

01/03
21 August 2002

DRAFT ASSESSMENT REPORT

APPLICATION A458

GLUCOSE OXIDASE AS A PROCESSING AID

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:
18 September 2002
(See “Invitation for Public Submissions” for details)

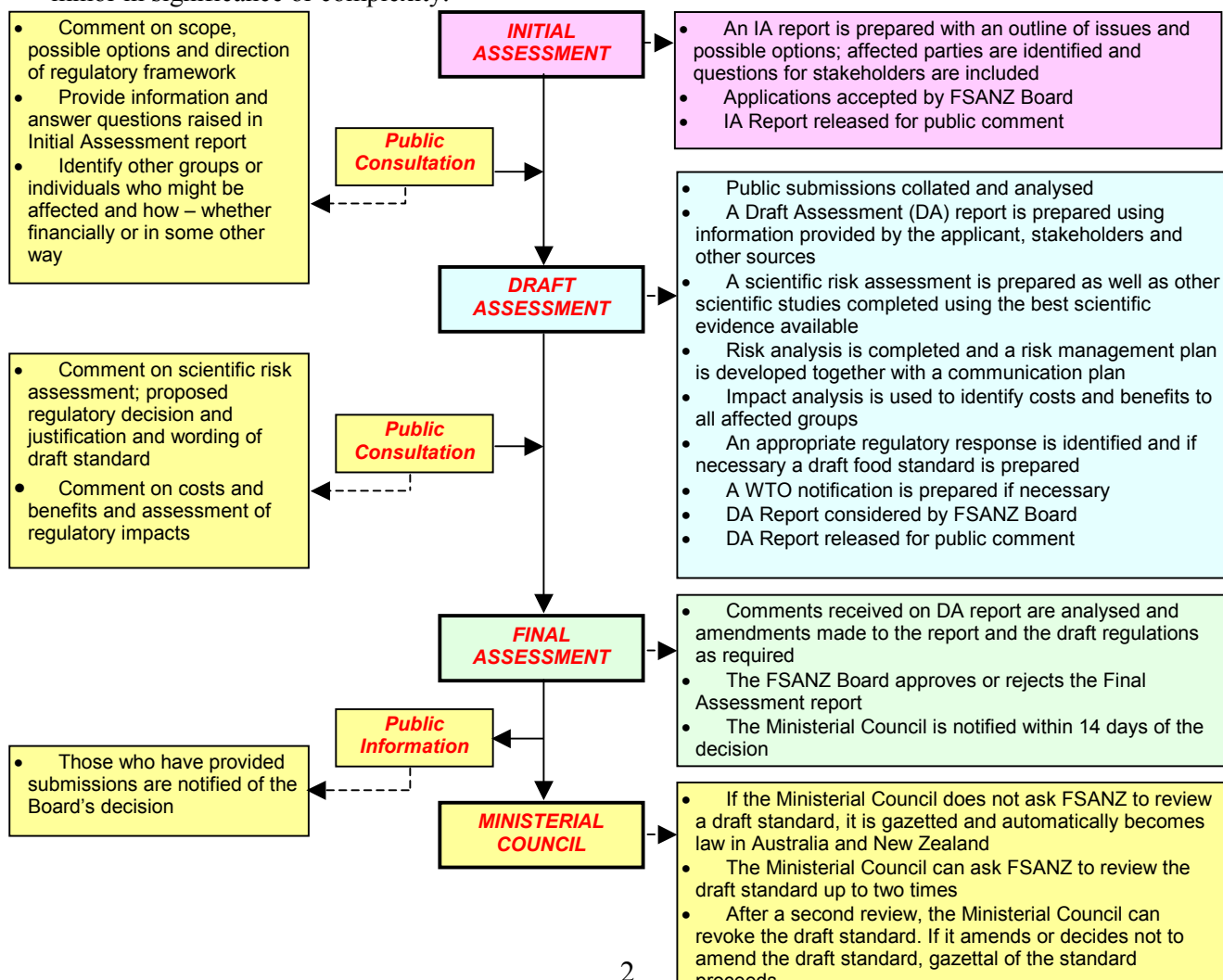
FOOD STANDARDS AUSTRALIA NEW ZEALAND

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia FSANZ also develops food standards for food safety, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

The Authority has prepared a Draft Assessment Report of Application A458 – Glucose oxidase as a processing aid; and prepared a draft variation to Volumes 1 and 2 of the *Food Standards Code*.

The Authority invites public comment on this Draft Assessment Report, the draft variation to Volumes 1 and 2 of the *Food Standards Code*; and the Regulation Impact Statement for the purpose of preparing an amendment to the *Food Standards Code* for approval by the FSANZ Board.

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

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

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

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

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

Submissions should be received by **18 SEPTEMBER 2002**.

