8 November 2000
09/01

FULL ASSESSMENT AND REGULATORY IMPACT ASSESSMENT

APPLICATION A373

PECTINESTERASE AS A PROCESSING AID

EXECUTIVE SUMMARY

- The Australia New Zealand Food Authority (ANZFA) received an application on 1 March 1999, from Novo Nordisk for the approval of the enzyme, pectinesterase (IUB 3.1.1.11), for use as a processing aid for fruit and vegetable processing. The variation would constitute an addition of an enzyme obtained from a genetically modified strain of *Aspergillus oryzae*, carrying the gene coding for pectinesterase isolated from *Aspergillus aculeatus*. The commercial name for the enzyme product is Rheozyme.

- Eleven submissions were received in response to the Section 14 gazette notice. Four submitters supported the application. The Office of Regulation Review submitted comments pertaining to Regulatory Impact Assessment. One submitter did not express any preference. Four submitters did not support the use of an enzyme derived from a genetically modified source organism, and on this basis did not support the application.

- The main issues raised by submissions were the labelling of processing aids obtained from genetically modified organisms (GMOs); and, the importance of safety assessment for the new organism and the enzyme product.

- The scientific evaluations concluded that the use of pectinesterase, produced by *Aspergillus oryzae,* from a pectinesterase gene isolated from *Aspergillus aculeatus,* is technologically justified and poses no additional risk to public health and safety. No significant concerns were raised in the public comment regarding the actual use or approval of the processing aid. Concerns were raised in regard to approval of foods produced from GMOs. ANZFA's section 10 objectives are not compromised by the proposed change to Standard A16. It is recommended that the draft variation should come into effect on the date of gazettal.

- The Regulatory Impact Statement concluded that the amendment to Standard A16 of the *Food Standards Code* to permit pectinesterase from the new source organism *Aspergillus oryzae* carrying the donor gene from *Aspergillus aculeatus,* is necessary, cost effective and of benefit to both producers and consumers.

- A consequential amendment to Standard 1.3.2 - Processing Aids, in the joint Australia New Zealand Food Standards Code will be required to include the enzyme in the joint Code.

BACKGROUND

ANZFA received an application on 1 March 1999, from Novo Nordisk for the approval of the enzyme, pectinesterase (IUB 3.1.1.11), for use as a processing aid for fruit and vegetable processing. The variation would constitute an addition of an enzyme obtained from a genetically modified strain of *Aspergillus oryzae*, carrying the gene coding for pectinesterase isolated from *Aspergillus aculeatus*. The commercial name for the enzyme product is Rheozyme.

A related processing aid, pectinase multicomponent enzyme (IUB 3.2.1.15), when sourced from the organisms *Aspergillus niger, A. oryzae* or *Trichoderma reesei*, is currently permitted for use as a processing aid in Standard A16 in the Australian *Food Standards Code*. The applicant seeks to vary the list of approved enzymes in Standard A16 - Processing Aids, by adding the enzyme pectinesterase (IUB 3.1.1.11).

Standard A16 makes provision for the appropriate use of approved processing aids in food manufacture. A processing aid is a substance used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food.

No comparable standard for processing aids exists in the *New Zealand Food Regulations* 1984. Processing aids are either regulated as food additives or are not specifically regulated. Under the Review of the *Food Standards Code*, a joint processing aids standard for Australia and New Zealand was proposed (P188). The ANZFA Board recommended that the Ministerial Council adopt Standard 1.3.2 as part of the joint Australia New Zealand *Food Standards Code*.

OBJECTIVE

To promote innovation in the food industry, while protecting public health and safety.

RELEVANT PROVISIONS

Australian Food Standards Code

Standard A11 – Specifications for the identity and purity of food additives, processing aids, vitamins, minerals and other added nutrients.

Standard A16 - Processing Aids

New Zealand Food Regulations

There is no comparable standard for processing aids in the NZFR. A limited number of substances are identified in *the New Zealand Food Regulations 1984* as processing aids, and these are exempt from the general labelling provisions.

Codex Alimentarius Commission

Codex have developed an *Inventory of Processing Aids*, which is not intended to be a complete or 'positive' list of permitted processing aids.

REGULATORY OPTIONS

Option 1

The status quo would be maintained and no specific permission would be given in the *Food Standards Code* for the use of pectinesterase from the source organism *Aspergillus oryzae*.

Option 2

The *Food Standards Code* would be amended to specifically permit the use of pectinesterase from the source organism *Aspergillus oryzae*.

The proposed variation to the *Food Standards Code* constitutes a minor technical change and is not envisaged to affect trade for either technical or sanitary or phytosanitary reasons. Therefore a notification to the World Trade Organization is not required.

PUBLIC CONSULTATION

The preliminary assessment report for A373 was released for public comment between 23 June 1999 and 4 August 1999. Eleven submissions were received in response to the public notification. Four submitters supported the application to extend the list of approved source organisms in Standard A16. The Office of Regulation Review submitted comments pertaining to Regulatory Impact Assessment. One submitter, the Victorian Food Safety Council did not express any preference, but simply noted that ANZFA would be undertaking a Full Assessment of the issue. Four submitters did not support the inclusion of a genetically modified source organism, and therefore did not support the application. A table summarising the comments from public submissions is included as an attachment to this report (Attachment 3).

ASSESSMENT

TOXICOLOGICAL EVALUATION

Pectinesterase from recombinant *Aspergillus oryzae*

Aspergillus oryzae has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in 1987 that this organism is a traditionally accepted constituent of food.

