EXPLANATORY STATEMENT

APPLICATION A606

ASPARAGINASE AS A PROCESSING AID

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 its 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 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.

Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

Purpose

The Applicant 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.

Decision

FSANZ recommends the 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 *A. oryzae* expressing the *A. oryzae* asparaginase gene.

Reasons for Decision

This Application has been assessed against the requirements for Final Assessment in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). FSANZ recommends the 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 Draft 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 draft variation is consistent with the FSANZ objectives specified in section 18 of the FSANZ Act.

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. The Draft Assessment Report was available for public comment from 12 December 2007 till 6 February 2008. Six submissions were received with the issues addressed in this report. Five submitters supported the Application (though one had a number of issues which have been addressed in this report) while one submitter opposed the Application based on a general opposition to GM foods.

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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 of the food 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[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 December 2007 (revision 11) 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.

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[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 7 April 2008.

[2] The CIAA Acrylamide "Toolbox", Rev 11, December 2007, http://www.ciaa.eu/documents/brochures/toolbox%20rev11%20nov%202007final.pdf, accessed 7 April 2008.

[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 7 April 2008.

[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 at pH 7, with its activity rapidly decreasing at temperatures above 60ºC. The thermal inactivation of the enzyme is dependent on the pH, time, temperature and food matrix however it can be expected that the enzyme will be inactivated between 70-80ºC for most uses. Certainly when enzyme treated foods are baked or fried above 120ºC (high temperatures are required to form high levels of acrylamide) there should be no enzyme activity. 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 Applicant states that the asparaginase enzyme preparation has a typical enzyme activity of 3,500 ASNU (asparaginase units)/g. One ASNU has been defined by the Applicant to be the amount of the enzyme that produces 1 micromole of ammonia per minute under specific conditions.

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 was submitted in France 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-eighth meeting, 19-28 June 2007 in Geneva.

[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 7 April 2008.

The full report of this evaluation has recently become available which concluded 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 has recently received another Application seeking approval for another form of the asparaginase enzyme sourced from a different genetically modified micro-organism (*A. niger* expressing the asparaginase gene from *A. niger*) from DSM Food Specialties. This new Application number is A1003, with the Application received on 7 January 2008. 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 separate applications are required. 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:

[6] Joint FAO/WHO Expert Committee on Food Additives, Sixty-eighth meeting, Safety evaluation of certain food additives and contaminants, 19-28 June 2007, WHO, Geneva, 2008, Asparaginase from *Aspergillus Oryzae* expressed in *Aspergillus Oryzae,* p55-63 http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf accessed on 31 March 2008.

[7] US FDA Agency response Letter GRAS Notice No. GRN 000214, http://www.cfsan.fda.gov/~rdb/opa-g214.html, accessed on 7 April 2008.

- 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.

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 a 90-day toxicity study in rats;
- in the 90-day study in rats, the No Observable Effect level (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 their 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 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.

JECFA has assigned an ADI of 'not specified' to the enzyme so there is no requirement to assess dietary exposure to the asparaginase enzyme preparation, therefore FSANZ has not performed its own dietary exposure assessment.

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 a 90-day feeding study in rats 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. As expected because of its inherent acid lability and sensitivity to destruction through the action of proteases in the gastrointestinal tract no adverse effects of asparaginase were observed at the highest tested dose in rats. This conclusion 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). Consequently in the absence of an ADI a dietary risk assessment does need not be considered because it would not be meaningful.

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)[10] and the Food Chemicals Codex[11].

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[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 assessed on 4 April 2008.

[10] 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.

[11] 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.

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. Results reported in the Application of trials undertaken by Novozymes provided in a Technology Sheet 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%.

There have only been a small number of peer-reviewed research articles which have independently assessed and reported the reduction in the formation of acrylamide due to the use of asparaginase. A major reason for this is that the Applicant's enzyme preparation has only been commercially available for a relatively short period of time so most of the confirmation work has been performed in-house by the Applicant or in pilot plant and laboratory trials. However, there seems to be interest in commercialising the use of the enzyme as a potential mitigating factor in reducing acrylamide formation if food, so a number of articles have expressed the fact that use of the enzyme should be pursued and trialled once it is approved by regulators[12,13,14].