Submissions received after this date may not be considered unless the Project Manager has given prior agreement for an extension. Submissions may also be sent electronically through the FSANZ website using the [Food Standards](#) tab and then through [Documents for Public Consideration](#). Assessment reports are available for viewing and downloading from the FSANZ website or alternatively paper copies of reports can be requested from the Authority’s Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au including other general enquiries and requests for information. Questions relating to making submissions or the application process can be directed to the Standards Liaison Officer at the above address or by emailing slo@foodstandards.gov.au.

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Executive Summary

The Australia New Zealand Food Authority (ANZFA - now Food Standards Australia New Zealand (FSANZ)) received an application from Novozymes A/S to amend the Australia New Zealand *Food Standards Code* to approve the use of the enzyme, glucose oxidase, as a processing aid under Standard A16 (Volume 1) and Standard 1.3.3 (Volume 2) of the *Food Standards Code*. The enzyme was produced using recombinant DNA techniques from the host bacterial strain, *Aspergillus oryzae*, and contains a donor gene coding for glucose oxidase from *Aspergillus niger*. There is already a permission in the *Food Standards Code* for glucose oxidase [1.1.3.4] sourced from *Aspergillus niger*.

The Application was received on 26 October 2001 and work was commenced on 12 December 2001.

A total of 4 submissions were received in response to the first round of public consultation at Initial Assessment – two were supportive and two were not supportive. The main issues raised in the submissions that were not supportive were (i) the labelling of processing aids obtained from genetically modified organisms (GMOs), (ii) lack of technological justification and (iii) the similarity to previous applications.

The scientific evaluations concluded that approval of the use of glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*, is technologically justified and poses no significant risk to public health and safety. None of the section 10 objectives in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act) are compromised by the proposed change to the *Food Standards Code*. It is recommended that the draft variation should come into effect on the date of gazettal.

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.

FSANZ will now seek further input from the public during the second round of public consultation before Final Assessment.

Regulatory Problem

Enzymes used in food preparations are regulated as processing aids under Standards A16 and 1.3.3 of the *Food Standards Code* and require pre-market safety assessment and approval before use. Glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*, is currently not approved in Australia and New Zealand (under Standard A16 (Volume 1) and Standard 1.3.3 (Volume 2)).

Objective

To determine whether the *Food Standards Code* can be changed to permit the use of glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*.

Issues considered during assessment of the Application

A range of issues were considered during the assessment; namely, the safety and technological need for the processing aid.

Options

FSANZ identified two options, namely:

1. Not to permit the use of glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*; or
2. Permit the use of glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*.

Impact Analysis

- Option 1, which supports *status quo* by not giving specific permission in the *Food Standards Code* for the use of this enzyme, has no perceived benefits to the stakeholders, government, consumers and industry.
- Option 2, which supports approval to allow an alternative safe source of glucose oxidase with no additional costs to government, industry or consumers, is the preferred option.

Consultation

The Application was released for the first round of public consultation following Initial Assessment from 13 March 2002 until 24 April 2002.

FSANZ is seeking public comment in order to assist in assessing this application at Final Assessment. Comments that would be useful could cover:

- Safety of glucose oxidase of from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*.
- Technological need for of glucose oxidase from genetically modified host bacterial strain, *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger*.

Statement of Reasons

The Statement of Reasons explains the basis of the Authority's decision to adopt draft variations to Standard A16 of Volume 1 and Standard 1.3.3 of Volume 2 of the *Food Standards Code*. The variations concern the inclusion of a new source of the enzyme glucose oxidase for use as a processing aid in the food industry.

The Draft variation to the *Food Standards Code* is recommended because:

- The safety evaluation of the glucose oxidase produced by *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger* found that, the source organism has a long history of safe use, the glucose oxidase gene is stably integrated into the host organisms, the enzyme preparation complies with the Joint Expert Committee on Food Additives (JECFA) specifications and there are no public health and safety concerns associated with the enzyme preparation.
- Use of glucose oxidase sourced from *Aspergillus oryzae*, that carries a gene coding for glucose oxidase isolated from *Aspergillus niger* is technologically justified.
- The proposed changes to Volume 1 and Volume 2 of the *Food Standards Code* are consistent with the section 10 objectives of the FSANZ Act.
- The Regulatory Impact Statement showed that for *Aspergillus oryzae*, carrying a gene coding for glucose oxidase isolated from *Aspergillus niger*, the benefits outweighed the costs in relation to the proposal to amend Standard 1.3.3 – Processing Aids. Approval would allow an alternative safe source of glucose oxidase with no additional costs to government, industry or consumers.