A related processing aid, pectinase multicomponent enzyme (IUB 3.2.1.15), when sourced from the organisms *Aspergillus niger, A. oryzae* or *Trichoderma reesei*, is currently permitted for use as a processing aid in Standard A16 in the Australian *Food Standards Code*.

Nutritionally, there are no positive or negative effects associated with the use of pectinesterase. The active enzyme will not be present in the final food, because any residue is found in the form of inactivated enzyme that is metabolised as protein.

Pectinesterase (IUB 3.1.1.11) produced from the source organism, *A. oryzae* carrying a donor gene from *Aspergillus aculeatus*, complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC), 4th ed., 1996. It also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).

Three toxicological studies were submitted in support of this application. These consist of a bacterial mutagenicity assay, an *in vitro* chromosomal damage test and a 13-week oral toxicity study in the rat.

Pectinesterase produced from the genetically modified source organism *A. oryzae* carrying a donor gene from *A. aculeatus*, did not exhibit any toxicological effects that would be associated with its use as a processing aid for the following reasons:

- A closely related enzyme, (sourced from *A. niger*) has been used for many years in the food industry with no safety problems. It is an approved processing aid in Australia. It has the same technical applications in food processing.
- *A. oryzae*, the source organism, has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in 1987 that this organism is a traditionally accepted constituent of food.
- *A. aciduleus,* the donor organism, belongs to a well-known group of fungi and is not related to any known pathogenic species.
- Details of the construction of the recombinant organism, including the DNA sequence of the gene coding have been provided by the applicant (commercial-in confidence) along with details of the manufacturing and purification process (also commercial-in-confidence) and these do not raise any matter of concern.
- The enzyme complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC), 4th Ed., 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).
- There are no nutritional issues associated with use. The active enzyme will not be present in the final food, because any residue is found in the form of inactivated enzyme that is metabolised as protein.
- The three toxicological studies on the preparation, namely, a bacterial mutagenicity assay, *in vitro* chromosomal aberration assay in mammalian cells and a 13-week oral toxicity study in the rat, did not raise any safety concerns.

The full toxicological evaluation is available as an attachment to this full assessment (Attachment 4).

FOOD SCIENCE AND TECHNOLOGY REPORT

The food science and technology report is available as an attachment to this full assessment (Attachment 5).

ISSUES RAISED IN PUBLIC SUBMISSIONS

1.1 Labelling of Processing Aids

Seven submitters raised concerns that processing aids from GMOs are not required to be labelled under current regulation.

Evaluation

Recently, the labelling of processing aids was addressed in the Review of Ingredients Lists (Proposal P143), which was completed in February 1999. Processing aids were proposed to be generally exempt from the requirements to be declared in ingredient lists, unless they contain substances that require a mandatory declaration of their presence in food (proposed Standard 1.2.1 Mandatory Information, and 1.2.4 Labelling of Ingredients). Proposal P161 proposes the mandatory declaration of a list of foods and food additives that may cause severe adverse reactions. If the processing aid is one of these foods or a derivative of one of these foods then it will be required to be declared in the label.

The approach taken by the general review of processing aids would apply to the products within this application.

The labelling of foods produced using gene technology, including whether there is a need for processing aids derived from GMOs was recently decided. The Australia New Zealand Food Standards Council (ANZFSC) decided not to require labelling of processing aids derived from GMOs unless source DNA and/or novel protein was detectable in the final food.

1.2 Processing aids from GMOs

The main issues raised by submissions were about the specific use of genetic modification to obtain the new source organism. There are concerns regarding the safety of such technology and the resulting products. Submitters were concerned that the pectinesterase enzyme itself is genetically modified.

Background

A related processing aid, pectinase multicomponent enzyme (IUB 3.2.1.15), when sourced from the organisms *Aspergillus niger, A. oryzae* or *Trichoderma reesei*, is currently permitted for use as a processing aid in Standard A16 in the Australian *Food Standards Code*. In this application, it is obtained from a related microorganism *Aspergillus oryzae* (the source) that has been genetically modified, using recombinant DNA techniques, to carry a gene from another fungus *Aspergillus aculeatus* (the donor). *A. oryzae* is a traditionally accepted constituent of food.

Evaluation

While the processing aid is the product of the genetic modification of a microorganism, it is not itself modified. The method of using recombinant technology to modify a source

organism, allows for more efficient production of pectinesterase, and therefore a cheaper final product.

The enzyme product (pectinesterase) is collected during and after fermentation by the microorganisms. There would be no microorganisms remaining in the collected product, when added into a food manufacturing process. Any enzymes remaining in the food in which they are used as a processing aid are no longer biologically active as enzymes are used at very low concentrations and are usually inactivated, or even removed before the finished food is sold. Remaining inactivated enzymes would be metabolised as protein.

1.3 Toxicological evaluation

Three submitters urged that a toxicological evaluation on the new combination be undertaken to establish if any public health and safety threats exist from either the enzyme or the microorganism.

Evaluation

Toxicological evaluations form part of the usual ANZFA assessment procedure for any new food additive, processing aid or similar type of product. The results of the toxicological evaluation undertaken as part of this assessment indicate that there are no concerns relating to either the toxicity or pathogenicity of *Aspergillus oryzae* carrying the *Aspergillus aculeatus* gene. The results of the evaluation are in the scientific evaluation section of this paper, below.

REGULATORY IMPACT ASSESSMENT

The objective of regulatory impact assessment is to examine labelling and other issues arising from permission to use pectinesterase as a processing aid in Standard A16. A cost/benefit approach is undertaken to meet ANZFA's objectives as described in section 10 of the *Australia New Zealand Food Authority Act* 1991.

