8 November 2000
09/01

FULL ASSESSMENT AND REGULATORY IMPACT ASSESSMENT

SUBJECT: A402

LIPASE FROM GENETICALLY MODIFIED ASPERGILLUS ORYZAE

EXECUTIVE SUMMARY

- The Australia New Zealand Food Authority (ANZFA) received an application (A402) on 12 November 1999, from Novo Nordisk for the approval of the enzyme lipase (IUB 3.1.1.3), for use as a processing aid in the dairy industry. The applicant seeks to include provision for lipase sourced from a strain of *Aspergillus oryzae (A. oryzae)*, which carries the gene coding for a lipase isolated from *Rhizomucor miehei (R. miehei).* The commercial name for the enzyme product is palatase.

- Eleven submissions were received in response to the public consultation. Three submitters supported the proposal to amend the Food Standards Code to widen the existing permission for lipase. However, one of these submitters commented that there they would only support the proposal if certain conditions were met. Five submissions generally disagreed with the application and proposed that the status quo be maintained. Three submissions either did not state a position on the proposed application or indicated that they would comment later in the consultation process.

- 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 lipase produced in *A. oryzae* carrying the donor gene from *R. miehei,* is technologically justified and poses no additional risk to public health and safety. None of ANZFA's section 10 objectives are compromised by the proposed change to Standard A16 - Processing Aids. 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 - Processing Aids to permit lipase from the new source organism *A. oryzae* carrying the donor gene from *R. miehei,* is cost effective and of benefit to both producers and consumers.

BACKGROUND

ANZFA received an application (A402) on 12 November 1999, from Novo Nordisk for the approval of the enzyme, lipase (IUB 3.1.1.3), for use as a processing aid in the dairy industry. The applicant sought to include a provision for lipase sourced from a strain of *A. oryzae*, which carries the gene coding for a lipase isolated from *R. miehei.* The commercial name for the enzyme product is palatase.

The enzyme lipase is currently permitted for use as a processing aid, when sourced from a genetically manipulated strain of *A. oryzae* containing the gene for lipase isolated from *Humicola lanuginosa,* in Standard A16 - Processing Aids. The applicant seeks to vary the list of approved source organisms in Standard A16 - Processing Aids, for the enzyme lipase. The variation would constitute an extension of recognised source organisms to include another genetically modified strain of *A. oryzae*, carrying the gene coding for lipase isolated from *R miehei*.

Standard A16 - Processing Aids 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(*NZFR). Under the review of food regulations, a joint Standard, 1.3.3, Processing Aids for Australia and New Zealand was proposed
(P188 Processing Aids).

OBJECTIVE

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

RELEVANT PROVISIONS

*Australian Food Standards Code (*AFSC)

Standard A16 - Processing Aids

NZFR

There is no comparable standard for processing aids in the NZFR. Processing aids are generally not treated in a uniform manner in New Zealand. A limited number of substances are identified in the NZFR as processing aids, and these are exempt from the general labelling provisions, except for products containing chymosin (as an enzyme).

Codex

Codex has 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

Maintain the status quo and provide no specific permission in the A*FSC* for the use of lipase from the genetically modified source organism *A. oryzae* containing the gene isolated from *R. miehei*.

Option 2

Amend the A*FSC* would be amended to specifically permit the use of lipase from the genetically modified source organism *A. oryzae* containing the gene isolated from *R. miehei*.

The proposed variation to the A*FSC* constitutes a minor technical change and is not envisaged to effect trade for either technical or sanitary or phytosanitary reasons. A notification to the World Trade Organization is not required.

PUBLIC CONSULTATION

The preliminary assessment report for A402 was released for public comment between 23 February 2000 and 5 April 2000. Eleven submissions were received in response to the public consultation. Three submitters supported the proposal to amend the Code to widen the existing permission for lipase. However, one of these submitters commented that there they would only support the proposal if certain conditions were met. Five submissions generally disagreed with the application and proposed that the status quo be maintained. Three submissions either did not state a position on the proposed application or indicated that they would comment later in the consultation process. A table elaborating the comments from public submissions is included as an attachment to this report (Attachment 2).

ASSESSMENT

TOXICOLOGICAL EVALUATION

Application A402 to approve the use of lipase from a genetically modified microorganism involves the use of two organisms - *A. oryzae* (the source organism) and *R miehei* (the donor organism). Both these strains are currently listed in Standard A16 as microbes permitted for use in the production of certain enzymes, and have a history of safe use. In this case the lipase gene from *R. miehei* has been transferred to *A. oryzae.*

There are no nutritional issues associated with the use of lipase produced using recombinant DNA technology. The enzyme is used as a processing aid only, and is not usually expected to be present in the final food. An assay (detection limit of 1 ng DNA/g) carried out on the test batch of enzyme found no recombinant DNA present. Any residue in the food would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The safety of the source organism is an important consideration in the safety assessment for recombinant lipase. *A. oryzae* is not considered to be pathogenic, is widely distributed in nature and is commonly found in foods. Enzymes from

A. oryzae is extensively used in food processing, and have been for many years. The organism from which the lipase gene is derived (*R. miehei*) is likewise regarded as non-pathogenic, and has been used for many years for the production of lipase and proteases.