There has been two recent studies on the efficacy of the Applicant's asparaginase in reducing acrylamide formation in food. A recent report in the literature has performed trials using the Applicant's asparaginase enzyme (termed by them the 'experimental asparaginase from Novozymes') on the reduction in the formation of acrylamide in hazelnut biscuits on pilot plant trials. Their results indicated reductions between 68-83% depending on incubation time, and up to 90% with high levels of enzyme preparation added[15]. Another study investigated the use of asparaginase on French fries[16]. Levels of up to 60% less acrylamide were reported in treated French fries compared to control.

A number of other references have also reported reductions in acrylamide formation in treated foods, though a number of these studies have been performed on laboratory enzymes, to confirm the mechanism of acrylamide formation and also the postulation that using asparaginase would in fact reduce the formation of acrylamide in treated products. Reductions have been reported in acrylamide formation for gingerbread[17] and wheat crackers[18] treated with asparaginase.

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.

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[12] Amrein, T.A., Andres, L., Escher, F. and Amadò, R. (2007) Occurrence of acrylamide in selected foods and mitigation options. *Food Add. Contamin.*, **24**(1):13-25

[13] Foot, R.J., Haase, N.U., Grob, K. and Gondé, P. (2007) Acrylamide in fried and roasted potato products: A review on progress in mitigation. *Food Add. Contamin*, **24**(1):37-46

[14] Konings, E.J.M., Ashby, P., Hamlet, C.G. and Thompson, G.A.K. (2007) Acrylamide in cereal and cereal products: A review on progress in level reductions. *Food Add. Contamin*, **24**(1):47-59

[15] Amrein, T.A., Andres, L., Escher, F. and Amadò, R. (2007) Occurrence of acrylamide in selected foods and mitigation options. *Food Add. Contamin*, **24**(1):13-25

[16] Pedreschi, F. Kaack, K. and Granby, K. (2008) The effect of asparaginase on acrylamide formation in French fries. *Food Chemistry* **109**:386-392.

[17] Amrein, T.M., Schönbächler, B., Escher, F. and Amadò, R. (2004)Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **52**:4282-4288

[18] Vass, M., Amrein, T.M., Schönbächler, B., Escher, F. and Amadò, R (2004) Ways to reduce the acrylamide formation in cracker products. *Czech. J. Food Sci.* **22**:19-21

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. A preliminary assessment of costs and benefits was undertaken and submitted to the Office of Best Practice Regulation (OBPR). As it is deemed to be a minor application in terms of impacts no further analysis is required.

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 provides:

> *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 was considered that this Application was a routine matter. Therefore, FSANZ has applied a basic communication strategy. This involved 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 have been notified at each stage of the assessment of the Application. Once the FSANZ Board has approved the draft variation to the Code, the decision will be notified to 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. Public comment on the Draft Assessment Report for this Application was sought from 12 December 2007 until 6 February 2008. Six submissions were received, of which five submissions supported the Application (though one submission raised a number of issues which they wished to be considered and addressed at Final Assessment), while one submission opposed the Application due to its GM aspects. **Attachment 4** summarises the submissions received during the first and second rounds of public comment.

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

9.1 Issues raised in submissions

9.1.1 Inactivation of the enzyme in all treated foods

One submission asked about potential residues of the enzyme in the final processed food and whether the enzyme will always be inactivated in the treated food, and if not then maybe a limit on which foods can be treated with the enzyme needs to be applied. If not then the enzyme should be treated as a food additive and not as a processing aid.

9.1.1.1 FSANZ evaluation

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.

The inactivation temperature for the asparaginase enzyme is lower than 120°C, being essentially between 70-80°C, depending on time, pH and the food matrix. The processing of all foods that could be expected to be treated with the asparaginase enzyme will be treated at temperatures higher than the inactivation temperature so there will be no active enzyme left in the final food. In this case the asparaginase enzyme is considered to be a processing aid, where is has performed its technological function to reduce the formation of acrylamide during the processing of the food, it is inactivated and has no further technological function in the final food.

Furthermore, the formation of acrylamide in food is increased with high temperature as a reaction product between the amino acid asparagine and reducing sugars. Therefore, the mitigation process of using asparaginase to treat food to limit the formation of acrylamide will be for products which undergo high temperature cooking and which will also inactivate the enzyme after it has performed it technological function.