As the use of pectinesterase from the source organism *Aspergillus oryzae* requires pre–market approval it is not appropriate to consider non–regulatory options for the Regulation Impact Statement. Currently processing aids used in Australia are listed in Standard A16. New entries in the schedule to Standard A16 are required to undergo an evaluation to ensure there are no health and safety concerns with permitting their use. The standard is intended to reflect current use and prohibit inappropriate use of processing aids.

IDENTIFICATION OF AFFECTED PARTIES

Parties affected by the options listed above include:

- State, Territory and New Zealand Health Departments;
- manufacturers and producers of food products that use pectinesterase as a processing aid; and
- consumers.

OPTION 1

The status quo would be maintained and no specific permission would be given in the *Food Standards Code* for the use of pectinesterase from the source organism *Aspergillus oryzae*.

BENEFITS

Government No perceived benefits.

Consumers No perceived benefits.

Industry No perceived benefits.

COSTS

Government No perceived cost at present. However, in the future, if other countries approve pectinesterase from the new source organism, lack of approval in Australia may be construed as a non-tariff barrier to trade. This Option is also inconsistent with the existence of a standard for processing aids.

Industry Industry may be denied the availability of this processing aid, which may affect their ability to save on production costs in this area.

Consumers Consumers may be denied cheaper food products that would be a result of reduced costs to food industry.

OPTION 2

The *Food Standards Code* would be amended to specifically permit the use of pectinesterase from the source organism *Aspergillus oryzae*.

BENEFITS

Government Approval of pectinesterase as a processing aid may in the future promote international trade and reduction of technical barriers to trade, while continuing to protect public health and safety.

Industry	Promotes fair trade in food. This option will allow manufacturers to use a cheaper more efficiently obtained processing aid in food production.
Consumers	Consumers may have greater access to cheaper products.

COSTS

Government	Cost of amending the *Food Standards Code*.
Industry	Possible loss in sales from consumer reaction to food, which has been produced using a processing aid, derived from a genetically modified organism.
Consumers	Consumers who object to the use of processing aids derived from genetically modified organisms in food may have reduced food choices. This is a commercial matter, which manufacturers will need to address.

Evaluation

Option 1

Parties disadvantaged by the current state of regulation, which would not permit this particular processing aid are the manufacturers of pectinesterase and producers who may use it in the manufacture of their final food products. This option would essentially deny Australian industry and consumers access to a possibly cheaper product.

Option 2

This is the preferred option. The assessment indicates that this application raises no new issues that would preclude pectinesterase from being included in Standard A16 – Processing Aids.

The amendment to Standard A16 of the *Food Standards Code* to permit pectinesterase from the source organism *Aspergillus oryzae* carrying the donor gene from *Aspergillus aculeatus,* is necessary, cost effective and of benefit to both producers and consumers. It is consistent with the current existence of a Standard to regulate processing aids

ASSESSMENT AGAINST ANZFA OBJECTIVES

Protection of public health and safety

Toxicological evaluation of pectinesterase from the new source organism *Aspergillus oryzae* indicates that there are no significant public health and safety concerns identified with its use, that are associated with either the enzyme, or the source or donor organisms. This is addressed in full by the Toxicology Report (in Attachment 4) and in the issues raised in public submissions.

The provision of adequate information relating to food to enable consumers to make informed choices and to prevent fraud and deception

Currently, there is no general requirement within the Australian *Food Standards Code* for the declaration of processing aids in ingredient lists. This is because their presence, if any, in the food is incidental to the final product. The labelling of processing aids was addressed under Proposal P143 – Review of Ingredient Lists. Processing aids are proposed to be generally exempt from requirements to declare their presence in ingredient lists unless they contain substances that require a mandatory declaration of their presence in food, eg if they may cause severe adverse reactions.

ANZFSC decided the labelling of food produced using gene technology, including food produced using processing aids derived from GMOs.

Promotion of fair-trading in food

Approval for the use of pectinesterase in the manufacture of food will be a provision available for all manufacturers and should not impact on fair-trading in food.

Promotion of trade and commerce in the food industry

If approved, this application would aid promotion of trade and commerce in the food industry, through the availability of a more efficient and cost-effective methods of production to manufacturers of processing aids. This saving would arguably be passed on to consumers.

Promotion of consistency between domestic and international food standards

There are no international standards that are relevant to the scope of this application.

OTHER RELEVANT MATTERS

Currently ANZFA is undertaking a review of Standard A16 and Standard A11 as part of the overall development of a Joint *Food Standards Code* for Australia and New Zealand. The proposed variation to A16 if accepted would finally appear in the joint provisions for the regulation of processing aids.

WORLD TRADE ORGANIZATION (WTO) NOTIFICATION

Australia and New Zealand are members of the WTO and are bound as parties to WTO agreements. In Australia, an agreement developed by the Council of Australian Governments (COAG) requires States and Territories to be bound as parties to those WTO agreements to which the Commonwealth is a signatory. Under the agreement between the Governments of Australia and New Zealand on Uniform Food Standards, ANZFA is required to ensure that food standards are consistent with the obligations of both countries as members of the WTO.

In certain circumstances Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards that may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists).

A variation in the Code to extend the listed recognised source organisms of the processing aid pectinesterase constitutes a minor technical change. This change will not effect trade issues for either technical or sanitary or phytosanitary reasons. Therefore a notification to the WTO on grounds relating to the Technical Barrier to Trade Agreement or Sanitary or Phytosanitary Agreement is not required.

CONCLUSIONS

The full assessment report concludes that approval of the use of pectinesterase from a new source organism is technologically justified and poses no additional risk to public health and safety.