The genetic modification process involved the transfer of the lipase gene from *R. miehei* to *A. oryzae*. A gene encoding resistance to ampicillin was also transferred during the modification process, but no gene expression is activated due to the absence of the expression signal. The recombinant organism was found to be stable during production fermentations. Southern blotting was used to investigate the stability of the integration of the lipase gene after large-scale fermentation, and found that the inserted DNA was stably integrated into the host genome.

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic and, as long as good manufacturing practice is followed, the enzyme produced should be safe.
Lipase from the source organism, *A. oryzae* carrying the gene from *R. miehei* has been shown to comply with the recommended purity specifications for food grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC, 1996).

Three toxicological studies were submitted in support of this application. These consist of a 13-week oral toxicity study in rats, a bacterial mutagenicity assay (Ames Test) and a human lymphocyte cytogenetic assay. The tests were conducted in accordance with the Organisation for Economic Co-operation and Development (OECD, 1984) Guidelines for the Testing of Chemicals and in accordance with the Toxicological Principles for the Safety Assessment of Direct Food Additives and Colour Additives in Food (USFDA, 1982). The test material was produced in the same manner as the commercial preparations. Enzyme activity was found to be 1,200,000 LU/g (defined as the activity of one gram of pure enzyme protein), and the total organic substance (TOS) content 83.6%. Results were published in 1994 (Broadmeadow et al. 1994).

Lipase produced from both *A. oryzae* and *R. miehei* have already been shown to be safe for use as processing aids for food. This assessment of the genetically modified lipase produced by *A. oryzae* carrying the lipase gene produced by *R. miehei* found that:

- Both source and donor organisms have a long history of safe use;
- The lipase gene is stably integrated into the host genome;
- The enzyme preparation contains no contaminants;
- The enzyme causes no mutagenic or cytogenic effects in *in vitro* studies;
- The NOEL from sub-chronic rat feeding studies is 1600 ppm.

From the information available, it is concluded that the use of the genetically modified lipase as a processing aid in food would pose no significant risk to human health.

The full toxicological evaluation is available as an attachment to this full assessment (Attachment 3).

ISSUES ARISING FROM PUBLIC SUBMISSIONS

Eleven submissions were received in response to the public consultation. Three submitters supported the proposal to amend the Code to widen the existing permission for lipase. However, one of these submitters commented that there they would only support the proposal if certain conditions were met. Five submissions generally disagreed with the application and proposed that the status quo be maintained. Three submissions either did not state a position on the proposed application or indicated that they would comment later in the consultation process.

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.

1. Labelling

Issue

The **Food Technology Association Australia Inc**, the **National Council of Women of Australia** and the **Ministry of Health** all questioned whether processing aids derived from genetically modified organisms will require labelling. Submissions from the **Food Technology Association Australia Inc** and the **New Zealand Ministry of Health** specifically requested that this issue be addressed in the Full Assessment report. The **National Council of Women of Australia** stated that public comment indicated that mandatory labelling would be the preferred option regardless of whether or not the genetically modified organisms are in the final food.

Background

Processing aids are not currently required to appear in ingredient lists under general labelling provisions in the FSC and the NZFR. Established international food standards also exempt processing aids, either conventional or GM-derived from labelling. There are numerous GM processing aids used by the food industry. Processing aids are generally present to fulfil a technological purpose relating to treatment or processing, but do not perform a technological function in the final food.

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 1.2.2 Mandatory Information, and 1.2.4 Labelling of Ingredients). Proposal P161 proposed the mandatory declaration of a list of foods and food additives that may cause severe adverse reactions. The approach taken by the general review of processing aids would apply to the products within this application, therefore all comments regarding the labelling of processing aids whether from GMOs or not, have been referred to that review project.

Evaluation and Conclusion

The labelling of foods produced using gene technology, was decided on at the Australia New Zealand Food Standards Council (ANZFSC) meeting on 28 July 2000. The ANZFSC decided to exempt processing aids and food additives except where novel DNA and/or protein is present in the final food.

2. Safety Issues

Safety issues relating to lipase from genetically modified *A. oryzae* related to concerns about public health and safety, product assessment, scientific justification, and whether DNA is present in the lipase in the final food.

2(a) General public health and safety concerns

Issue

Barbarah Baragwanath, Natalie Baragwanath, the **National Council of Women of Australia** and the **National Council of Women of New Zealand** specifically commented that they had public health and safety concerns about the proposed standard which need to be addressed.

Evaluation and Conclusion

The scientific evaluations have concluded that the use of genetically modified lipase as a processing aid in food, is technologically justified and poses no additional risk to public health and safety.