The Australia New Zealand Food Authority (ANZFA) to FSANZ transitional requirements for an application/proposal at preliminary (initial) stage provide that FSANZ is taken to have made an initial assessment of the Application. Any submissions received by ANZFA about an Application in response to a notice given under section 13A or 14 of the *Australia New Zealand Food Authority Act 1991*, are taken to be submissions made to FSANZ about the application/proposal in response to a notice under section 13A or 14 under the *Food Standards Australia New Zealand Act 1991*.

1. INTRODUCTION

Food Standards Australia New Zealand (FSANZ) is a bi-national statutory body responsible for developing draft food standards and draft variations of standards, in order to make recommendations to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council), and to review standards. The Ministerial Council then may decide to adopt the standards or draft variations of standards, which results in their incorporation into food laws of the Australian States and Territories and New Zealand.

On 24 November 2000, the Ministerial Council adopted the *Australia New Zealand Food Standards Code* (known as Volume 2 of the *Food Standards Code*) that applies both in Australia and New Zealand. A two-year transitional period has been implemented at the conclusion of which Volume 2 of the *Food Standards Code* will be the sole code for both countries. In the interim, for the majority of food standards, there are two standards operating in Australia and three in New Zealand (including the New Zealand Food Regulations).

The application from Novozymes A/S is seeking to amend Standard A16 (Volume 1) and Standard 1.3.3 of the joint *Australia New Zealand Food Standards Code* (Volume 2) to approve a new source of the enzyme, glucose oxidase (EC 1.1.3.4), as a processing aid.

1.2 Transitional Requirements

This Application reached preliminary (initial) assessment stage under the operation of the *Australia New Zealand Food Authority Act 1991* (ANZFA Act), and will be finalised in accordance with the provisions of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act).

FSANZ therefore:

1. is taken to have made an initial assessment of the application; and
2. any submissions made to ANZFA about the application in response to a notice under section 13A or 14 have effect as if those submissions had been made to FSANZ in response to a notice under section 13A or 14 under the FSANZ Act.

2. REGULATORY PROBLEM

Standards A16 (Volume 1) and Standard 1.3.3 (Volume 2) of the *Food Standards Code* make 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. There is currently no permission for the use of glucose oxidase sourced from *Aspergillus oryzae* which carries a gene coding for a glucose oxidase isolated from *Aspergillus niger*.

3. OBJECTIVE

The objective of this assessment is to determine whether to amend the *Food Standards Code* and permit the use of another source of the enzyme glucose oxidase. Such an amendment to the *Food Standards Code* will need to be consistent with the section 10 objectives of FSA NZ Act.

In developing or varying a food standard, FSA NZ is required by its legislation to meet three primary objectives which are set out in Section 10 of the *Food Standards Australia New Zealand Act 1991*. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSA NZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. BACKGROUND

ANZFA (now FSA NZ) received an application (A458) on 26 October 2001, from Novozymes A/S seeking to amend Standard A16 (Volume 1) and Standard 1.3.3 of the recently adopted joint *Australia New Zealand Food Standards Code* (Volume 2) to approve a new source of the enzyme, glucose oxidase (EC 1.1.3.4), as a processing aid. The applicant sought to include a provision for glucose oxidase sourced from a strain of *A. oryzae*, and containing a donor gene coding for glucose oxidase from *A. niger*. The application has already completed the Initial Assessment (Preliminary Assessment – section 13) stage and has now progressed to the Draft Assessment.

4.1 Relevant Provisions

Food Standards Code

Standard A16, Table IV- Enzymes, Group III- Microbial Origin (Volume 1) and Standard 1.3.3 - Processing Aids, Table to clause 17 – Permitted Enzymes of Microbial Origin (Volume 2) of the *Food Standards Code* do not include glucose oxidase produced by *Aspergillus oryzae*, carrying the gene coding for glucose oxidase isolated from *Aspergillus niger*.

4.2 Codex

There is no Codex Standard for glucose oxidase produced by *Aspergillus oryzae*, carrying the gene coding for glucose oxidase isolated from *Aspergillus niger*.

4.3 Commercial in Confidence (C-I-C) data

Commercial-in-confidence claims have been made in relation to this application. These relate to the genetic modification and the method of production of the enzyme. Whilst the formal procedure for granting the approval of the CIC request has not been finalised, it is likely that the Authority will grant the applicant's request for CIC data.

5. REGULATORY OPTIONS

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, and the food industry and governments in both Australia and New Zealand. The benefits and costs associated with the proposed amendment to the *Food Standards Code* have been analysed in a Regulatory Impact Assessment.

Parties affected by the options listed below include:

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

The regulatory options currently under consideration are:

Option 1. Not approve the use of glucose oxidase produced by *Aspergillus oryzae*, carrying the gene coding for glucose oxidase isolated from *Aspergillus niger*.