Approval for use will provide Australian manufacturers with a processing aid that the Regulatory Impact Statement has concluded would be more cost-effective and technologically efficient to manufacture and use.

The issue of labelling of processing aids derived from genetically modified organisms was decided by ANZFSC.

The draft variation should come into force on gazettal.

ATTACHMENTS:

1 Draft Variation to the Food Standards Code.

2 Draft Explanatory Notes.

3 Summary of Public Comment.

4 Toxicological Report.

5 Food Technology Report – Pectinesterase

Attachment 1

DRAFT VARIATION TO THE AUSTRALIAN FOOD STANDARDS CODE

To commence: On gazettal

Standard A11 of the *Food Standards Code* is varied by inserting in columns 1 and 2 respectively of the Table in the Schedule, after the entry for "Pectinase (*Aspergillus niger*)" –

Pectinesterase	FCC p107 (enzyme preparations)

Standard A16 of the *Food Standards Code* is varied by:

(a) inserting in columns 1 and 2 respectively of the Table IV, Group III of the Schedule, after the entry for "Pectinase multicomponent enzyme" -

Pectinesterase [EC 3.1.1.11]	*Aspergillus oryzae*[13]

And;

(b) inserting in the footnotes to Table IV, Group III of the Schedule, after footnote 12 –

[13] Pectinesterase may be produced from a genetically manipulated strain of *Aspergillus oryzae* containing the gene for pectinesterase isolated from *Aspergillus aculeatus*.

Attachment 2

EXPLANATORY NOTES

A373 - PECTINESTERASE AS A PROCESSING AID

EXECUTIVE SUMMARY

- The Australia New Zealand Food Authority (the Authority) received an application (A373) on 1 March 1999, from Novo Nordisk for approval of the enzyme, pectinesterase (IUB 3.1.1.11), for use as a processing aid during fruit and vegetable processing, when produced in *Aspergillus oryzae* from a pectinesterase gene isolated from *Aspergillus aculeatus*. The commercial name for the enzyme product is Rheozyme.

- Eleven submissions were received in response to the Section 14 gazette notice. Four submitters supported the application. The Office of Regulation Review submitted comments pertaining to Regulatory Impact Analysis. One submitter did not express any preference. Four submitters did not support the use of an enzyme derived from a genetically modified source organism, and on this basis did not support the application.

- The main issues raised by submissions were the labelling of processing aids obtained from genetically modified organisms (GMOs); and the importance of safety assessment for the new organism and the enzyme product.

- The scientific evaluations have concluded that the use of pectinesterase produced in *Aspergillus oryzae,* from a pectinesterase gene isolated from *Aspergillus aculeatus,* is technologically justified and poses no additional risk to public health and safety. No significant concerns were raised in the public comment regarding the actual use or approval of the processing aid. None of the Authority's section 10 objectives are compromised by the proposed change to Standard A16. It is recommended that the draft variation should come into effect on the date of gazettal.

- The Regulatory Impact Statement concluded that the amendment to Standard A16 of the *Food Standards Code* to permit pectinesterase from the new source organism *Aspergillus oryzae* carrying the donor gene from *Aspergillus aculeatus,* is necessary, cost effective and of benefit to both producers and consumers.

REGULATORY IMPACT ANALYSIS

The Authority is required, in the course of development of regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

To assist in this process, comment on potential impacts or issues pertaining to these regulatory options are sought from all interested parties in order to complete the development

of the regulatory impact statement. Public submissions should clearly identify relevant impact(s) or issues and provide support documentation where possible.

The Regulatory Impact Assessment has concluded that there are no costs to industry or the consumer and a negligible cost to government associated with bringing about these changes to the Code.

WORLD TRADE ORGANIZATION (WTO) NOTIFICATION

This matter does will not be notified to the WTO as a Sanitary or Phytosanitary notification or a Technical Barrier to Trade (TBT) notification because the proposed variation to the Code constitutes a minor technical change to the Code and will have no effect on trade issues for either technical or sanitary or phytosanitary reasons.

FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered into an Agreement in December 1995 establishing a system for the development of joint food standards. As a result of this Agreement and Commonwealth legislative changes, the National Food Authority became the Australia New Zealand Food Authority in July 1996. The Authority is now working towards the development of a joint *Australia New Zealand Food Standards Code*, which will be the one source of compositional and labelling food standards in both Australia and New Zealand.

Until the joint *Australia New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either the Australian *Food Standards Code*, as gazetted in New Zealand, or the New Zealand *Food Regulations 1984,* but not a combination of both. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the New Zealand *Food Regulations* 1984.

- **Food imported into Australia other than from New Zealand** must comply solely with the Australian *Food Standards Code.*

- **Food imported into New Zealand from Australia** must comply with either the Australian *Food Standards Code* or the New Zealand *Food Regulations* 1984, but not a combination of both.

- **Food imported into Australia from New Zealand** must comply with the Australian *Food Standards Code*. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may be imported into Australia from New Zealand if it complies with the New Zealand *Food Regulations* 1984 or *Dietary Supplements Regulations* 1985.

- **Food manufactured in Australia and sold in Australia** must comply solely with the Australian *Food Standards Code*, except for exemptions granted in Standard T1.

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act* 1986 and all food sold in Australia must comply with the Australian *Trade Practices Act* 1974, and the respective Australian State and Territory *Fair Trading Acts.*

Any person or organisation may apply to the Authority to have the *Food Standards Code* amended. In addition, the Authority may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. The Authority can provide advice on the requirements for applications to amend the *Food Standards Code*.

INVITATION FOR PUBLIC SUBMISSIONS

The Authority has completed a full assessment of the application, prepared draft variations to the Australian *Food Standards Code* and will now conduct an inquiry to consider the draft variations and its regulatory impact.