2(b) Product assessment

Issues

Food Technology Association Victoria Inc questioned whether the current precedent for GMO produced enzymes in Standard A16 is going to continue under the proposed standard. **The National Council of Women of Australia** commented that no further expansion of any genetically engineered product should be undertaken until such time as the Office of the Gene Technology Regulator is established and matters dealing with gene technology pass through that office first.

Evaluation and Conclusions

At present toxicological evaluations form part of the usual ANZFA assessment procedure for any new food additive, processing aid or similar type of product. Permission for use of GMO produced enzymes under the joint FSC will also undergo a toxicological assessment to ensure there are no concerns relating to either the toxicity or pathogenicity of genetically modified enzymes. ANZFA has a team of highly qualified biologists with expertise in the fields of genetics plant, insect and bacterial biology, toxicology, food science, human health and medical research who are responsible for full safety assessments of all genetically modified foods for retail sale. ANZFA proposes to ensure that the current precedent for GMO produced enzymes in Standard A16 will continue through to the joint FSC.

2(c) Scientific justification for permitting lipase from genetically modified *A. oryzae*

Issues

Several submissions stated that the following considerations need to be taken into account before permitting lipase from genetically modified *A. oryzae*. **Natalie Baragwanath** commented that no genetically modified substances should be given approval until the New Zealand Royal Commission has fully inquired into the implications of genetic manipulation for food safety, health, environmental safety, biosecurity and the right of consumers to make informed decisions. Research from Dr Arpad Pusztai about the effect of genetically manipulated foods on animals was also cited.

The **National Council of Women of Australia** commented that there are no human studies carried out with genetically engineered foods, additives, or enzymes and until such studies are done and the results evaluated, any risks remain unknown. **The National Council of Women of New Zealand** questioned the need for another source of lipase given that the current sources are adequate and commented that they hoped for sound technical justification for permitting the lipase from genetically modified *A. oryzae* compared with the justification put forward in Application A371 which they believe was inadequate.

The **Ministry of Health** requested that ANZFA send the Ministry information on the following aspects including:

- details of the isolation of the lipase gene *R. meihei* and the vehicle used to insert the isolated gene to the *A. oryzae* strain of bacteria;

- information on the chemical characteristics of the enzyme derived by this method. The Ministry claim that this is essential (this reveals the specificity of the enzyme toward mono, di or triacylglycerols of saturated fatty acids);

- information about the process by which the purification of lipase from *A. oryzae* takes place including the possibility that some bacterial residues may be present in the purified enzyme. They also commented that a series of toxicological data (long-term toxicity studies, reproduction toxicity studies, mutagenicity and carcinogenicity studies) are required to document the safety of *A. oryzae*, use of and its metabolites, beta nitropropionic acid; potential to cause mutagenicity and chromosomal aberrations; pathogenicity of *A. oryzae*; and human exposure levels through estimated dietary intake.

ANZFA can reassess new information as a result of the Royal Commission's findings as they arise and has the responsibility to ensure that the permission for genetically modified lipase is scientifically justified. In addition, all genetically modified foods must be assessed by ANZFA, determined to be safe and approved by the ANZFSC before they can be legally sold in Australia or New Zealand.

The Ministry of Health had a number of questions relating to the application to permit genetically modified lipase, including labelling issues. The studies requested by the Ministry of Health are not normally required to establish safety of processing aids by ANZFA. ANZFA considers the studies submitted in the Safety Assessment are adequate to establish the safety

of the enzyme . Furthermore, the chemical characteristics of the enzyme derived by this method, as the chemical characteristics are the same as those of lipase which are currently permitted in Standard A16. Concerns about the process of purification of lipase and the lack of toxicological data relating to *A. oryzae* were also raised by the Ministry of Health. The lipase proposed complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC) 4th, 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The safety of *A. oryzae* is discussed in the toxicological assessment (Attachment 4 to this report). Enzymes *from A. oryzae* have been extensively used in food processing and have been for many years. The **National Council of Women of New Zealand** questioned the need for another source of lipase given that the current sources are adequate. The **National Council of Women of Australia** talked about the Office of the Gene Technology Regulator and how all matters dealing with genetically modified organisms pass through the office before there is any further expansion of any genetically modified product. ANZFA considers that the additional source of lipase is safe, will allow for fair trade, will promote international trade, allow manufacturers to use a cheaper more efficiently obtained processing aid and give consumers greater access to cheaper products.

2(d) Residue from the processing aid (enzyme) in the final food

Issue

Informed Systems Limited and the **National Council of Women of New Zealand** expressed concern that there may be residue from the processing aid (enzyme) in the final food and that this may be a concern for consumers. Furthermore, **Informed Systems** commented that if the enzyme were not free of DNA then they would only support the application if the modified organism did not have an antibiotic resistance marker gene or similar marker gene present and active.

Evaluation

While the processing aid is the product of the genetic modification of a micro-organism, it is not itself modified. The resulting enzyme lipase is the same enzyme that would be obtained from the already approved *Humicola lanuginosus*. In addition, there was no DNA found in a test batch of enzyme. There would be no microorganisms remaining in the collected product, when added into a food manufacturing process. Any enzymes remaining in the food would be 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.