9.1.2 Issues relating to enforcement

One jurisdiction submission indicated that their analytical services laboratory does not currently have suitable analytical methodology 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.

It would be up to individual jurisdictions whether there is the need for analyses for the enzyme, the source micro-organism and/or acrylamide. If such analyses were required then added analytical costs would likely be incurred by the jurisdictions, either to develop capability to perform analyses (methods would be available from the Applicant) or to get them performed by external agencies.

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[19].

[19] 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

9.1.3 Confirmation of Applicants claims on efficacy to reduce acrylamide formation

A submitter expressed concern that FSANZ was not able to assess or validate the claims made in the Application relating to efficacy of the asparaginase enzyme to reduce the formation of acrylamide in treated foods. This fact concerned the submitter since the decision to approve the use of the enzyme should be based on evidence supporting the technological claims made by the Applicant.

9.1.3.1 FSANZ response

There have only been a small number of peer-reviewed research articles which have independently assessed and reported the reduction in the formation of acrylamide due to the use of asparaginase. A major reason for this is that the Applicant's enzyme preparation has only been commercially available for a relatively short period of time so most of the confirmation work has been performed in-house by the Applicant or in pilot plant and laboratory trials by interested companies which have not been published. However, there is interest in commercialising the use of the enzyme as a potential mitigating factor in reducing acrylamide formation if food once it is approved by regulators. Various interested companies will need to perform more detailed trials and evaluations to ensure maximum acrylamide reductions for their products, in commercial plant production, as well as ensuring no deleterious quality changes to their product or unacceptable production parameters are required.

There has been two new studies available since the Draft Assessment, reported in peer-reviewed scientific journals which have independently assessed and verified the efficacy of the Applicant's asparaginase in reducing the formation of acrylamide. One of these is in French fries[20]. Levels of up to 60% less acrylamide were reported in treated French fries compared to controls. The second recent reference reported the reduction in the formation of acrylamide in a hazelnut biscuits[21]. Use of the asparaginase enzyme reduced acrylamide formation by greater than 60% using standard incubation times of 15 mins and 450 ASNU of asparaginase per kg of the dough. Greater reductions were found when higher levels of the enzyme, and longer incubation times were used (between 68-83%, and up to 90% for very high levels of the enzyme.

Further information on the reduction in acrylamide in treated food with asparaginase are reported in section 5.4 and in the Food Technology Report (**Attachment 3**).

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.

[20] Pedreschi, F. Kaack, K. and Granby, K. (2008) The effect of asparaginase on acrylamide formation in French fries. *Food Chemistry* **109**:386-392.
[21] Amrein, T.A., Andres, L., Escher, F. and Amadò, R. (2007) Occurrence of acrylamide in selected foods and mitigation options. *Food Add. Contamin.* **24**(1):13-25

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 or Sanitary and Phytosanitary Measures Agreements.

CONCLUSION

10. Conclusion and Preferred Approach

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

Decision

FSANZ recommends the 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 *A. oryzae* expressing the *A. oryzae* asparaginase gene.

10.1 Reasons for Decision

FSANZ recommends the 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 draft variation is consistent with the FSANZ objectives specified in section 18 of the FSANZ Act.

11. Implementation and Review

Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Food Technology Report
4. 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 Code[22].

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.

[22] 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 *E. 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:

Blumenthal, C.Z. (2004) Production of toxic metabolites in Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regul.Toxicol Pharmacol* 39(2):214-228.

Burdock, G.A., Soni, M.G. and Carabin, I.G. (2001a) Evaluation of health aspects of kojic acid in food. *Regul.Toxicol Pharmacol* 33(1):80-101.

Burdock, G., Carabin, I. and Soni, M. (2001b) Safety assessment of B-nitropropionic acid: a monograph in support of an acceptable daily intake in humans. *Food Chemistry* 75:1-27.

JECFA (2001) *General specifications and considerations for enzyme preparations used in food processing. GFAO Food and Nutrition Paper 52,Add.9,pp.37-39.*

JECFA (2007a) *Asparaginase from Aspergillus oryzae expressed in Aspergillus oryzae*. http://jecfa.ilsi.org/evaluation.cfm?chemical=ASPARAGINASE%20FROM%20ASPERGILLUS%20ORYZAE%20EXPRESSED%20IN%20ASPERGILLUS%20ORYZAE&keyword=ASPARAGINASE. Accessed on 9 October 2997a.