Written submissions containing technical or other relevant information which will assist the Authority in undertaking a full assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. The Authority's policy on the management of submissions is available from the Standards Liaison Officer upon request.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any confidential information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it in confidence. The *Australia New Zealand Food Authority Act* 1991 requires the Authority to treat in confidence trade secrets relating to food and any other information relating to food, the commercial value of which would be or could reasonably be expected to be, destroyed or diminished by disclosure.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A373** at one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra Mail Centre ACT 2610
AUSTRALIA
Tel (02) 6271 2222 Fax (02) 6271 2278

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942 Fax (04) 473 9855

Submissions should be received by the Authority by: **20 December 2000.**

General queries on this matter and other Authority business can be directed to the Standards Liaison Officer at the above address or by Email on <slo@anzfa.gov.au>. Submissions should not be sent by Email as the Authority cannot guarantee receipt. Requests for more general information on the Authority can be directed to the Information Officer at the above address or by Email <info@anzfa.gov.au>.

Attachment 3

PUBLIC COMMENT RECEIVED AT FULL ASSESSMENT

A373 – Pectinesterase as a Processing Aid

List of Submitters

1 Ms Elaine Attwood
2 Enzafoods New Zealand
3 Food Technology Association of Victoria Inc
4 InforMed Systems Ltd
5 National Council of Women of Australia
6 Office of Regulation Review
7 Ms Donella Peters
8 Victorian Food Safety Council Standards Sub-Committee
9 Mr Arnold Ward
10 Western Australia Food Advisory Committee

Submitter	Position	Comments
Ms Elaine Attwood	Opposes	Same as for National Council for Women of Australia: Labelling for increased consumer awareness is important. With the current disquiet surrounding all aspects of gene technology related to food no new permissions for any GT product should be granted. Consumers should be able to make informed choices and cannot whilst PA's are exempt from labelling.

Submitter	Position	Comments
Enzafoods New Zealand	Supports	Note that the use of this enzyme as a processing aid may have significant positive impact on yield and throughput in commercial fruit juice processing. This will advantage domestic food producers in enabling them to compete in an international market.
Food Technology Association of Victoria Inc	Supports, conditions. (See comments)	Accepts the application provided that: the toxicological safety assessment is satisfactory and consideration is undertaken to address the labelling of genetically modified processing aids.
InforMed Systems Ltd.	Supports, with conditions. (See comments)	Provided it can be shown that adequate documentation is provided about the safety of this product in the human diet. Agrees that no scientific justification exists for labelling, but cautions that such a requirement would be advisable in the present climate of public fear.
National Council of Women of Australia	Opposes	Consider the exclusion from labelling for processing aids to be contrary to consumers having an informed choice. As this product is derived from gene technology it should not be allowed until consumers have information freely available. Applications for foods from GT should be rejected until the foods are tested, approved and labelled accordingly.

Submitter	Position	Comments
Office of Regulation Review (ORR)	Do not state a position	Provide comment on developing the Regulatory Impact Statement. Suggest that if the products are genetically modified that this is made more explicit. The RIS should indicate that Govts have intervened in the market for processing aids for reasons of Public Health and Safety, and hence manufacturers must seek amendment to seek new market access. Widening permission would be consistent with treatment given to other applicants and enzymes.
Ms Donella Peters	Opposes	As genetic engineering is a new and very untested technology, and we don't know what effects we may see from it some years down the track, this should not be allowed. There is too much potential for it to prove detrimental to our health and food producers should not be using us as guinea pigs.
Victorian Food Safety Council Standards Sub-Committee	Do not state a position	Issues: hopefully safety will be addressed during the Full Assessment. Seek inclusion of detail of the source of the enzymes in Standards A16 indicating that they may be derived from recombinant strains and an indication of how genes are inserted (this comment also referred to the review team for Standard A16) Note that ANZFA are undertaking a full assessment.

Submitter	Position	Comments
Mr Arnold Ward	Opposes	As the processing aids are already in use and are based on the natural organism, what possible reason can there be for introducing a genetically modified version? Whenever there is a genetic modification of a natural organism there is a always the potential for something to go wrong. Provides excerpts from the literature and media. Gives the example of L-tryptophan and FDA findings. Discusses the faults of the substantial equivalence concept. Requests copies of the tests performed by ANZFA that indicates that products made using the processing aids are absolutely safe. If not why not?
Western Australia Food Advisory Committee	Supports	The Western Australian Food Advisory Committee feel that as species of *Aspergillus* have been used as processing aids without public health and safety incident, there should be no toxicological concerns.

Attachment 4

TOXICOLOGICAL ASSESSMENT

PECTINESTERASE – PROCESSING AID FOR FRUIT, VEGETABLES AND JUICE

Summary

The applicant has submitted three studies - a bacterial mutagenicity assay, an *in vitro* chromosomal aberration assay and a 13-week oral toxicity study in the rat - testing the toxicological potential of the enzyme, pectinesterase (IUB 3.1.1.11).

The submitted studies indicated that pectinesterase did not demonstrate mutagenic potential and did not cause chromosomal aberrations under the conditions of the tests. Rats exposed to pectinesterase through the diet indicated no toxicological consequences under the conditions of the study. The NOEL for this study was greater than 10 mg/kg/day.

Introduction

An application has been received from Novo Nordisk for the approval of the enzyme, pectinesterase (IUB 3.1.1.11) for use as a processing aid for fruit, vegetables and juice. The product is commercially known as Rheozyme.