Conclusion

Lipases produced from both *A. oryzae* and *R. miehei* have been assessed as safe for use as processing aids.

REGULATORY IMPACT ANALYSIS

The objective of regulatory impact analysis is to examine labelling and other issues arising from permission to use lipase, from a new source organism, 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 lipase from source organism *A. 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 lipase as a processing aid; and
- consumers.

OPTION 1

The status quo would be maintained and no specific permission would be given in the A*FSC* for the use of lipase from genetically modified *A. oryzae* carrying the *R. miehei* gene.

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 lipase from the new genetically modified source organism, lack of approval in Australia or New Zealand may be construed as a non-tariff barrier to trade.

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 A*FSC* would be amended to specifically permit the use of lipase from *A. oryzae* carrying the *R. miehei* lipase gene.

BENEFITS

Government	Approval of lipase from a new genetically modified source organism 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 *FSC*.
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 manufacturers will need to address. The issue of labelling of such products is under consideration by the ANZFSC.

Evaluation

OPTION 1

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

OPTION 2

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

The amendment to Standard A16 of the A*FSC* to permit lipase from the new genetically modified source organism *A. oryzae* carrying the donor gene from *R. miehei,* is cost effective and of benefit to both producers and consumers.

ASSESSMENT AGAINST ANZFA OBJECTIVES

Protection of public health and safety

Toxicological evaluation of lipase from the new genetically modified source organism *A. oryzae* indicates that there are no public health and safety concerns identified with its use, relating to either the enzyme itself, 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 enzyme lipase is already approved as a food-grade processing aid.

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 *FSC* 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 is being 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. The labelling of food produced using gene technology, including food produced using processing aids derived from GMOs, is an issue under consideration by ANZFSC.

Promotion of fair trading in food

Approval for the use of lipase from *A. oryzae* carrying the *R. miehei* gene 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 method of production to manufacturers of processing aids. This saving could 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

ANZFA is currently undertaking a review of both Standards A16 and A11 as part of the overall development of a Joint *FSC* for Australia and New Zealand. The proposed variation to A16, if accepted, would require a consequential amendment to the proposed joint Standard 1.3.3 for processing aids.

WORLD TRADE ORGANISATION (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 lipase 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 lipase from a new source organism is technologically justified and poses no significant risk to public health and safety.

Approval for use will provide Australian manufacturers with a processing aid which is claimed to be more cost-effective and technologically efficient to manufacture and use.

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

The draft variation should come into force on gazettal.

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 A402** 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>.

ATTACHMENTS:

1 Draft Variation to the Food Standards Code.

2 Summary of Public Submissions.

3 Toxicological Report.

Attachment 1

DRAFT VARIATION TO THE AUSTRALIAN FSC

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 "Lipase (*Aspergillus niger*)" -

Lipase (*Aspergillus oryzae)*	AMFEP Appendix 1

Attachment 2

SUMMARY OF PUBLIC SUBMISSIONS RECEIVED
A402 – LIPASE AS A PROCESSING AID

No.	Organisation	Position	Comments
1	**Consumers Association of South Australia Inc**	Support Option 1	Supports comments from Ms Elaine Attwood, National Council of Women of Australia.
2	**Informed Systems**	Support basic concept but has conditions that would need to be met.	Support basic concept, but note there is no information about the purity of the product. Questions whether the enzyme will be effectively free of DNA from the organism. If the enzyme is not free of DNA the applicant would only support the application if the modified organism does not have an antibiotic-resistance marker gene or similar marker gene present and active. If the enzyme is free of DNA from the organism then the applicant would support the application.
3	**Food Technology Association, Victoria Inc**	Do not state a position	FTA endorses the following comments of the Technical Sub Committee. The Committee requested clarification of two issues before it can fully assess the application: 1. Although there are precedents for GMO produced enzymes in Standard A16 is this situation going to continue? 2. What is the situation with this application and Draft Standard A18 and new Processing Aids based on GMO that requires declaration of these types of processing aid? Request that they be maintained on the circulation lists for further changes to this application.
4	**New Zealand Dairy Board**	Supports Option 2	Supports Option 2 to amend the code as lipase is already an approved processing aid
5	**Barbara Baragwanath**	Support Option 1	Expresses concern about the extent of the use of additives/chemicals in food.

			Comment that ANZFA should be using the precautionary principal and putting health first. Refuses to buy any food containing genetically engineered or genetically modified organisms and refuses to see future generations damaged by the current generations pursuit of profit before commonsense. Is alarmed to observe the pressure ANZFA is put under by powerful trade interests.
6	**Natalie Baragwanath**	Supports option 1	Strongly objects to any alteration to the food standard until there is substantial proof of safety of genetically engineered organisms. Cites research by Dr Arpad Pusztai, United Kingdom whose experimental research findings have been published in the journal The Lancet. The submitter commented that the research indicated that genetically manipulated foods can when fed to animals in reasonable amounts cause gradual organ damage and immune system damage. The submitter states that 20 top scientists have peer reviewed the research and state the conclusions are justified. Comment that no genetically modified substances should be given approval until the Royal Commission has fully inquired into the implications of genetic manipulation for food safety, health, environmental safety and biosecurity and the right of consumers to make informed decisions.
7	**Ministry of Health**	Do not state a position	Request that ANZFA send the Ministry information on the following aspects of the full assessment: The report does not indicate the details of the isolation of the lipase gene from *R miehei* and the vehicle used to insert the isolated gene to the *A. oryzae* strain of bacteria.