JECFA (2007b) *Technical Specification for Asparaginase from Aspergillus oryzae expressed in A. oryzae*. http://www.fao.org/ag/agn/jecfa-additives/details.html?id=920. Accessed on 9 October 2007b.

NAS FNB (2004) *Food Chemical Codex, 5th edition*. In: National Academy of Sciences, F.a.N.B.C.o.F.C.C. eds. National Academy Press, Washington DC.

Pariza, M.W. and Johnson, E.A. (2001) Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Regul.Toxicol Pharmacol* 33(2):173-186.

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 multiple copies of the gene coding for asparaginase, also from *Aspergillus 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 reported trials in their Application 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%. Two recent independent peer-reviewed articles confirmed that the Applicant's asparaginase enzyme reduced the formation of acrylamide in hazelnut biscuits by 68-83%, while a separate study found its use reduced acrylamide levels in French fries by up to 60%.

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 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 *Australia New Zealand Food Standards Code* (the Code) to permit the use of the enzyme asparaginase sourced from *Aspergillus 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 *Aspergillus 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 produced using glycerol as the carrier and preserved using sodium benzoate and potassium sorbate.

Glycerol or glycerine (INS 422) is listed in Schedule 2 of Standard 1.3.1 – Food Additives of the Code 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. 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, with its activity decreasing rapidly at temperatures above 60ºC. The inactivation temperature for the asparaginase enzyme preparation is essentially between 70-80ºC, depending on the time, pH and the food matrix. 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 unstandardised enzyme taken from the Application is provided below compared to the JECFA specification.

Criteria	**JECFA specification**	**Results for asparaginase**
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).

The inactivation temperature for the asparaginase enzyme preparation is essentially between 70-80ºC, depending on the time, pH and the food matrix so baking and frying the processed foods inactivates the asparaginase enzyme so that the final food does not contain any of the active enzyme, just inactivated protein.

The Applicant states that the asparaginase enzyme preparation has a typical enzyme activity of 3,500 ASNU (asparaginase units)/g. One ASNU has been defined by the Applicant to be the amount of the enzyme that produces 1 micromole of ammonia per minute under specific conditions.

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 mg 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 (these calculations include moisture and mass losses so that 1 kg potatoes produces 0.42 kg French fries and 0.33 kg sliced potato chips).

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.

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).

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. water treatment only

There have only been a small number of peer-reviewed research articles which have independently assessed and reported the reduction in the formation of acrylamide due to the use of asparaginase. A major reason for this is that the Applicant's enzyme preparation has only been commercially available for a relatively short period of time so most of the confirmation work has been performed in-house by the Applicant or in pilot plant and laboratory trials. However, there seems to be interest in commercialising the use of the enzyme as a potential mitigating factor in reducing acrylamide formation if food, so a number of articles have expressed the fact that use of the enzyme should be pursued and trialled once it is approved by regulators.

There have been two recent reports in the literature that have performed trials using the Applicant's' asparaginase enzyme (termed by one the 'experimental asparaginase from Novozymes') on the reduction in the formation of acrylamide in food. One evaluated the reduction in acrylamide in hazelnut biscuits from pilot plant trials. Their results indicated reductions between 68-83% depending on incubation time, and up to 90% with high levels of enzyme preparation added (Amrien *et al*, 2007). The second report investigated the treatment of French fries and reported reductions in acrylamide of up to 60% (Pedreschi *et al*, 2007).

A number of other references have also reported reductions in acrylamide formation in treated foods, though a number of these studies have been performed on laboratory enzymes, to confirm both the mechanism of acrylamide formation and also the postulation that using asparaginase would reduce the formation of acrylamide in treated products. For example reductions have been reported in acrylamide formation for gingerbread (Amrein *et al*, 2004) and wheat crackers (Vass *et al*, 2004) treated with asparaginase.

The Confederation of the Food and Drink Industries of the EU (CIAA) Acrylamide 'Toolbox' (revision 11, December 2007) also makes reference to the reduction in acrylamide formation for a number of asparaginase treated foods. Specifically it refers to using asparaginase to reduce acrylamide formation in cereal based products including biscuits, and crispbread, potato dough based products and potentially coffee and coffee mixtures.