The Schedule to Standard A16 – Processing Aids, permits the use of pectinase multicomponent enzyme produced by *Aspergillus* oryzae. The applicant is seeking an amendment to the Australian *Food Standards Code* (AFC) to include pectinesterase, which has a specific pectinase activity.

The enzyme is produced using a genetically manipulated strain of *Aspergillus oryzae* carrying the gene coding for the enzyme from *Aspergillus aculeatus*. *Aspergillus oryzae* has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA), 1987 concluded in 1987 that this organism is a traditionally accepted constituent of food.

Nutritionally, there are no positive or negative effects associated with the use of pectinesterase. The active enzyme will not be present in the final food because any residual is found in the form of inactivated enzyme that is metabolised as protein.

Purity of enzyme preparation and proposed specifications

Pectinesterase (IUB 3.1.1.11) produced from the source organism, *A. oryzae*, complies with the purity criteria recommended for enzyme preparations as described in Food Chemicals Codex (FCC), 4th ed., 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).

Evaluation of the submitted studies

Three toxicological studies were submitted in support of this application, namely, a bacterial mutagenicity assay, *in vitro* chromosomal aberration assay and a 13-week oral toxicity study in the rat.

Pectinesterase (Batch Number: PPJ 5402): Testing for mutagenic activity with *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 in a treat and plate assay. Novo Nordisk Study No. 968033. Author: P B Pederson, Novo Nordisk A/S, Denmark, August 1996.

Pectinesterase (Batch Number PPJ 5402) was examined for mutagenic activity in histidine-dependent auxotrophs of *Salmonella typhimurium* (TA1535, TA100, TA1537 and TA98). Bacteria were exposed in a liquid culture assay to six doses of the test substance in two complete and independent experiments, in the presence or absence of metabolic activation (S9 mixture). The experiments complied with OECD Guidelines for testing chemicals, 'Bacterial Reverse Mutation Test'. Proposal for replacement of Guidelines 471 and 472 (1996).

The test material was a crude enzyme preparation containing an abundance of various nutrients, including a growth medium to test bacteria. This means that comparison of variable counts between exposed cultures and control culture reflects growth simulation/inhibition, as well as cell death.

Positive controls possessed sensitivity for crystal violet (rfa-character) and for Mytomycin C (uvrB), and were resistant to ampicillin (pKM101), tested in the presence and absence of metabolic activation. All positive control chemicals induced significant increases in revertant colony numbers.

The maximum concentration of test material used was 10 mg/ml. Bacteria were exposed to six doses of the test substance in a phosphate buffered nutrient broth. After an incubation of three hours, the test substance was removed by centrifugation prior to plating. The plates were incubated for 64 hours, after which the number of revertants to prototrophy and viable cells were estimated.

No dose-related or reproducible increases in revertants to prototrophy were obtained with any of the bacterial strains exposed to pectinesterase (Batch Number PPJ 5402), either in the presence or absence of metabolic activation, at concentrations ranging from 313 μg to 10 mg/ml.

Conclusion

The test material, pectinesterase, did not exhibit any mutagenic activity under the conditions of the test.

Pectinesterase: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Novo Nordisk Study No. 966008. Author: R Marshall, Corning Hazleton (Europe), England, October 1996.

The potential of pectinesterase (Batch Number PPJ 5402, purity stated as 88.8 KPMU/g) to damage the chromosomal structure was tested in human lymphocyte culture *in vitro*. The methodology used in this study complied with the OECD Test Guideline 473 (1983), UK EMS Test Guidelines (1990) and EEC Annex V Test B 10 (1993).

Forty-eight hour cell cultures established from whole human blood were exposed to the test substance, in the presence and absence of metabolic activation (S9 mixture), as follows:

- 3 hours treatment plus 17 hour recovery with metabolic activation (rat liver-derived Aroclor 1254 induced S9 mixture)
- 3 hours treatment plus 41 hours recovery with metabolic activation
- 20 hours treatment plus 0 hours recovery without metabolic activation
- 44 hours treatment plus 0 hours recovery without metabolic activation

Sterile purified water was added to cultures and designated as negative controls. The positive control chemicals, 4-nitroquinoline 1-oxide (final concentration 2.5 μg/ml) and cyclophosphamide (final concentrations 12.5 and 25 μg/ml) were dissolved in sterile anhydrous analytical grade dimethyl sulphoxide, in the absence or presence of S-9 mixture, respectively.

The doses of pectinesterase selected for cytogenetic analysis were determined by a cell toxicity pretest. The doses chosen for analysis were 2813, 3750, and 5000 μg /ml.

One and one half hours before the harvesting, colchicine was added to a final concentration of approximately 1 μg to arrest dividing cells in metaphase. From each culture and the controls, 100 metaphases were scored for chromosome aberrations.

Treatment with pectinesterase PPJ 5402, did not produce biologically or statistically significant increases in the frequency of metaphase with aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation. No significant increase in polyploid, endoreduplicated or hyperdiploid cells was noted. Positive controls gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

Conclusion
Pectinesterase PPJ 5402 showed no clastogenic potential under the test conditions.

Pectinesterase, SP 572, Batch PPJ 5402 13 week toxicity study in rats with administration by gavage. Novo Nordisk Study No. 956031. Authors: S A Walker an S Clubb, Inveresk Research, Scotland, 29 August 1996.

Sprague-Dawley rats (10/sex/group) were dosed with pectinesterase SP572 (Batch No. PPJ 5402) daily by gavage at doses of 0, 1, 5, or 10 mg/kg/day (equivalent to 0, 0.12, 0,6 or 1.19 total TOS/kg/day) for 13 weeks.