			Information on the chemical characteristics of the enzyme derived by this method is essential (this reveals the specificity of the enzyme toward mono, di or triacylglycerols of saturated fatty acids. The process by which the purification of lipase from *A. oryzae* takes place should also be discussed and the possibility that some bacterial residues may be present in the purified enzyme. A series of toxicological data (long-term toxicity studies, reproduction toxicity studies, mutagenicity and carcinogenicity studies) are required to document the safety of *A. oryzae* in use of and its metabolites, beta nitropropionic acid; potential to cause mutagenicity and chromosomal aberrations; pathogenicity of *A. oryzae*; and human exposure levels through estimated dietary intake. The labelling of foods produced using gene technology, including whether there is a need for processing aids derived from GMOs to be labelled, is currently a matter under consideration by the ANZFSC. This will need to be discussed in the full assessment report.
8	**National Council of Women of Australia**	Support Option 1	Does not consider this technology has been sufficiently tested to ensure its safety in the food supply. In addition it is a processing aid and as such is not labelled. Public comment indicates that mandatory labelling is preferred whether the genetically modified organisms are in the final food or not. There are no human studies carried out with genetically engineered foods, additives or enzymes and until such studies are done and the results evaluated, any risk remains unknown. It is difficult to determine whether this application would be technologically

			justifiable, as the Authority gave no reason for why phytase (in A371) was considered technically justifiable. Believe there should be no further expansion of any genetically engineered product until such time as the Office of the Gene Technology Regulatory is established and all matters dealing with GMOs pass through that office first. The National Council of Women of Australia Inc, represents 500 affiliated organizations plus a large individual membership. It coordinates the views of more than 3M women throughout Australia and is non-party political and non-sectarian.
9	**Dieticians Association of Australia**	Supports option 2	Supports application.
10	**National Council of Women of New Zealand**	Support option 1 but provided their issues are addressed, the submitter would be guided at full assessment by ANZFA.	Submitter is an umbrella organization representing 46 nationally organised societies. It has 36 branches spread throughout the country to which women from some 150 societies are affiliated. Questions the need for another source of lipase and take the view that current sources are adequate. Concerned that consumers have no guarantee that there is no residue from processing aids in the final food. Question whether the use of such a product would be acceptable in New Zealand at this time. Believe there are public health and safety concerns. State that provided the above issues are addressed, then the submitter would be guided by the full assessment by ANZFA.
11	**Office of Regulation Review**		Did not want to comment at this stage, but look forward to seeing a draft Regulatory Impact Statement.

Attachment 3

TOXICOLOGICAL ASSESSMENT

1 Introduction

Application A402 to approve the use of lipase from a genetically modified microorganism involves the use of two organisms - *A. oryzae* (the source organism) and *R. miehei* (the donor organism). Both these strains are currently listed in Standard A16 as microbes permitted for use in the production of certain enzymes, including lipase, and have a history of safe use. The only difference in this case is that the lipase gene from *R. miehei* has been transferred to *A. oryzae.*

There are no nutritional issues associated with the use of lipase produced using recombinant DNA technology. The enzyme is used as a processing aid only, and is not present in the final food. An assay (detection limit of 1 ng DNA/g) carried out on the test batch of enzyme found no recombinant DNA present. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

2 The source (production) organism - *A. oryzae*

The safety of the source organism is an important consideration in the safety assessment for recombinant lipase. *A. oryzae* is not considered to be pathogenic, is widely distributed in nature and is commonly found in foods (Barbesgaard et al, 1992). Enzymes from *A. oryzae* are extensively used in food processing, and have been for many years (Rogers, 1977).

3 The donor organism – *R miehei*

The organism from which the lipase gene is derived is likewise regarded as non-pathogenic, and has been used for many years for the production of lipase and proteases (Broadmeadow et al, 1994).

4 Nature of the genetic modification

The genetic modification process involved the transfer of the lipase gene from *R. miehei* to *A. oryzae*. A gene encoding resistance to ampicillin was also transferred during the modification process, but no gene expression is possible due to the absence of expression signal. The recombinant organism was found to be stable during production fermentations. Southern blotting was used to investigate the stability of the integration of the lipase gene after large-scale fermentation, and found that the inserted DNA was stably integrated into the host genome.

5 Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic and, as long as good manufacturing practice is followed, the enzyme produced should be safe. The detailed specifications to which the preparation was found to conform are laid out in Table 1.

