001; JECFA, 2007b).

One batch was analysed and the results reported in Table 1.

Table 1: Analytical data for a batch of asparaginase enzyme

Criteria	PPV 24743
Asparaginase activity (ASNU/g)	4440
Total viable count/g	$<2 \times 10^2$ /g
Total coliforms/g	< 10 /g
Enteropathogenic E. coli/25 g	negative
Salmonella/25 g	negative
Production strain/g	negative
Heavy Metals	3.9 ppm
Arsenic	< 0.1 ppm
Lead	< 1 ppm
Cadmium	< 0.05 ppm
Mercury	< 0.03 ppm

Asparaginase produced by *A. oryzae* has a theoretical molecular weight of approximately 37 kDa. The Applicant states that the amino acid sequence of asparaginase expressed in this production strain is identical to the wild-type *A. oryzae* asparaginase.

3. The production organism – *Aspergillus oryzae*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as processing aids. The primary issue is the toxigenic potential of the production organism, that is, the possible synthesis by the production strain of toxins, and the potential for the carryover of these into the enzyme preparation (Pariza and Johnson, 2001).

A. oryzae is not considered to be a pathogen and has a history of safe use as a production organism for food enzymes and is a permitted source of a number of enzymes in the *Australia New Zealand Food Standards Code*[14].

The strain of *A. oryzae* in which asparaginase is produced is designated pCAHj621/BECh2#10, and the enzyme preparation known as PPV2473. This strain was produced by transformation of the BECh2 strain with the asparaginase expression plasmid pCaHj621.

[14] The following enzymes sourced from *A. oryzae* are permitted in the Code: aminopeptidase; α-amylase; carboxyl proteinase; β-glucanase; glucoamylase; a-glucosidase; xylanase; lactase β –galactosidase; triacylglycerol lipase; metalloproteinase; mucorpepsin; pectin methlyesterase; 6-phytase; polygalacturonase; serine proteinase; and phospholipase A1.

BECh2 was produced from strain IFO 4177 (synonym A1560) obtained from the Institute for Fermentation, Osaka (IFO), by a series of gene deletions and mutations to remove the ability to produce unwanted side activities (including amylase and protease activities) and secondary metabolites.

This was done because certain strains of *A. oryzae* may produce one or more of the moderately toxic secondary metabolites cyclopiazonic acid, kojic acid and β-nitropropionic acid (Burdock *et al*., 2001a; Burdock *et al*., 2001b; Blumenthal, 2004). *A. oryzae* strains used in the production of food enzymes need to be routinely screened for the production of cyclopiazonic acid and other undesirable metabolites. Also, *A. oryzae* is closely related to the aflatoxin-producing fungus *Aspergillus flavus*, and contains some genes from the aflatoxin biosynthesis pathway. However, these genes are inactive in *A. oryzae* and it is generally agreed that *A. oryzae* does not produce aflatoxin (Blumenthal, 2004).

In BECh2, it has been shown that one arm of a chromosome, containing the genes suspected to be involved in cyclopiazonic acid synthesis and the whole aflatoxin gene cluster has been deleted, making BECh2 unable to produce, or revert to a strain that is capable of producing, these mycotoxins. The synthesis of kojic acid is also impaired in this strain. Although BECh2 has the metabolic pathway necessary to produce β-nitropropionic acid, it appears that it is expressed only very weakly under specific circumstances.

This was verified by testing the ability of *A. oryzae* strains IFO 4177 and BECh2 to produce cyclopiazonic acid, β-nitropropionic acid and kojic acid when grown on optimal media. While strain IFO 4177 produced cyclopiazonic acid, β-nitropropionic acid and traces of kojic acid, only kojic acid was detected from the fermentations with the BECh2 strain, estimated to be present in quantities of only 15% of the level detected in IFO 4177.

Absence of these secondary metabolites under enzyme production conditions was confirmed for the asparaginase production strain. One batch of asparaginase was analyzed and the results shown below.

Analysis of Asparaginase produced in *A. oryzae* PPV2473

	Batch 1
Kojic Acid (<1.4 mg/kg)	ND
3-Nitropropionic acid (<0.6 mg/kg)	ND

a) Limit of detection is given in brackets
b) ND = Not Detected

It is concluded that the production strain does not produce secondary metabolites of toxicological concern to humans. Further, *A. oryzae* strains have a history of safe use in the production of food enzymes. The use of *A. oryzae* to produce asparaginase does not pose any concern to human health and safety.

4. Characterisation of the genetic modification

A. oryzae strain pCaHj621/BECh2#10 was produced by transformation of BECh2 with the *A. oryzae* expression plasmid pCAHj621. This plasmid contains the asparaginase gene from *A. oryzae* strain IFO 4177 (synonym A1560).