Option 2. Approve the use of glucose oxidase produced by *Aspergillus oryzae*, carrying the gene coding for glucose oxidase isolated from *Aspergillus niger*.

This option would allow an alternative safe source of glucose oxidase with no cost to government, industry or consumers.

Approval of glucose oxidase from a new genetically modified source organism would promote international trade and reduce technical barriers to trade, while continuing to protect public health and safety. From the industry point of view, this option will promote fair trade in food and will allow manufacturers to use an alternative source of glucose oxidase.

6. IMPACT ANALYSIS

The objective of regulatory impact analysis is to examine the impact of the permission to use glucose oxidase from a new source organism, as a processing aid in Standard 1.3.3.

As the use of glucose oxidase from genetically modified source organism *A. niger* requires pre-market approval it is not appropriate to consider non-regulatory options to address this application. Processing aids used in Australia and New Zealand are required to be listed in Standard 1.3.3. – Processing Aids.

There are no perceived benefits to the stakeholders, government, consumers and industry, by maintaining the *status quo* and not giving specific permission in the *Food Standards Code* for the use of this enzyme.

Although there is no perceived cost for the government at present, if, in the future, other countries approve glucose oxidase 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 would also suffer from the non-availability of an alternative source of glucose oxidase.

Parties disadvantaged by not permitting this particular processing aid, are the manufacturers of glucose oxidase and producers who wish to use it in the manufacture of their final food products.

Option 2, which supports the use of glucose oxidase produced by *Aspergillus oryzae* carrying the glucose oxidase gene from *Aspergillus niger* is the preferred option, as approval would allow an alternative safe source of glucose oxidase with no cost to government, industry or consumers.

7. CONSULTATION

7.1 Public Consultation

One round of public consultations has been carried out. During the first round, the Initial Assessment report for A458 was released for public comment between 13 March 2002 and 28 April 2002. Four submissions were received in response to the public consultation. Two submitters supported the proposal to amend the *Food Standards Code* to widen the existing permission for glucose oxidase. Two submitters disagreed with the application and proposed that the *status quo* be maintained. A table elaborating the comments from public submissions from the two rounds is included as an attachment to this report (Attachment 2).

FSANZ is now seeking public comment in order to assist in assessing this application at Final Assessment. Comments that would be useful could cover:

- Scientific aspects of this application;
- Other issues.

Under the transitional provisions these submissions which were made to ANZFA have effect as if they had been made to FSANZ.

7.2 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).

It is considered that this change to the *Food Standards Code* is a liberalising measure under the Sanitary/Phytosanitary Agreement. Matters raised in this Draft Assessment therefore will be notified to the WTO.

8. ISSUES ADDRESSED DURING ASSESSMENT

8.1 Safety of glucose oxidase from genetically modified *Aspergillus oryzae* containing a donor gene coding from glucose oxidase from *Aspergillus niger*.

Application A458 to approve the use of glucose oxidase from a genetically modified microorganism involves the use of two organisms - *Aspergillus oryzae* (the source organism) and *Aspergillus niger* (the donor organism). Both *Aspergillus oryzae* and *Aspergillus niger* are currently listed in Standard 1.3.3- Processing Aids as a microorganisms permitted for use in the production of certain enzymes, and have a history of safe use.

There are no nutritional issues associated with the use of glucose oxidase produced using recombinant DNA technology. The enzyme is used as a processing aid only, and is not expected to be present in the final food as a result of its proposed food uses. If a residue did occur in the food it would be in the form of inactivated enzyme, and in any case would be metabolised like any other protein.

The safety of the source organism is an important consideration in the safety assessment for recombinant glucose oxidase. Both *A. oryzae* and *A. niger* are not considered to be pathogenic, are widely distributed in nature and are commonly found in foods. Enzymes from *A. oryzae* and *A. niger* are extensively used in food processing, and have been for many years. Furthermore, only a limited and well-characterized DNA fragment from the donor strain is used in the construction of the genetically modified strain. In addition, the production strain is not detectable in the final enzyme product and the toxicology data also confirmed the safety of this product. The DNA used for transforming the *A. oryzae* host strain does not contain antibiotic resistant genes.

The genetic modification process involves the transfer of the glucose oxidase gene from *A. niger* to *A. oryzae*. The recombinant organism was found to be stable during production fermentations. Southern blotting technique was used to investigate the stability of the integration of the glucose oxidase 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 arising from the host organism. 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.

Glucose oxidase from the source organism, *A. oryzae* carrying the gene for glucose oxidase from *A. niger* complies with the recommended purity specifications for food-grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)¹.

Two toxicological studies were submitted in support of this application. These consist of a bacterial mutagenicity assay (Ames Test) and an *in vitro* cytotoxicity test : neutral red uptake in L929 monolayer culture. The Ames test was conducted in accordance with the Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals no. 471 (1997).