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 potato based and wheat dough based products which are baked or fried, to reduce the formation of acrylamide in the final products.

References

Amrein, T.M., Schönbächler, B., Escher, F. and Amadò, R. (2004) Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **52**:4282-4288

Amrein, T.M., Andres, L., Escher, F. and Amadò, R. (2007) Occurrence of acrylamide in selected foods and mitigation options. *Food Additives & Contaminants*, **24**(1):13-25

Konings, E.J.M., Ashby, P., Hamlet, C.G. and Thompson, G.A.K. (2007) Acrylamide in cereal and cereal products: A review on progress in level reductions. *Food Additives & Contaminants*, **24**(1):47-59

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.

Foot, R.J., Haase, N.U., Grob, K. and Gondé, P. (2007) Acrylamide in fried and roasted potato products: A review on progress in mitigation. *Food Additives & Contaminants*, **24**(1):37-46

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

Pedreschi, F. Kaack, K. and Granby, K. (2008) The effect of asparaginase on acrylamide formation in French fries. *Food Chemistry* **109**:386-392.

The CIAA Acrylamide "Toolbox", Rev 11, December 2007, http://www.ciaa.eu/documents/brochures/toolbox%20rev11%20nov%202007final.pdf, accessed 24 January 2008.

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

Vass, M., Amrein, T.M., Schönbächler, B., Escher, F. and Amadò, R (2004) Ways to reduce the acrylamide formation in cracker products. *Czech. J. Food Sci.* **22**:19-21

Attachment 4

Summary of Submissions on the Draft Assessment Report

Submitter Organisation	Name
New Zealand Food Safety Authority	Carole Inkster
Food Technology Association of Australia	David Gill
Queensland Health	Tenille Fort
Australian Food and Grocery Council	Kim Leighton
New South Wales Food Authority	David Cusack
Private	Ivan Jeray

Submitter	Position supports	Comments
New Zealand Food Safety Authority	Supports progression	It is satisfied that the use of the enzyme is technologically justified and that no public health or safety concerns were identified.
Food Technology Association of Australia	Supports progression	No further comments
Queensland Health	General support for permitting the enzyme, however have a number of issues	• Is the enzyme inactivated when used in all food products, or potential food products? If not then the enzyme may need to be treated as a food additive and not a processing aid. • Would like the results of the efficacy studies relating to reductions in acrylamide reported in the Application and mentioned in the Food technology Report to be verified. It is uncomfortable if FSANZ is unable to verify the Applicant's claims. • Enforcement agencies may need to analyse enzyme treated food for the presence of the enzyme, the source organism and the concentration of acrylamide formed. This will be an added costs on the enforcement agencies.
Australian Food and Grocery Council	Supports progression	• Supports the conclusion that use of the enzyme does not raise in public health and safety concerns, noting that JECFA also assessed the enzyme in 2007 and established an ADI of 'not specified". • Pointed out a recent reference (Pedreschi et al, Food Chemistry 2007) reported on the effectiveness of the Applicant's enzyme to reduce the formation of acrylamide in fried potato strips. The use of the asparaginase to reduce the formation of acrylamide in certain foods is technologically justified on the potential health and safety benefits for consumers. • Notes that food manufacturers may wish to make claims about reduced levels of acrylamide for treated food once the enzyme is permitted. For such claims to be substantiated enforcement agencies may need to undertake analytical assessments. AFGC further states that there are NATA accredited laboratories capable of performing such analyses for acrylamide, such as the National Measurement Institute. • Supports FSANZ's assessment that enforcement

Submitter	Position supports	Comments
		agencies will not need to undertake analysis work to check for the presence of the enzyme in the final food. • Supports the current GM labelling requirements of the Code. The GM aspect of this Application relates to inserting DNA into the host organism and not the actual enzyme. The refinement process removes any traces of the production organism from the enzyme preparation so there is no novel DNA in the enzyme or left in the treated food.
New South Wales Food Authority	Supports progression	No further comments
Ivan Jeray	Opposed to the Application	• Opposed to approving the enzyme due to the GM aspects of the Application. • Opposes it on unproven safety, economical, environmental and ethical grounds. He raises a number of opposition points to GM food and the GM labelling requirements in general. • Also is concerned that it was not obvious in the FSANZ Notification Circular that the enzyme had GM aspects to it, which he notes with suspicion.