In addition to the asparaginase gene, other genetic information contained on plasmid pCaHj621 to enable the efficient expression of this gene in *A. oryzae* includes:

- the neutral amylase II promoter (NA2) from *Aspergillus niger* strain BO-1;
- the 5' untranslated leader of the triose phosphate isomerase (TPI) gene, which is synthetic and corresponds to the sequence of the *Aspergillus nidulans* TPI gene; and
- the glucoamylase transcriptional terminator from *A. niger* strain BO-1.

In addition to the asparaginase gene and regulatory elements, pCaHj621 contains two marker genes, *amdS* and *URA3*. The acetamidase gene (*amdS*) from *A. nidulans* allows selection in *A. oryzae* as it allows growth on media with acetamide as the sole nitrogen source. The *URA3* gene from *Saccharomyces cerevisiae* allows selection in *E. coli* as it confers uridine prototrophy in auxotrophic *pyrF E. coli*. The *A. oryzae* expression plasmid also contains a bacterial origin of replication (from pUC19).

The gene cassette does not contain any antibiotic resistance marker genes.

BECh2 protoplasts were transformed with plasmid pCaHj621. Transformants were selected by growing on a medium with acetamide as the sole nitrogen source and screening for co-expression of asparaginase activity. The selected transformant was designated pCAHj621/BECh2#10 and this is the strain that is used for enzyme production.

Genetic stability

The applicant states that *A. oryzae* pCAHj621/BECh2#10 is stable during production fermentation, as the inserted DNA is integrated into the chromosome. This was tested after large-scale fermentation. The strain stability during fermentation was analyzed by Southern blotting and no instability of the strain was observed.

5. Evaluation of the safety studies

A bioinformatics analysis for homology of the asparaginase protein sequence with known protein toxins and allergens was submitted in support of this application, as were three toxicological studies. These were:

1. a 90-day sub-chronic oral toxicity study in rats;
2. a Salmonella/*E. coli* Reverse Mutation Assay (Ames test); and
3. a human lymphocyte assay for chromosomal aberrations.

5.1 Potential toxicity and allergenicity of asparaginase

The *A. oryzae* asparaginase protein sequence was compared to the sequences of known toxins and allergens to assess if there was any significant sequence homology.

The sequence with which asparaginase shared the greatest homology (28.1%) was a putative L-asparaginase protein. This protein had only been included in the toxin database as part of a full genome sequence, referred to in a published article with the word 'toxin' in its title. The next most similar sequence shared only 16.2% homology and was not considered to be a significant match.

No matches greater than six contiguous residues were found between known allergens and asparaginase. It has been reported that an immunologically significant sequence similarity requires a match of at least eight contiguous identical residues.

These data demonstrate that asparaginase is unlikely to share structurally or immunologically relevant sequence similarities with known protein toxins or allergens.

5.3 Sub-chronic toxicity

Asparaginase, PPV 24743 Toxicity Study by Oral Administration to CD Rats for 13 weeks. Study Director: N Hughes. Study No: NVZ0037/054031, Sponsor Reference No: Novozymes Ref No 20066001. 17 October 20064

Test material	Asparaginase, PPV 24743 (4440 ASNU/g, Total Organic Solids 8.4% w/w)
Control and vehicle material	R.O. purified water
Test Species	Crl:CD® (SD) BR rats (4 groups of 10 males and 10 females)
Dose	0, 1, 3.3 or 10 mL liquid enzyme concentrate/kg bw/day (equivalent to 0, 0.088, 0.29, 0.88 g TOS/kg bw/day) by gavage
GLP	OECD (1997)
Guidelines	OECD guideline 408 (1998)

Results

There were no deaths during the treatment period.

Forelimb and hind limb strength in males receiving 3.3 or 10 mL/kg bw per day were slightly high compared to control males, however this was statistically significant only for the high dose group. All values were within the historical control range, and in the absence of similar findings in females, or of any behavioural signs in the males, this difference was considered to be of no toxicological significance.

Food consumption was slightly low throughout the treatment period for females in the high dose group, with an overall food consumption that was approximately 0.93 x that of the control group. This difference was small and males in the high dose group did not demonstrate similar reduced feed consumption. Water consumption was slightly high in females in the medium and high dose groups, but within the normal range and no similar changes were observed in males. These changes were therefore not considered toxicologically important.

Males in the medium and high dose groups had slightly low haematocrit and haemoglobin concentrations; however there was no dose-relationship or similar changes in females. Only one male had individual values that were below the reference range.

All treated females had low total white blood cell counts, but this was not dose-related, was within the historical range, and males were unaffected. Basophil counts were also low in treated females compared to the control females; however this was attributed to high values (close to the upper limit of the historical range) in the control group.