The assessment of the genetically modified glucose oxidase produced by *A. oryzae* carrying the glucose oxidase gene produced by *A. niger* found that:

- the source organism has a long history of safe use;
- the glucose oxidase gene is stably integrated into the host genome;
- the enzyme preparation complies with the JECFA specifications;
- the enzyme preparation causes no mutagenic or cytogenic effects in *in vitro* studies.

Because the host organism is safe and because the genetic modifications are well characterised and specific utilising well-known plasmids for the vector constructs, and the introduced genetic material does not encode and express any toxic substances, it is concluded that the use of genetically modified glucose oxidase 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 final assessment (Attachment 3).

8.2 Technological Justification

The use of enzyme glucose oxidase as a processing aid in the food industry is technologically justified and is not expected to result in its presence in food. A detailed Food Technology report is attached (Attachment 4).

The applicant has indicated that the enzyme is to be used in the baking industry as a processing aid to strengthen gluten in dough systems. It causes a more elastic and stronger gluten network similar to that obtained by traditional oxidising agents such as potassium bromate or ascorbic acid. The enzyme is active in the dough and the leavening of the unbaked bread, but normally inactivated by high temperatures during the baking. The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

¹ Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992) - FAO (1992) General Specifications for Enzyme Preparations. Compendium of Food Additives Specifications, Vol. 1, Annex 1.

The Applicant has stated that glucose oxidase complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC) 4th Edition, 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992.

8.3 Labelling of food when glucose oxidase is used

Processing aids are not currently required to appear in ingredient lists under general labelling provisions in the *Food Standards Code* and the *New Zealand Food Regulations*. 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 foods produced using gene technology, was decided on at the Australia New Zealand Food Standards Council (now the Australia and New Zealand Food Regulation Ministerial Council) meeting on 28 July 2000. The ANZFSFC decided to exempt processing aids and food additives from labelling requirements except where novel DNA and/or protein from the processing aid or food additive is present in the final food (see clause 4, Standard 1.5.2). While the gene coding for the glucose oxidase enzyme from the donor strain is novel, neither the gene nor the enzyme is expected to be present in the final food.

9. TRANSITIONAL ISSUES

This Application reached Preliminary (initial) Assessment stage under the operation of the ANZFA Act, and will be finalised in accordance with the provisions of the FSANZ Act. Under the transitional provisions, FSANZ is considered to have made an Initial Assessment of the Application under the FSANZ Act. Any submissions received under the ANZFA Act have the same effect as if they had been received under the FSANZ Act.

10. OTHER RELEVANT MATTERS

10.1 Workplan Classification

FSANZ's initial assessment of this proposal for placement on the workplan was Group 2, Category 3 (see FSANZ website for further information about the workplan and the different groups and categories).

11. CONCLUSION AND RECOMMENDATION

The draft assessment report concludes that approval of the use of glucose oxidase from a new source organism is technologically justified and poses no significant risk to public health and safety.

Adoption of the draft variation, giving approval for the use glucose oxidase as a processing aid in Australia and New Zealand, is recommended for the following reasons:

- The safety evaluation of the glucose oxidase produced by *Aspergillus oryzae*, containing a donor gene coding for glucose oxidase from *Aspergillus niger* found that, the source organism has a long history of safe use, the glucose oxidase gene is stably integrated into the host organisms, the enzyme preparation complies with the Joint Expert Committee on Food Additives (JECFA) specifications and there are no public health and safety concerns associated with the enzyme preparation.
- Use of glucose oxidase sourced from *Aspergillus oryzae*, that carries a gene coding for glucose oxidase isolated from *Aspergillus niger* is technologically justified.
- The proposed changes to Volume 1 and Volume 2 of the *Food Standards Code* are consistent with the section 10 objectives of the FSANZ Act.
- The Regulatory Impact Statement showed that for *Aspergillus oryzae*, carrying a gene coding for glucose oxidase isolated from *Aspergillus niger*, the benefits outweighed the costs in relation to the proposal to amend Standard 1.3.3 – Processing Aids. Approval would allow an alternative safe source of glucose oxidase with no additional costs to government, industry or consumers.

ATTACHMENTS

- 1 Draft Variations to the *Food Standards Code*.
- 2 Summary of Public Submissions.
- 3 Toxicological Report.
- 4 Food Technology Report

ATTACHMENT 1

DRAFT VARIATIONS TO *FOOD STANDARDS CODE*

To commence: On gazettal

[1] *Standard A16 of Volume 1 is varied by –*

[1.1] *inserting in Table IV – Enzymes, Group III – Microbiological Origin, as a source for the enzyme Glucose oxidase EC [1.1.3.4] –*

<i>Aspergillus oryzae</i> ¹⁴

[1.2] *inserting in the footnotes to Table IV – Enzymes, Group III – Microbiological Origin –*

¹⁴Glucose oxidase may be produced from a genetically manipulated strain of *Aspergillus oryzae* containing the gene for glucose oxidase isolated from *Aspergillus niger*.