Table 1. Standard for identity of Palatase preparation

Criteria	Specification
Heavy Metals	not more than 30 ppm
Lead	not more than 5 ppm
Arsenic	not more than 3 ppm
Total viable count	not more than $5x10^4$
Total coliforms/g	not more than 30
Enteropathogenic E. coli/25g	negative by test
Salmonella/25g	negative by test
Antimicrobial activity	negative by test
Mycotoxins	negative by test
Production organism	negative by test

Lipase from the source organism, *A. oryzae* carrying the gene from *R. miehei* has been shown to comply with the recommended purity specifications for food grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives, JECFA (FAO, 1992) and the Food Chemicals Codex (FCC, 1996).

6 Evaluation of the submitted studies

Three toxicological studies were submitted in support of this application. These consisted of a 13-week oral toxicity study in rats, a bacterial mutagenicity assay (Ames Test) and a human lymphocyte cytogenetic assay. The tests were conducted in accordance with the OECD Guidelines for the Testing of Chemicals (OECD, 1984) and in accordance with the Toxicological Principles for the Safety Assessment of Direct Food Additives and Colour Additives in Food (USFDA, 1982). The test material was produced in the same manner as the commercial preparations. Enzyme activity was found to be 1,200,000 LU/g (defined as the activity (LU) of one gram of pure enzyme protein), and the total organic substance (TOS) content 83.6%. Results were published in 1994 (Broadmeadow et al. 1994).

6.1 Toxicity study by dietary administration to CD rats for 13 weeks. Life Science Research Ltd. Author: A. Broadmeadow, 7 March 1990.

Methods

Groups of CD rats (20/sex/group) were administered a diet containing lipase at concentrations of 0, 1600, 8000 or 40000 ppm, for 13 weeks. This resulted in average intake of 0, 120.6, 600 or 2892 mg/kg/bodyweight/day respectively.

Rats were observed twice daily for evidence of systemic toxicity or ill health and were palpated once weekly. Body weight and food consumption was recorded weekly and the food conversion ratio calculated. Water consumption was recorded over a three-day period in weeks 1, 6 and 13. An eye examination of all animals was conducted before the study period and on all control and high dose animals during weeks 6 and 13 of the study. Haematological,

coagulation, and blood chemistry parameters were also measured, and urinalysis carried out, in weeks 6 and 13 of the study. After 13 weeks all animals were killed and subjected to a detailed necropsy, including organ weight analysis and histopathology.

Results

The detailed results of changes associated with treatment can be seen in Table 2. There were no premature deaths in any of the rats receiving lipase. There were no clinical signs shown during the 13-week study period, although at necropsy facial staining was seen in the high dose group.

Food consumption was reduced in the high dose group only, for the first 3 and 5 weeks respectively for the male (-7%) and female (-10%) groups. Thereafter the amount consumed was similar to that of the control group, and over the 13 week period there were no significant differences between control animals and those receiving lipase ($p \geq 0.05$). The amount of food scattered was comparable between all groups, suggesting that all diets were palatable, even at the highest Lipase dose, and the initial reduction in food consumption did not significantly affect weight gain or food conversion ratios. There was no effect on water consumption, and no treatment-related ocular changes at week 13.

Some slight haematological differences were noted in the animals receiving lipase:

- Slightly prolonged prothrombin times for rats in the medium and high dose groups after 6 and 12 weeks in males, and after 6 weeks in females;
- Higher alanine and aspartate amino-transferase and acetylcholinesterase activities in male rats receiving medium and high doses;
- Higher ornithine carbamyl transferase activity in high-dose male rats;
- Marginally low albumin concentration in high-dose females, and lower plasma protein in medium and high-dose females.

Higher urinary specific gravity, often associated with low volume, was noted in medium and high-dose groups.

Histopathological differences were as follows:

- Chronic myocarditis in high-dose males;
- Hyperplasia of the gastric glands in medium-dose males, and high-dose males and females (only statistically significant in the high-dose male group);
- Hyperkeratosis and acanthosis in the keratinised region of the stomachs of medium and high-dose males and females (though not at a statistically significant level).

The only difference in organ weights identified at necropsy was slightly higher % relative kidney weight in female rats receiving the high dose lipase diet.

Discussion and conclusions

Sub-chronic administration of lipase at the doses mentioned above was associated with effects upon food intake, the stomach, heart, and hepatic and renal function.
Effects on the stomach, as well as the lack of appetite seen in high dose animals are difficult to explain, but may represent an adaptive response to the administration of an irritant compound present in the enzyme preparation.

Cardiac and other related changes (myocarditis and high plasma aspartate amino-transferase activities) are normal in aging rats, but are known to be exacerbated by a number of materials (Gopinath et al 1987). As such, they are of little toxicological significance.

Plasma changes (high alanine and aspartate amino-transferase, acetylcholinesterase, ornithine carbamyl transferase and glutamyl transpeptidase activities and low total plasma protein concentration) were indicative of minor changes in liver metabolism, although no changes in liver weight were seen. In addition inter-group differences in urine reflected minor changes in renal function. These were associated with a slight increase in kidney weight (but no morphological change), in high-dose animals. Since these slight changes were seen in animals receiving doses of 8000 and 40000 ppm, the NOEL for lipase is 1600 ppm (120.6 mg/kg bw/day).