Similarly, treated females in all groups had low activated partial thromboplastin times compared to the control group, which had values close to the upper limit of the background range. No inter-group differences were seen in prothrombin times or in the treated males. Consequently, these findings were considered to be of no toxicological significance.

After 13 weeks, slightly high plasma potassium concentrations compared to control animals were observed in males in the medium dose group and males and females in the medium and high dose groups. No other changes in electrolyte concentrations were observed. In the absence of other findings in the blood or kidneys that might indicate a renal change, the variation in plasma potassium was not considered toxicologically relevant.

All other statistically significant inter-group differences in blood chemistry were minor, lacked dose-relationship or were confined to one sex and were therefore considered to represent normal biological variation.

Macroscopic and microscopic examination of organs and tissues revealed no treatment related findings. All reported findings were considered to be within the background incidence of findings reported in rats of this age and strain and were considered incidental and of no toxicological significance.

Conclusion

No treatment related changes were observed in rats treated with up to 10 mL PVV 24743/ kg bw per day for 13 weeks (880 mg TOS).

Under the conditions of this study, the NOEL of asparaginase was shown to be 880 mg/kg bw per day, based on the maximum dose tested in this study. This is equivalent to 10 mL liquid enzyme concentrate (or approximately 46576 ASNU/kg bw per day).

5.4 Genotoxicity studies

Asparaginase, PVV 24743: Test for Mutagenic Activity with Strains of *Salmonella typhimurium* and *Escherichia coli*. Study Director Peder Bjarne Pedersen. Safety and Toxicology, Novozymes A/S. Study no. 20068039. 18 March 2006.

Test article

Asparaginase (Batch number PVV 24743) liquid enzyme concentrate sterilized and standardized at 5% w/v dry matter.

Study design

Asparaginase was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a two strain of *Escherichia coli* (WP2uvrA and WP2uvrApKM101). Experiments were performed with and without metabolic activation using liver S9 fraction from chemically pre-treated rats.

Like many crude enzyme preparations, the asparaginase preparation contains the free amino acids histidine and tryptophan, which confound the standard Ames test, based on histidine auxotrophy in the *S. typhimurium* strains (and tryptophan auxotrophy in the *E. coli* strains).

A slightly different procedure was therefore used, known as 'treat and plate', where bacterial cultures are exposed to the test article in liquid culture for 3 hours, before being washed and plated on minimal glucose agar plates. All investigations with the histidine requiring Salmonella strains used this method. Initially the standard plate incorporation tests were conducted with the *E. coli* strains, however asparaginase significantly supported growth of tryptophan-requiring *E. coli*, so two independent experiments were conducted with *E. coli* strain WP2uvrApKM101 using the 'treat and plate' assay. The study was conducted in accordance with OECD guideline 471, however the exposure of the culture to the test substance in liquid culture is not specifically described in any guideline.

The study comprised of negative and positive controls with and without S9 metabolising system. Viability 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/incubation 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 (2-nitrofluorene, 9-aminoacridine, n-methyl-n'-nitro-nitrosoguanidine, n-ethyl-n'-nitro-n-nitrosoguanidine, 2-aminoacnthracene).

Results and conclusion

No toxicity of the test article was observed. No dose-related increases in mutation frequency were observed in the strains tested. It was concluded that asparaginase produced by *A. oryzae* did not exhibit mutagenic activity under the conditions of the test.

Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Study director James Whitwell, Novozymes. Covance Study no. 1974/46. Novozymes reference number 31 March 2006.

Test article

Asparaginase liquid enzyme preparation (PPV24743) with a purity of 4440 ASNU/g.

Study design

Asparaginase was tested in an *in vitro* cytogenetics assay using human lymphocyte cultures prepared from the pooled blood of three female donors in two independent experiments. Treatment was performed in the absence and presence of metabolic activation (S9). The study was conducted in accordance with OECD guideline 473 (1997).

In the first experiment, treatment was for 3 hours followed by a 17-hour recovery period prior to harvest. The dose levels (see table 1) were selected by evaluating the effect of asparaginase on mitotic index. The highest concentration chosen for analysis induced approximately 44% and 33% mitotic inhibition in the absence and presence of S9 respectively.

In the second experiment, treatment in the absence of S9 was continuous for 20 hours. Treatment in the presence of S9 was for 3 hours followed by a 17-hour recovery period. Three dose levels were chosen (430, 838 and 1311 µg/mL without S9 and 3200, 4000 and 5000 µg/mL with S9) based on mitotic inhibition at the highest dose of 53% and 0% at the highest doses with and without activation.