[2] *Standard 1.3.3 of Volume 2 is varied by inserting in the Table to clause 17, as a source for the enzyme Glucose oxidase [1.1.3.4] -*

<i>Aspergillus oryzae</i> , containing the gene for glucose oxidase isolated from <i>Aspergillus niger</i>
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ATTACHMENT 2

SUMMARY OF PUBLIC SUBMISSIONS RECEIVED A458 – GLUCOSE OXIDASE AS A PROCESSING AID

Round One

No.	Organisation	Position	Comments
1	National Council of Women of Australia	Supports Option 1	Considers that without labelling the genetically engineered processing aid will deceive public. Considers that this application is not technologically justifiable as other non-GE glucose oxidase is available. Use of this glucose oxidase should not be approved because of its similarity to other applications.
2	Consumers' Association of South Australia Inc.	Supports Option 1	Endorses the views of the National Council of Women of Australia.
3	Food Technology Association, Victoria Inc.	Supports option 2.	Supports this application.
4	Fonterra	Supports option 2	Supports this application.

SAFETY ASSESSMENT REPORT

A458 – GLUCOSE OXIDASE AS A PROCESSING AID

INTRODUCTION

Application A458 to approve the use of glucose oxidase from a genetically modified microorganism involves the use of two organisms – *A. oryzae* (the source organism) and *A. niger* (the donor organism).

The enzyme is to be used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The source (production) organism - *Aspergillus oryzae*

The safety of the source organism is an important consideration in the safety assessment for recombinant glucose oxidase. *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 production of a variety of foods such as syrups, alcohol, fruit juices, brewing, chocolate syrup, baking and meat tenderising , and have been for many years (Rogers, 1977).

The donor organism – *Aspergillus niger*

The organism from which the glucose oxidase gene is derived is *Aspergillus niger*. Glucose oxidase from a non-genetically modified *Aspergillus niger* is already permitted by Standard 1.3.3 of the Australia New Zealand *Food Standards Code* and has been evaluated by JECFA (http://apps3.fao.org/jecfa/additive_specs/docs/0/additive-0206.htm).

Nature of the genetic modification

The genetic modification process involved the transfer of the glucose oxidase gene from *A. niger* to *A. oryzae*. The applicant has provided information to indicate that the recombinant organism was found to be stable during production fermentations. Southern blotting was used to investigate the stability of the integration of the glucose oxidase gene after large-scale fermentation, and found that the inserted DNA was stably integrated into the host genome. The DNA used for transforming the *A. oryzae* host strain does not contain antibiotic resistance genes.

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.

TOXICOLOGY STUDIES

1. Gluzyme, Batch PPX 7029 In Vitro Cytotoxicity test: Neutral Red Uptake in L929 monolayer Culture

Study No.: 20018027

Ninna Berg Study Director, Novozymes. October 23, 2001.

Test Material

Gluzyme, batch PPX 7029, a brown liquid at room temperature

Received from recovery Plant, Novozymes A/S, 27 February 2001

Specific gravity: 1,061 g/ml

Enzyme activity: 4790 GODU-FIA/g; 20600 CIU/g

TOS: 10.7% w/w; pH: 7.0; Osmolality: 311 mOsm/kg

Study initiated: 6 March 2001

Study Completed: 14 March 2001

Study performed according to GLP described in OECD Principles of GLP, Dok. C(81) 30 (1981)

Test Method

The purpose of the study was to screen for the cytotoxic potential of the present enzyme preparation. The basis of the test system is that a cytotoxic substance regardless of site or mechanism of action will interfere with the viability and growth of the continuously dividing fibroblasts and result in a reduction of the cell number. The degree of inhibition of growth, related to the concentration of the test substance, provides an indication of toxicity.

The neutral red uptake assay is a quantitative, colorimetric method to measure the cell viability. Neutral red is actively taken up by the cells and retained in the lysosomes/endosomes. The amount of neutral red taken up by the cells after exposure to the test substance is an indication of the number of viable cells and thus provides a measure of general toxicity.

L929 is an established mouse fibroblast cell line, selected for the ease for which these cells are maintained and grown as monolayer culture. It is commonly used as the first order test system for general cytotoxicity. L929 was grown in EMEM with 10% foetal calf serum (FCS). Cell culture (150 µl – 5x10⁵ cells per well) was added to 96-well microplates and incubated for 24 hours at 37°C to establish a near confluent monolayer.