Table 2. Changes associated with treatment in the 13 week toxicity study

Group: Level (mg/kg diet)	1 M 0	2 M 1600	3 M 8000	4 M 40000	1 F 0	2 F 1600	3 F 8000	4 F 40000
Food Consumed (g) Wks 1-3 Wks 1-5	577 975	607 1031	598 1018	535 925	431 735	436 746	439 755	380 662
Prothrombin time(s) Wk 7 Wk 13	14.4 ± 0.6 16 ± 1.0	14.3 ± 0.7 15.6 ± 0.6	14.8 ± 0.4[c] 16.4 ± 0.7[b]	15.0 ± 0.6[c] 16.5 ± 0.7[b]	13.8 ± 0.5 17.5 ± 3.2	13.8 ± 0.4 17.2 ± 4.6	14.2 ± 0.4[b] 17.3 ± 1.7	14.4 ± 0.3[c] 18.0 ± 3.9
ALT (iu/l) Wk 7 Wk 13	29 ± 4 36 ± 5	30 ± 5 37 ± 5	34 ± 5[b] 35 ± 5	34 ± 5[b] 40 ± 6[a]	28 ± 5 35 ± 19	27 ± 4 28 ± 5	27 ± 4 32 ± 7	28 ± 5 33 ± 11
AST (iu/l) Wk 7 Wk 13	81± 8 84 ± 10	83 ± 8 88 ± 11	87 ± 7[b] 92 ± 11[a]	88 ± 8[b] 97 ± 11[b]	76 ± 11 79 ± 44	78 ± 8 74 ± 11	73 ± 9 73 ± 12	76 ± 9 78 ± 14[a]
Acetyl CHE (iu/l) Wk 7 Wk 13	866 ± 126 812 ± 130	897 ± 133 849 ± 99	923 ± 104 898 ± 105[a]	963 ± 164[a] 901 ± 129[a]	3824 ± 808 4635 ± 961	3418 ± 926 4115 ± 1023	3682 ± 882 4570 ± 907	3351 ± 981[a] 4310 ± 1253
OCT (iu/l) Wk 7 Wk 13	9.1 ± 2.0 6.5 ± 1.1	9.8 ± 2.3 5.1 ± 1.5	8.6 ± 2.3 5.6 ± 1.6	10.7 ± 1.6[b] 7.9 ± 1.6[b]	12.5 ± 3.8 6.3 ± 3.1	13.3 ± 5.9 7.7 ± 1.9	11.4 ± 3.9 7.8 ± 2.0	15.9 ± 5.6 6.2 ± 2.8
Total protein (g%) Wk 7 Wk 13	6.8 ± 0.3 7.2 ± 0.4	6.7 ± 0.3 7.1 ± 0.2	6.7 ± 0.2 7.1 ± 0.2	6.7 ± 0.3 7.2 ± 0.3	7.3 ± 0.3 7.8 ± 0.4	7.1 ± 0.4 7.6 ± 0.3	7.2 ± 0.3 7.6 ± 0.3[a]	7.2 ± 0.3 7.5 ± 0.3[b]
Albumin (g%) Wk 7 Wk 13	3.2 ± 0.2 3.7 ± 0.3	3.4 ± 0.2 3.6 ± 0.3	3.2 ± 0.1 3.6 ± 0.4	3.4 ± 0.2[b] 3.6 ± 0.2	4.3 ± 0.3 4.3 ± 0.5	4.2 ± 0.3 4.3 ± 0.3	4.4 ± 0.3 4.4 ± 0.4[a]	4.2 ± 0.3 4.0 ± 0.3[a]
Urinary Volume(ml) Wk 5 Wk 11	6.5 ± 1.5 7.0 ± 2.0	6.0 ± 1.5 7.0 ± 2.0	6.0 ± 1.5 6.0 ± 2.0	5.0 ± 1.5[b] 6.5 ± 2.0[a]	5.0 ± 1.5 5.0 ± 1.5	4.5 ± 1.5 4.0 ± 1.5[a]	4.5 ± 2.0 4.5 ± 1.0	4.0 ± 1.0 3.0 ± 1.0[c]
Urinary SG Wk 5 Wk 11	1046 ± 9 1043 ± 7	1049 ± 8 1047 ±10	1054 ± 9[b] 1052 ± 10	1056 ± 9[c] 1049 ± 8	1046 ±13 1047 ± 9	1053 ± 13 1054 ± 11	1054 ±13 1051 ±11	1060 ±12[b] 1064 ±10[c]
Kidney Weight (% bodyweight)	0.79 ± 0.07	0.79 ± 0.04	0.80 ± 0.05	0.81 ± 0.07	0.76 ± 0.07	0.80 ± 0.07	0.78 ± 0.08	0.83 ± 0.07[c]

Facial Staining	0/20	1/20	3/20	6/20[a]	1/19	4/20	3/20	10/20[b]
Histopathology:								
Chronic	1/20	3/20	6/20	12/20[c]	1/19	-	-	0/20
myocarditis	2/20	2/20	7/20	10/20[a]	0/19	0/20	0/20	4/20
Hyperplastic gastric glands	0/20	0/20	3/20	4/20	0/19	0/20	3/20	2/20
Hyperkeratosis & acanthosis in the keratinised stomach								

Values shown after ± are standard deviations.