In both experiments all treatments were performed in duplicate. 4-nitroquinoline 1-oxide (-S9) and cyclophosphamide (+S9) were used as positive controls.

Following harvesting, lymphocytes were fixed and slides prepared and stained. Slides were examined microscopically and cells with structural aberrations (including and excluding gaps) and polyploid, endoreduplicated or hyperdiploid cells were scored.

Table 1: Doses used in chromosome aberration test

Experiment	*Concentration*	*Metabolic activation*	*Result*
1	1187, 2813 and 5000 µg/mL	-	Negative
	582, 2109 and 5000 µg/mL	+	Negative
2	430, 838 and 1311 µg/mL	-	Negative
	3200, 4000 and 5000 µg/mL	+	Negative

Results and Conclusion

Treatment with or without metabolic activation did not increase the frequency of cells with structural chromosomal aberrations. The aberrant cell frequency of all treated cultures fell within current historical negative control ranges. With the exception of single cultures at concentrations of 5000 µg/mL and 3200 µg/mL following the 3 +17 hour (+S9) in experiments one and two respectively, the frequencies of cells with numerical aberrations for all asparaginase treated cultures fell within historical negative control values. The slight increases noted above were marginal and not considered of biological importance.

Positive controls induced significant increased in the number of cells with structural aberrations, confirming the sensitivity of the test procedure.

It was concluded that asparaginase did not induce chromosome aberrations in cultured human peripheral blood lymphocytes either in the presence or absence of S9 under the conditions of this study.

6. JECFA consideration of asparaginase

Asparaginase was evaluated by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) at its meeting in 2007 (JECFA, 2007a). At this time, the Committee allocated an ADI of 'not specified' for asparaginase from this recombinant strain of *A. oryzae*, used in the applications specified and in accordance with good manufacturing practice. This means that asparaginase is considered to be of very low toxicity. New specifications were prepared at this time (JECFA, 2007b).

7. Conclusion

Following the safety assessment of asparaginase from *A. oryzae*, it was concluded that:

- the production organism has a history of safe use as a production strain for food-grade enzyme preparations and has been shown not to produce toxic metabolites;
- the recombinant DNA in the production organism is considered to be stable and poses no safety concern;

- the enzyme preparation complies with international specifications;
- there was no evidence of toxicity the 90-day toxicity study in rats;
- the NOEL from the 90-day toxicity study was greater than 880 mg/kg bw per day, the highest dose level. This is equivalent to 10 ml liquid enzyme concentrate (or approximately 46576 ASNU)/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 asparaginase as a processing aid in food would pose no public health and safety risk. This is consistent with the findings of JECFA, which established an ADI of 'not specified' at their meeting in 2007, confirming that asparaginase is a substance of very low toxicity.

References:

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Attachment 3

Food Technology Report

A606 – Asparaginase as a Processing Aid

Summary

Novozymes has developed an asparaginase enzyme preparation produced from a submerged batch-fed fermentation of a selected genetically modified strain of *Aspergillus oryzae*. This production micro-organism contains a gene coding for asparaginase, also from *A. oryzae* which increases the production of asparaginase from the source micro-organism The commercial asparaginase preparation complies with internationally recognised specifications for the production of enzymes; the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium of Food Additive Specifications and the Food Chemicals Codex.

The asparaginase enzyme preparation is proposed to be used to treat wheat dough based products such as biscuits and crackers and processed products based on potato such as French fries and potato chips. The Applicant claims that the asparaginase preparation reduces the formation of acrylamide, which can be formed from reactions involving the amino acid asparagine and reducing sugars in products which are heated above 120°C by baking or frying.

Novozymes reported results of trials undertaken using the enzyme preparation indicating reductions of acrylamide in potato and wheat dough based heat processed foods (which are high contributors to acrylamide exposure in the diet) in the range from 40% up to more than 90%. These reports have not yet been independently assessed or published in a peer-reviewed scientific article.

The use of the asparaginase enzyme sourced from *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene as a processing aid is technologically justified to treat some potato based and wheat dough based products which are baked or fried. The enzyme preparation is claimed to reduce the formation of acrylamide in the final products.

Introduction

Novozymes Australia Pty Limited submitted an Application to FSANZ seeking to amend the Code to permit the use of the enzyme asparaginase sourced from *A. oryzae* expressing a gene encoding for asparaginase from *A. oryzae*. An amendment to the Table to clause 17 of Standard 1.3.3 – Processing Aids will be required to permit this enzyme from this microbial source organism for use in food manufacture.

The Applicant is seeking permission to use this asparaginase enzyme for food manufacture as asparaginase can reduce the formation of acrylamide formed during processing of potato based products such as potato chips and French fries, and wheat dough based products such as biscuits, crackers, crisp breads, tortilla chips, pretzels and bread.