Gluzyme was added neat at concentrations of 300, 1,000, 3,000, 10,000 and 30,000 µg/ml growth medium (EMEM 10% FCS). The positive control was 80, 100 and 120 µg SDS per ml growth medium. The information time of exposure was 24 h.

Results

The concentration of the test substance required to reduce the viability of the treated test system to 50% of that of the untreated control test system was determined as the endpoint (NRU₅₀). The NRU₅₀ value for gluzyme, batch PPX 7029, was estimated to be >30 mg/ml. The positive control (SDS) met the criteria of a valid test.

The results (Table 1) indicate that the sample of gluzyme is non-cytotoxic *in vitro* in the Neutral red Uptake assay in the mouse fibroblast cell line L929.

Table 1

	Concentration µg/ml	Viability %	NRU ₅₀ mg/ml
Gluzyme Batch PPX 7029	0	100	>30
	300	82	
	1000	75	
	3000	75	
	10,000	75	
	30,000	71	
	Concentration µg/ml	Viability %	NRU ₅₀ mg/ml
SDS	0	100	96
	80	92	
	100	39	
	120	1	

2. Gluzyme (Batch No: PPX 7029) Test for Mutagenic Activity with Strains of *Salmonella typhimurium* and *Escherichia coli*. PB Pedersen and A Bergman. Department of Toxicology, Novozymes A/S Krogshøjvej 36, DK-2880 Bagsvaerd

Test Material

Gluzyme, batch PPX 7029, a brown liquid at room temperature with a 13:1 % (w/w) dry matter. Gluzyme was inactivated by heat treatment for 30 mins at 60°C at PH 2 and subsequent adjustment to neutral pH. Thereafter it was diluted in deionised water corresponding to a final concentration of 5% w/v (dry matter). This solution was sterilised by filtration through a 0.8 µm, 0.45 µm and 0.2 µm membrane filter, successively. The sterility was confirmed by plate counting.

Received from recovery Plant, Novozymes A/S, 27 February 2001

Study initiated: 22 March 2001

Study Completed: 4 April 2001

The study was completed in compliance with OECD Guideline for testing of chemicals, No. 471: Bacterial Reverse Mutation Assay (July 1997), European Commission Annex V. Test Method B14, and the Japanese guideline: “Concrete Operation Procedure of Mutagenicity Study Using Bacteria. Ministry of Labour, 1988”

Test Method

Gluzyme (Batch: PPX 7029) was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA in accordance with OECD Guidelines (No. 471, July 1997). Crude enzyme preparations like gluzyme contain free amino acids. This condition is not consistent with the test principle of a “plate incorporation assay” if the amount of histidine or tryptophan in the test substance exceeds a critical concentration. In preliminary investigations the applicant states that Gluzyme significantly increased the growth of the histidine requiring *Salmonella* strains following direct plate incorporation. Further Gluzyme caused pronounced cytotoxicity and dose related increases of induced mutations, when a plate incorporation assay as well as a liquid culture assay (“treat and plate”) is applied. The principal enzyme activity of Gluzyme is a glucose oxidase. In the presence of glucose hydrogen peroxide is produced by the catalytic action of glucose oxidase. Hydrogen peroxide is a well known cytotoxic and mutagenic compound *in vitro*. Therefore, in this study the glucose oxidase was inactivated by the heat treatment for 30 minutes at 60°C at pH 2 and subsequent adjustment to neutral pH.

To overcome the problems caused by free histidine in the test substance all Salmonella strains were exposed to Gluzyme in liquid culture assay, and bacteria exposed to six doses of the test substance in a phosphate buffered broth for three hours with 5 mg/ml as the highest concentration. After incubation the test substance was removed by centrifugation, plated, and the number of both revertants to prototrophy and viable cells estimated.

The part of the study comprising *E. coli* was conducted using the direct plate incorporation assay. Six doses of the test substance were applied with 5 mg/plate (dry matter) per plate as the highest dose level followed by successive bi-sections between doses.

The study was carried out both in the presence and absence of metabolic activation (in the form of a liver preparation, S-9, pre-treated with Aroclor 1254, 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, N-methyl-N-nitro- N-guanidine, N-ethyl-N-nitro-N-guanidine, and 2-Nitrofluorene).

Results

No dose-related or reproducible increases in revertants to prototrophy were obtained with any of the bacterial strains exposed to Gluzyme (Batch PPX 7029) either in the presence or absence of metabolic activation. A repeat experiment confirmed these results. It was concluded that the test material Gluzyme did not exhibit any mutagenic activity under the conditions of the test.