The enzyme activity of the test batch was stated to be 88.8 KPMU/g and had a total organic solid (TOS) content of 11. 9%, a dry matter content of 87.6% and an ash content of 0.5%. The vehicle was sterile water. Stability of the test substance during the 13 week study was demonstrated.

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice as determined by the United Kingdom Department of Health and as accepted by International Regulatory Authorities throughout the European Community, United States of America and Japan. This study was designed to follow EU guidelines.

Rats were observed twice daily for clinical signs of toxicity and were palpated once weekly. A weekly record of body weights and food and water consumption was maintained. An eye examination of all animals was conducted before the study period and on all control and high dose animals after 12 weeks of dosing. Haematological, coagulation and clinical blood chemistry parameters were assessed in week 13 of the study. All animals were subjected to a detailed necropsy, including organ weight analysis and histopathology.

Five male study animals were killed ahead of the scheduled sacrifice - one control animal, one dose level 1 mg/kg/day animal, and three dose level 5 mg/kg/day animals. All were killed because of eye damage following collection of blood samples from the orbital sinus during week 13 of the study. The deaths were not attributed to treatment with pectinesterase.

The only clinical observations of note in five male animals were associated with eye damage following the orbital sinus bleeds. The affected animals were killed prematurely, as discussed above. In one female, observations included nosebleed, hunched appearance, irregular respiration, partially closed and pale eyes, pale ears and piloerection for several hours after the orbital sinus bleed. There were no other significant clinical changes in any of the groups treated with pectinesterase.

Body weights and food and water consumption were comparable between all study groups.

There were no ocular changes attributable to treatment with pectinesterase. Haematological analysis showed that 1 mg pectinesterase/kg/day resulted in a non-statistically significant increase in eosinophils in a single male animal. This aberration was not considered to be of toxicological significance. At 5 and 10 mg pectinesterase/kg/day, all findings were comparable with the control animals. In females, there was no evidence that treatment with pectinesterase adversely affected the measured haematological or coagulation parameters.

There were no significant effects on any of the clinical chemistry measurements in the male animals treated with pectinesterase. In females treated with pectinesterase at 1 and 5 mg/kg/day, there was a slight, non-statistically significant increase in the levels of aspartate and alanine aminotransferase. In the absence of any abnormal findings at 10 mg pectinesterase/kg/day, or any associated histopathological or organ weight findings, it is considered that the increases are not treatment-related. At the dose level of 10 mg/kg/day, all findings were comparable with the control group.

In males treated with 5 and 10 mg pectinesterase/kg/day, there was a slight, but statistically significant increase in heart weight ($p < 0.01$ and $p < 0.05$), respectively. However, the effect was not observed in females and there were no corroborative clinical chemistry, necropsy or histopathological findings. Therefore, it is considered unlikely that this finding is related to treatment with pectinesterase.

Females showed a slight decrease in lung weight in all groups treated with pectinesterase. Statistical analysis indicated a significant decrease in lung weight of $p < 0.05$ in the low and intermediate dose groups, whereas the high dose group attained a statistical significance of $p < 0.01$. However, the lung weight of one control female was noticeably higher than the rest of the group, causing an increase in the mean value. Also, these findings were not observed in male animals, nor were there any correlative necropsy or histopathological findings, indicating that these observations were not likely to be treatment-related.

There were no other significant intergroup changes in organ weights in either males or females.

Histological analysis showed no treatment related differences in either males or females.

Conclusion
Administration of pectinesterase SP572, at dosages up to 10 mg/kg/day for 13 weeks to rats was not associated with any significant toxicity. The NOEL for this study was greater than 10 mg/kg/day.

Attachment 5

FOOD TECHNOLOGY REPORT

A373 – Pectinesterase (IUB 3.1.1.11) as a processing aid

The Australia New Zealand Food Authority has received an application from Novo Nordisk BioIndustrial, seeking to amend Standard A16 - Processing Aids, to permit the use of the enzyme pectinesterase as a processing aid for use during fruit and vegetable processing.

Pectinesterase (IUB 3.1.1.11) is an enzyme that hydrolyses the ester linkages between methanol and galacturonic acid in esterified pectin. Alternative names for pectinesterase are pectin methylesterase, pectin demethoxylase and pectin methoxylase.

Pectinases, including pectinesterase, are widely used in the juice industry for clarification and improvement of yields[1, 2, 3, 4, 5, 6]. The use of pectinesterase for modification of the texture of plant-derived products has also been known for years[7, 8, 9, 10]. Pectinesterase occurs naturally in plants and in some microorganisms that degrade plant cell walls.

In this application, the biological source is *Aspergillus oryzae* carrying the gene coding for pectinesterase from *Aspergillus aculeatus*. The enzyme preparation is derived from a non-pathogenic and non-toxigenic source, and is produced under conditions that prevent the introduction of other microorganisms that could be a source of toxic materials. The manufacturing process is designed to ensure that no production organism is present in the final product.

Advantages of Rheozyme

The possibility to clone and express selected enzymes has facilitated a shift from enzyme mixtures towards utilisation of mono-component enzymes or of specifically boosted enzyme complexes. Pectinesterase is an example of such a mono-component enzyme, substantially free from interfering depolymerising activities such as polygalacturonases.

The enzyme may either be used as an efficient booster in combination with traditional multi-component pectinase products for clarification of fruit juice or wine, or alone with the aim of modifying the viscosity of fruit and vegetable based products, thus eliminating the need for adding exogenous pectin or other thickeners.

The enzymatic conversion of high methoxylated pectin to low methoxylated pectin makes an *in situ* viscosity increase or gel formation possible and may, in jam and tomato ketchup for example, render further addition of thickening agents unnecessary.