Characterisation of asparaginase

The International Union of Biochemistry and Molecular Biology (IUBMB) indicates that the enzyme asparaginase hydrolyses the amide in the amino acid L-asparagine to the corresponding acid L-aspartate (aspartic acid) and ammonia.

$$\text{L-asparagine} + H_2O = \text{L-aspartate} + NH_3$$

Common name: asparaginase
IUBMB systematic name: L-asparagine amidohydrolase
Enzyme Commission (EC) number: 3.5.1.1
Chemical Abstracts Service (CAS) number: 9015-68-3.
The commercial name of the Novozymes asparaginase enzyme preparation is Acrylaway® L.

The Applicant claims that there are no significant levels of side activities since the native amylase and protease genes have been deleted in the production strain of the micro-organism. The Applicant also states that apart from asparagine, asparaginase only acts on glutamine and has no activity on other amino acids. Asparaginase has no activity on asparagine residues in peptides or proteins.

The commercial preparation of the asparaginase enzyme is stated by the Applicant to typically have activity of 3500 ASNU (Asparaginase Units)/g. One ASNU has been defined as the amount of the enzyme that produces 1 micromole of ammonia per minute under specific defined conditions. This definition and the method to measure the enzyme activity is an in-house Novozymes method.

Production of the enzyme

The asparaginase enzyme preparation is produced by submerged fed-batch fermentation using a selected genetically modified production strain of *A. oryzae* containing a gene coding for asparaginase sourced from *A. oryzae*. The enzyme preparation is manufactured in accordance with Good Manufacturing Practices. Once the fermentation has been completed the desired enzyme is separated from the microbial biomass using centrifugation and filtration. The separated enzyme preparation is then concentrated (using ultra filtration and/or evaporation), standardised, preserved and stabilised. The final enzyme preparation is preserved using glycerol, sodium benzoate and potassium sorbate.

Glycerol or glycerine (INS 422) is listed in Schedule 2 of Standard 1.3.1 as a food additive approved in many processed foods to levels determined by Good Manufacturing Practice. Schedule 2 additives are also generally permitted processing aids. Sodium benzoate (INS 211) and potassium sorbate (INS 202) are permitted as preservatives in a number of foods specified in Schedule 1 of Standard 1.3.1, in particular for preparations of food additives to maximum levels of 1000 mg/kg (0.1%). There are no specific requirements for food additives in enzyme preparations in the Code.

The typical composition of the commercial asparaginase enzyme preparation as indicated in the Application is:

Enzyme solids (Total Organic Solids)	approximately 4%
Water	approximately 46%

Glycerol	approximately 50%
Sodium benzoate	approximately 0.3%
Potassium sorbate	approximately 0.1%

The enzyme preparation is a light brown liquid. The enzyme is stable between pH 5.0 to 9.0. The enzyme activity range occurs between pH 5.0 to 9.0, with its optimum activity at pH 7.0. The optimum temperature of use is 60ºC. The molecular weight of the enzyme was determined to be 36 kDa (US FDA GRAS No. 000201, 2006).

Specification

The Application states that the enzyme preparation complies with the international specifications relevant for enzymes, which are compiled by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications (2001) and the Food Chemical Codex (2004). These specification references are both primary sources of specifications listed in clause 2 of Standard 1.3.4 – Identity and Purity of the Code.

The specification of a batch of unstandardardised enzyme taken from the Application is provided below compared to the JECFA specification.

Criteria	**JECFA specification**	**Results for aspaginase**
Heavy metals as Pb	Not more than 40 ppm	3.9 ppm
Lead	Not more than 5 ppm	<1 ppm
Arsenic	Not more than 3 ppm	<0.1 ppm
Cadmium		<0.05 ppm
Mercury		<0.03
Total viable counts (cfu/g)	Not more than 50,000	<200
Total coliforms (cfu/g)	Not more than 30	<10
Enteropathogenic *E. coli* (/25 g)	Negative by test	ND
Salmonella (/25 g)	Negative by test	ND
Antibiotic activity	Negative by test	ND
Production strain (/g)		ND

ND – Not detected

The manufacturing process ensures that there are no production micro-organisms (the genetically modified *A. oryzae*) present in the final enzyme preparation.

Technological function of the enzyme

The asparaginase enzyme hydrolyses the amino acid L-asparagine to L-aspartic acid and ammonia. The asparaginase enzyme preparation is intended to be used during food manufacture to reduce the formation of acrylamide which is formed as a reaction product between asparagine and reducing sugars when food products are heated above 120°C. The amino acid asparagine and reducing sugars are found in many food raw materials (such as potatoes and wheat dough based products). Heating the processed food to temperatures above 120°C also inactivates the asparaginase enzyme so that the final food does not contain any of the active enzyme, just inactivated protein.