ALT – Alanine amino-transferase

AST – Aspartate amino-transferase

CHE – cholinesterase

OCT – Ornithine carbamyl transferase

GT – Glutamyl transpeptidase

SG – specific gravity

[a,b] or [c] denotes value statistically different from control (a=p< 0.05, b=p< 0.01, and c=p< 0.001)

6.2 Mutagenicity assay with strains of Salmonella typhimurium strain TA 98, TA 100, TA 1535 and TA 1537 in a liquid culture assay. Industrial Biotechnology R&D, Novo Industrial A/S Study 89070. Author: P. B. Pederson, 9 August 1989.

Lipase (the same preparation as for the subchronic study) was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537). A liquid culture assay was applied and bacteria exposed to five doses (ranging from 0.1 to 10 mg/ml) of the test substance in a phosphate buffered broth for three hours. After incubation the test substance was removed by centrifugation, plated, and the number of both revertants to prototrophy and viable cells estimated. The test was carried out both in the presence and absence of metabolic activation (in the form of a liver preparation, S-9, and co-factors required for mixed function oxidase activity). The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (2-Aminoanthracene, 9-Aminoacridine and 2-Nitrofluorene).
No dose-related or reproducible increases in revertants to prototrophy were obtained with any of the bacterial strains exposed to lipase either in the presence or absence of metabolic activation. A repeat experiment confirmed these results. It was concluded that the test material lipase PPW 2771 did not exhibit any mutagenic activity under the conditions of the test.

6.3 Chromosome aberration assay in cultured human lymphocytes. Study no. NOD 13/HLC. Author: R Marshall, Microtest Research Limited, York, UK, 15 August 1989.

The potential of lipase SP 388 (Batch PPW 2771) to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using duplicate human lymphocyte cultures from a

single female donor. Tests were carried out in the presence and absence of S-9 metabolic activation, over a broad range of doses. No evidence of mitotic inhibition was seen at any of the dose levels analysed. Cells receiving doses of 2113, 3250 and 5000 µg/ml were checked for chromosomal aberration.

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation (See Table 3). Positive controls (Methyl methanesulphonate and Cyclophosphamide) 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.

Table 3. Results of *in vitro* chromosome aberration assay.

		-S-9				+ S-9			
Treat-ment	Concen t-ration (µg/ml)	Mito tic inde x	% cells with aberra t-ions	% cells with structural & numerica l aberratio ns	% cells with structur al aberrati -ons	Mitoti c index	% cells with aberr ation s	% cells with structural & numerica l aberratio ns	% cells with structu -ral aberrat -ions
Control	0	2.6	4.5	2.5	1	1.9	2	0	0
SP388	2113	2.2	3	2	1	2.3	4	0.5	0
SP388	3250	2.3	3	1.5	0.5	2.6	3.5	1.5	0.5
SP388	5000	2.5	2.5	2	0.5	2.7	4	1	1
MMS	75	-	40	38	38				
CPA	12.5					-	68	64	64

7 Conclusions

Lipase produced from both *A. oryzae* and *R. miehei* have already been shown to be safe for use as processing aids for food. This assessment of the lipase produced by *A. oryzae* carrying the lipase gene from *R. miehei* found that:

- Both source and donor organisms have a long history of safe use;
- The lipase gene is stably integrated into the host genome;
- The enzyme preparation contains no contaminants of toxicological significance;
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;
- The NOEL from the sub-chronic rat feeding study is 1600 ppm (120.6 mg/kg bw/day).

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

8 References

Barbesgaard, P., Heldt-Hansen, H., and Diderichsen, B. (1992) On the safety of *A. oryzae:* a review. Appl Microbiol Biotechnol, 36: 569-572.

Broadmeadow, A., Clare, C. and de Boers, A. (1994) An overview of the safety evaluation of the *R miehei* lipase enzyme. Food Additives and Contaminants, 11(1): 105-119.

FCC (1996) Food Chemicals Codex 4th Edition, 1996.
Gopinath, C., Prentice, D. & Lewis, D (1987) In: Atlas of Experimental Toxicological Pathology, M.T.P. Press Ltd., 13, 11-21.

FAO (1992) General Specifications for Enzyme Preparations. Compendium of Food Additives Specifications, Vol. 1, Annex 1.

Rogers, R. W. (1977) Monograph on Microbial Enzymes. Informatics Inc. Rockville, MB, Prepared for the Food and Drug Administration, Washington DC, 1977.