The Australian and New Zealand fruits/vegetables/juice industry has expressed a desire for producing processed fruits with improved firmness with this new technology and also for obtaining improved pectinase preparations for use during juice and cider manufacture.

Pectins and Pectinases

Pectic substances are major components of the primary cell wall and middle lamella of the world's major crops, including fruit and vegetables. Pectins, depending on the length of the polymer chain and the degree of methoxylation, affect the viscosity of liquids in which they are present.

Pectin is a protective colloid that helps to keep insoluble particles in suspension. Cloudiness is required in some commercial products to provide a desirable appearance. For this reason, to maintain cloud stability in some fruit juices, high temperature short time (HTST) pasteurisation is used to deactivate pectolytic enzymes. The destruction of the high levels of pectinesterase during the production of tomato juice is vital. The pectinesterase will act quite rapidly once the tomato is broken. In the so-called hot-break method, the tomatoes are broken up at high temperature so the pectic enzymes are destroyed instantly [11].

In other applications the aim is to produce clear juice and enzyme preparations that break down pectins are used in the clarification process. Pectic enzymes are used commercially in the clarification of fruit juices and for aiding in the disintegration of fruit pulps. By reducing the large pectin molecules into smaller units and eventually into galacturonic acid, the compounds become water soluble and lose their suspending power, also their viscosity is reduced and the insoluble pulp particles rapidly settle out[11].

Commercial food grade pectic enzyme preparations usually contain several different pectic enzymes. The traditionally used multi-component pectinase enzyme acts mainly to reduce the large pectin molecules into smaller units thus lowering viscosity as described above. There is also an enzyme component that decreases methoxylation of the pectin molecules, which assists in the overall clarification process. It is this mono-component enzyme pectinesterase activity that the current application seeks to have approved.

The polygalacturonic acid residues in pectins have varying degrees of esterification with methanol, that is, varying degrees of methoxylation. Pectinesterase hydrolyses the ester linkage between the methanol and galacturonic acid in esterified pectin. This action of pectinesterase results in a pectin with a low level of methoxylation, which in the presence of calcium ions forms a strong gel. However this situation only comes about if the pectinesterase acts alone. The viscosity is lowered if the polygalacturonic acid backbone of the pectin is reduced to smaller units, as occurs when the traditionally used multi-component enzyme is used.

Thus pectinesterase can be used alone to modify viscosity in fruit and vegetable juice products such as aspics and fruit gels. When pectinesterase is transported into plant tissue, either by means of passive infusion or by vacuum infusion, it allows the conversion of HM pectin (high methoxylated pectin) to LM pectin (low methoxylated pectin) to take place and thus it may also have an application for firming fruit and vegetables.

Public Health Issues

Rheozyme (trade name) is a water-soluble liquid preparation of pectinesterase. It is standardised and stabilised by blending with water, sorbitol, glycerol, potassium chloride and potassium sorbate. The commercial preparation has a declared activity of 10 Pectinesterase Units/mL.

The applicant suggests that the level of use of Rheozyme, according to requirements for normal production (GMP), ranges from 0.05–15 mL Rheozyme per kg of fruit. This level of use corresponds to 0.5-150 Pectinesterase Units per kg fruit. Pariza & Foster [12] in their review of food enzyme safety concluded that:

- Food enzymes were inherently non-toxic since they are inactivated and hydrolysed during digestion of the food;
- Toxicity studies are conducted on enzyme preparations largely to examine the potential toxicity of possible contaminants and, for this purpose, mutagenicity tests and a short-term toxicity study are normally considered adequate; and that
- Routine testing for allergenicity is not required and that health and safety considerations should concentrate on the safety of the source and donor organisms and whether toxic substances were likely to be formed during manufacture.

A. oryzae, the source organism, has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in 1987 that this organism is a traditionally accepted constituent of food.

A. aculeatus, the donor organism, also has a history of safe use in the food industry. It belongs to the *A. niger* group and is classified as a Class 1 organism according to the National Institutes of Health (NIH) guidelines. Enzyme preparations from the *A. niger* group were evaluated by JECFA (joint FAO/HO Expert Committee on Food Additives) as having an ADI 'not limited'.

Rheozyme complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC), 4th Ed., 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).

The closely related enzyme pectinase multi-component enzyme from *A. niger* and from *A oryzae* are already permitted for use in standard A16 of the *Food Standards Code*. As mentioned above, the donor organism *A. aculeatus* belongs to the *A. niger* group. In addition, both the source and the donor organisms are used as sources of many other microbial enzymes already permitted in standard A16.

The evaluation of the toxicological studies accompanying the Full Assessment report for this application concluded there would were no additional safety concerns associated with permitting the use of this enzyme as a processing aid.

Use of Rheozyme in other jurisdictions

Approval for use of Rheozyme has been obtained in Denmark and permission is pending in the USA and in France.

Conclusions

- The use of the enzyme pectinesterase as a processing aid for fruit, vegetables and their products, including juices, is technologically justified.
- Use of pectinesterase has advantages over the traditionally used pectinase multicomponent enzyme because when used alone, handling of the product is reduced, wastage is reduced, yield is increased, and the need for added thickeners is minimised.
- Pectinesterase also can be used together with traditional multi-component pectolytic enzymes to improve the clarification of juice and juice concentrate during manufacture and during winemaking.
- There are no additional public health and safety concerns associated with the use of microbial pectinesterase in a variety of food processes. The methods of enzyme production ensure that no production organisms would be present in the enzyme product. Further, the methods in which this enzyme would be employed means that no active enzyme would be present in the final foods.

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