Wheat dough-based products

For wheat dough-based products Novozymes recommends that the enzyme preparation is added prior to the dough being baked. The recommended dosages vary from 200-2500 ASNU (corresponding to 60-700 g of the enzyme preparation) per kg of the processed food (in this case dough).

Potato based products

The Applicant recommends for potato based products that the cut potato strips be soaked or dipped into water baths containing the enzyme preparation, before the potato segments are further heat processed. It is recommended that an enzyme treatment bath is made up to 12,000 ASNU/litre of water (approx. 3.4 g of enzyme preparation/litre water). The Applicant has assumed a 5% water pick up of the treated potato product, giving 600 ASNU/kg treated potato. Mass balance calculations conducted by the Applicant indicate that the enzyme treatment for the final produced products are between 1400 and 1800 ASNU/kg final product.

Efficacy studies on acrylamide reduction

Novozymes provided an asparaginase Technology Sheet in the Application which reported some results of trials that they have conducted. The Applicant compared the reduction in the formation of acrylamide when their asparaginase enzyme preparation has been used compared to control foods not treated by the enzyme. The results are provided as summary tables and graphical representations of results with different treatments undergone by the food. Little detail of the trial protocols was provided.

FSANZ cannot assess or validate whether the results as presented are reasonable and reproducible, and the results have not been reported in peer-reviewed journals. JECFA indicated in 2005 in their assessment of acrylamide, that potato products such as French fries and potato chips and cereal based products such as biscuits and bread are some of the main contributors to acrylamide exposure from food (JECFA, 2005). FSANZ was also involved in an earlier Australian survey of acrylamide in carbohydrate-based foods which supported the same conclusion (Croft et al, 2004). Some further information on the dietary levels of acrylamide in food is provided in **Attachment 4.**

Table 1 is a summary table of the results of trials performed by Novozymes on the efficacy of using their asparaginase enzyme preparation to reduce the levels of acrylamide in the final food compared to a control (or in the case of French fries also to a blank which is the treatment with water only).

Table 1: Summary of reductions in acrylamide formation in food treated with Novozymes asparaginase enzyme preparation, taken from the Novozymes Application

Food Product	**Acrylamide reduction (%)**
Semi-sweet biscuits	80-85
Fabricated potato chips	80-98
Crisp bread	84-92
Ginger nut biscuits	64-79
Toast bread	~40
French fries	80 vs. a control 50-60 vs. a water treatment only blank

Conclusion

The use of the asparaginase enzyme sourced from *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene as a processing aid is technologically justified to treat some potato based and wheat dough based products which are baked or fried, to reduce the formation of acrylamide in the final products.

References

Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin.* **21**(8):721-736.

Food Chemical Codex (2004), National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex, 5th Edition, National Academy press, Washington DC, pp 146-152.

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

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2005) *Report on 64th meeting* (Rome, 8-17 February 2005), Acrylamide, pages 7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

US Food and Drug Administration (FDA) GRAS Notice No. GRN 000201, 24 November 2006, http://www.cfsan.fda.gov/~rdb/opa-g201.html

Attachment 4

Dietary Exposure Assessment Report

A606-Asparaginase as a processing aid (enzyme)

Summary

FSANZ has reviewed the dietary exposure assessment for the enzyme asparaginase which was provided by the applicant. The applicant's estimate of maximum dietary exposure for asparaginase was 0.35 mg total organic substances (TOS)/kg/day, assuming asparaginase contains 4% TOS. FSANZ considers this to be a conservative assessment (i.e. an overestimate) as it is based on the Budget Method, an internationally accepted methodology to screen food additives for safety concerns using very conservative assumptions. Based on claimed levels of acrylamide reductions provided by the applicant for foods that are major contributors to acrylamide dietary exposure, FSANZ concludes that it is likely the intended use of asparaginase as a processing aid will reduce total acrylamide dietary exposure.

Given the applicant is seeking the approval of the enzyme asparaginase, FSANZ considers a dietary exposure assessment for acrylamide is not necessary. An Australian survey of acrylamide in carbohydrate-based foods found major contributors to total dietary exposure to include hot potato chips, potato crisps, white toast, sweet biscuits, breads and breakfast cereals (wheat biscuit style)[15]. The 2005 JECFA evaluation of acrylamide found similar contributors to total dietary exposure assessment the Australian survey and indicated that coffee was an additional major contributor to total acrylamide dietary exposure [16].