CONCLUSION

The assessment of the genetically modified glucose oxidase produced by *A. oryzae* carrying the glucose oxidase gene produced by *A. niger* found that:

- the source organism has a long history of safe use;
- the glucose oxidase gene is stably integrated into the host genome;
- the enzyme preparation complies with the JECFA specifications;
- the enzyme preparation causes no mutagenic or cytogenic effects in *in vitro* studies.

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

FOOD TECHNOLOGY REPORT

GLUCOSE OXIDASE AS A PROCESSING AID

An application has been received from Novozymes A/S to amend the Australia New Zealand *Food Standards Code* to approve the use of the enzyme, glucose oxidase, as a processing aid under Standard A16 (Volume 1 – *Food Standards Code*) and Standard 1.3.3 (Volume 2 – Australia New Zealand *Food Standards Code*). The enzyme was produced using recombinant DNA techniques from the host bacterial strain, *Aspergillus oryzae*, and contains a donor gene coding for glucose oxidase from *Aspergillus niger*.

Glucose oxidase from a non-genetically modified *Aspergillus niger* is already permitted by Standard 1.3.3 of the Australia New Zealand *Food Standards Code* and has been evaluated by the Joint FAO/WHO Expert Committee for Food Additives (JECFA) for safety.

The applicant has indicated that the enzyme is to be used in the baking industry as a processing aid to strengthen gluten in dough systems. Its use results in a more elastic and stronger gluten network similar to that obtained by traditional oxidising agents such as ascorbic acid. The enzyme is active in the dough and the leavening of the unbaked bread during proofing, but normally inactivated by high temperatures during the baking. The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

Oxidising agents increase dough consistency after mixing and development. The gluten (coiled molecular complex) becomes stretched and ruptured by mechanical energy imparted at mixing, following enzymatic action with the onset of fermentation. Bonds become broken and disulphide links combine with hydrogen. The presence of oxidants in adequate concentrations then reform the disulphide links, conferring strength and elasticity to the gluten structure, the process being essentially an electron transfer. Oxidation requirements increase with milling extraction, owing to location and access to active groups in the wheat kernel. The sulphhydryl groups, occurring in large amounts in the aleurone layer and in the germ rather than in the endosperm.

Under-oxidized doughs tend to be weak, soft, sticky and extensible, making them difficult to machine and process. The finished loaves have reduced volume, weak crusts, uneven grain and texture, poor break and shred and loss of symmetry. Over-oxidized doughs are tight, firm, bucky and difficult to mould, tearing easily. They break on proofing owing to their inelasticity. Bread will be small-volumed, with a rough break and shred, uneven grain and large holes.

Glucose oxidase complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC) 4th Edition, 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992 (see Table 1.)

Glucose oxidase is listed in a compilation of microbially derived enzymes which the FDA recognized as GRAS in opinion letters issued in the early 1960's. The opinions are predicated on the use of non-pathogenic and non-toxicogenic strains of the respective organisms and on the use of current good manufacturing practice.

Table 1. GLUCOSE OXIDASE AND CATALASE FROM ASPERGILLUS NIGER VAR.

Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992)

SYNONYMS	1. Glucose oxyhydrase, glucose aerodehydrogenase, notatin, aero-glucose dehydrogenase; INS No. 1102 2. None
SOURCES	Commercial enzyme preparations are produced by the controlled fermentation of <i>Aspergillus niger</i> var.
ACTIVE PRINCIPLES	1. Glucose oxidase 2. Catalase
SYSTEMATIC NAMES AND NUMBERS	1. β-D-glucose: oxygen 1-oxidoreductase (EC 1.1.3.4) 2. Hydrogen-peroxide: hydrogen-peroxide oxidoreductase (EC 1.11.1.6)
REACTIONS CATALYZED	1. β-D-glucose + O ₂ --> D-glucono-delta-lactone + H ₂ O ₂ 2. H ₂ O ₂ + H ₂ O ₂ --> 2H ₂ O + O ₂
SECONDARY ENZYME ACTIVITIES	Invertase (EC 3.2.1.26)
DESCRIPTION	Off-white to brown liquids; soluble in water and practically insoluble in ethanol, chloroform and ether
FUNCTIONAL USES	Enzyme preparation Used in the preparation of and/or use in milk, cheese, eggs, beverages and salads
GENERAL SPECIFICATIONS	Must conform to the <i>General Specifications for Enzyme Preparations used in Food Processing</i>

Conclusion

Glucose oxidase from a non-genetically modified *Aspergillus niger* is already permitted by Standard 1.3.3 of the Australia New Zealand *Food Standards Code* and has been evaluated by JECFA for safety.

The use of the enzyme glucose oxidase as a processing aid is technological justified to aid to strengthen gluten in dough systems.

Reference

Stear C.A. Handbook of breadmaking technology. Elsevier Applied Science. London 1990.