Background

An application was received by FSANZ to amend the Australia New Zealand Food Standards Code to permit the use of the enzyme, asparaginase, as a processing aid. Asparaginase is produced from a strain of the host micro-organism *Aspergillus oryzae* expressing the *A. oryzae* asparaginase gene. It is claimed that the enzyme hydrolyses the amino acid asparagine to aspartic acid by hydrolysing the amide in asparagine to the corresponding acid, aspartic acid. The enzyme is intended for use as a processing aid during food manufacture to reduce acrylamide formation in baked or fried wheat dough based products such as biscuits and crackers and cut vegetable products such as sliced potato chips and French fries.

FSANZ has reviewed the dietary exposure assessment for the enzyme asparaginase which was provided by the applicant. Given the applicant is seeking the approval of the enzyme, asparaginase, FSANZ considers a dietary exposure assessment for acrylamide is not necessary. However, previous dietary exposure assessments for acrylamide are summarised below.

[15] Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin*. **21**(8):721-736

[16] Joint FAO/WHO Expert Committee on Food Additives, Report on Sixty-fourth meeting, Rome, 8-17 February 2005, Acrylamide, pages 7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

Dietary exposure assessment for asparaginase

The applicant has based the estimation of dietary consumption of asparaginase on the Budget Method[17] [18] as the enzyme may be used for a variety of food applications. This method is an internationally accepted 'worst case' or 'screening' method for food additive dietary exposure. The Budget Method estimates the levels of use of food additives that would result in dietary exposures within safety limits and relies on assumptions regarding physiological requirements for energy and liquid and assumptions on energy density of foods, rather than food consumption data[19]. The results of the Budget Method are not intended to represent a realistic estimate of dietary exposure.

In order to demonstrate a worst case calculation, the applicant has applied the following assumptions:

- all processed foods are produced using asparaginase as a processing aid at the highest recommended dose;
- a conservative estimate of ideal food consumption is 25 g per kg body weight per day of which processed food is 50% of the food consumption or 12.5 g per kg body weight per day (from the Budget Method); and
- asparaginase contains 4% TOS. The calculation assumes that all total organic substances (TOS) from the fermentation of the enzyme (mainly protein and carbohydrate components), remain in the final product.

The applicant recommends a maximum dosage of 70 g of asparaginase per 100 kg processed food. If asparaginase is assumed to contain 4% TOS, the maximum concentration would be 28 mg TOS/kg processed food. Based on the estimate for processed food consumption of 12.5 g per kg body weight per day, the maximum dietary exposure to asparaginase corresponds to 28 x 0.0125 = 0.35 mg TOS per kg body weight per day. This is likely to be a gross overestimate.

Dietary exposure assessments for acrylamide

In 2002 FSANZ undertook preliminary dietary exposure estimates for acrylamide for the Australian population. The preliminary assessments, based on the Swedish National Food Administration and UK Food Standards Agency analytical data and the 1995 National Nutrition Survey (NNS) data[20], resulted in estimated mean dietary exposures for consumers of acrylamide of 23 µg/day (0.4 µg/kg bw/day) for all Australians aged 2 years and above, and 20 µg/day (1.1 µg/kg bw/day) for children aged 2-6 years[21]. These estimates for the Australian population were in a similar range to other international estimates[22].

[17] Hansen, S.C (1966) Acceptable daily intake of food additives and ceiling on levels of use, *Food Cosmet Toxicol*, 4:427-432.
[18] Hansen, S.C (1979) Conditions for use of food additives based on a budget for an acceptable daily intake, *Food Protect,* 42:429-432.
[19] International Life Sciences Institute (1997) An evaluation of the Budget Method for screening food additive intake.
[20] McLenann, W. and Podger A. (1997), National Nutrition Survey 1995, Canberra Australian Bureau of Statistics.
[21] FSANZ (2002) Australian dietary exposure assessment for acrylamide, World Health Organization Consultation (unpublished).
[22] Joint Food and Agriculture Organization of the United Nations and the World Health Organization (2002) Consultation on the health implications of acrylamide in food.

Further to this preliminary estimate, an Australian survey of acrylamide in carbohydrate-based foods was undertaken. This study analysed composite samples from more than 100 carbohydrate-based foods in the Australian diet, including breads, cakes, breakfast cereals and snack foods. The dietary exposure was estimated by combining usual patterns of food consumption, as derived from 1995 NNS data, with the levels of acrylamide analysed in similar food samples. Nearly all NNS respondents (91%) consumed at least one of the foods containing acrylamide. The estimated mean dietary exposures for Australian consumers of acrylamide aged 2 years and above were between 22 and 29 µg/day (0.4 and 0.5 µg/kg bw/day) and between 73 and 80 µg/day (1.4 and 1.5 µg/kg bw/day) for 95th percentile consumers. Major contributors to total acrylamide dietary exposure included hot potato chips (main contributor), potato crisps, white toast, sweet biscuits, breads and breakfast cereals (wheat biscuit style). These foods are reflective of their popularity in the Australian diet[23].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook an evaluation of acrylamide at its sixty-fourth meeting in 2005, at the request of the Codex Committee on Food Additives and Contaminants. All regions were represented except Latin America and Africa where no dietary exposure estimates were available. National dietary exposure estimates were generally calculated using deterministic modelling by combining national individual consumption data with mean occurrence data obtained from national surveys and using the consumer body weight reported in consumption surveys. Mean dietary exposure estimates of acrylamide ranged from 0.3 to 2.0 µg/kg bw/day for the general population. For high percentile consumers (90th to 97.5th) dietary exposure estimates ranged from 0.6 to 3.5 µg/kg bw/day and up to 5.1 µg/kg bw/day for the 99th percentile consumer. Estimates of acrylamide dietary intake for children were around two to three times that of adults, expressed on a body weight basis, as expected as children have a higher food consumption per kilogram body weight due to growth needs. The major contributing foods to the total dietary exposures for most countries were hot potato chips, potato crisps, coffee, pastry and sweet biscuits and bread and rolls/toasts (JECFA 2005)[24].

Acrylamide reductions with asparaginase

The applicant has tested various food products in the laboratory for acrylamide reduction using asparaginase and provided the information shown in Table 1.

Table 1: Acrylamide reductions achieved with asparaginase in a variety of food products

Food product	Acrylamide reduction (%)
Semi-sweet biscuits	80-85%
Fabricated potato chips	80-98%
Crisp bread	84-92%
Ginger nut biscuits	64-79%
Toast bread	~40%
French fries	80% vs. a control 50-60% vs. a blank

[23] Croft, M.; Tong, P.; Fuentes, D. and Hambridge, T. (2004) Australian survey of acrylamide in carbohydrate-based foods. *Food Add. Contamin*. **21**(8):721-736

[24] Joint FAO/WHO Expert Committee on Food Additives, Report on Sixty-fourth meeting, Rome, 8-17 February 2005, Acrylamide, pages 7-17, http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

Based on the claimed acrylamide reductions for the food products provided in Table 1 and considering asparaginase will be used in processing of food items that are major contributors to the total acrylamide dietary exposure (as assessed by Croft et al and JECFA), FSANZ considers it is likely that the intended use of asparaginase as a processing aid will reduce total acrylamide dietary exposure.

Attachment 5

Summary of Submissions

Round one – Submissions on the Initial Assessment Report

Submitters

Submitter Organisation	Name
New Zealand Food Safety Authority	Carole Inkster
Food Technology Association of Australia	David Gill
Queensland Health	Gary Bielby
Australian Food and Grocery Council	Kim Leighton
New South Wales Food Authority	Jo Dellow

Submitter	Position supports	Comments
New Zealand Food Safety Authority	No position at this stage	No comments at this stage, may comment at Draft Assessment
Food Technology Association of Australia	Supports option 2, permit the enzyme	It supports permitting the enzyme and had no other comments.
Queensland Health	No position at this stage	No position at this stage but will review the position once the safety assessment and other data of the application has been assessed at Draft Assessment stage. Acknowledge that there is a potential health benefit in reducing acrylamide formation in foods. Asks that the Draft Assessment Report thoroughly evaluate two potential concerns. The first relates to confirming that residual asparaginase remaining with the food has been destroyed during the cooking process. The second possible safety risk they identify relates to residues of the genetically modified organism remaining in the enzyme preparation and then subsequently being incorporated into the treated food. In the cost/benefit analysis it wishes to acknowledge that Queensland Health Scientific Services currently does not have methodology available for testing for acrylamide, asparaginase activity or genetically modified *Aspergillus oryzae*.

Submitter	Position supports	Comments
Australian Food and Grocery Council	Supports option 2, permit the enzyme	It makes note of recent conclusions from JECFA (2005) and the US FDA (2006) relating to the risk assessment and dietary modelling of acrylamide in the diet. It also notes that the Confederation of the Food and Drink Industries of the EU (the CIAA) has been active and has recently developed an acrylamide toolbox to assist industry to minimise the formation of acrylamide in processed food. It considers the enzyme to be technologically justified to reduce the potential formation of acrylamide in wide variety of processed foods. It points out the safe commercial use of *A. oryzae*, and the fact that it is an approved microbial source for a wide number of approved enzymes in the Code.
New South Wales Food Authority	Supports further progression of the Application	Appears to be sufficient technological justification due to its potential to reduce acrylamide formation in some processed foods to support approving the enzyme.