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

**06/03**
**18 December 2002**

# FINAL ASSESSMENT REPORT

# APPLICATION A458

# GLUCOSE OXIDASE AS A PROCESSING AID

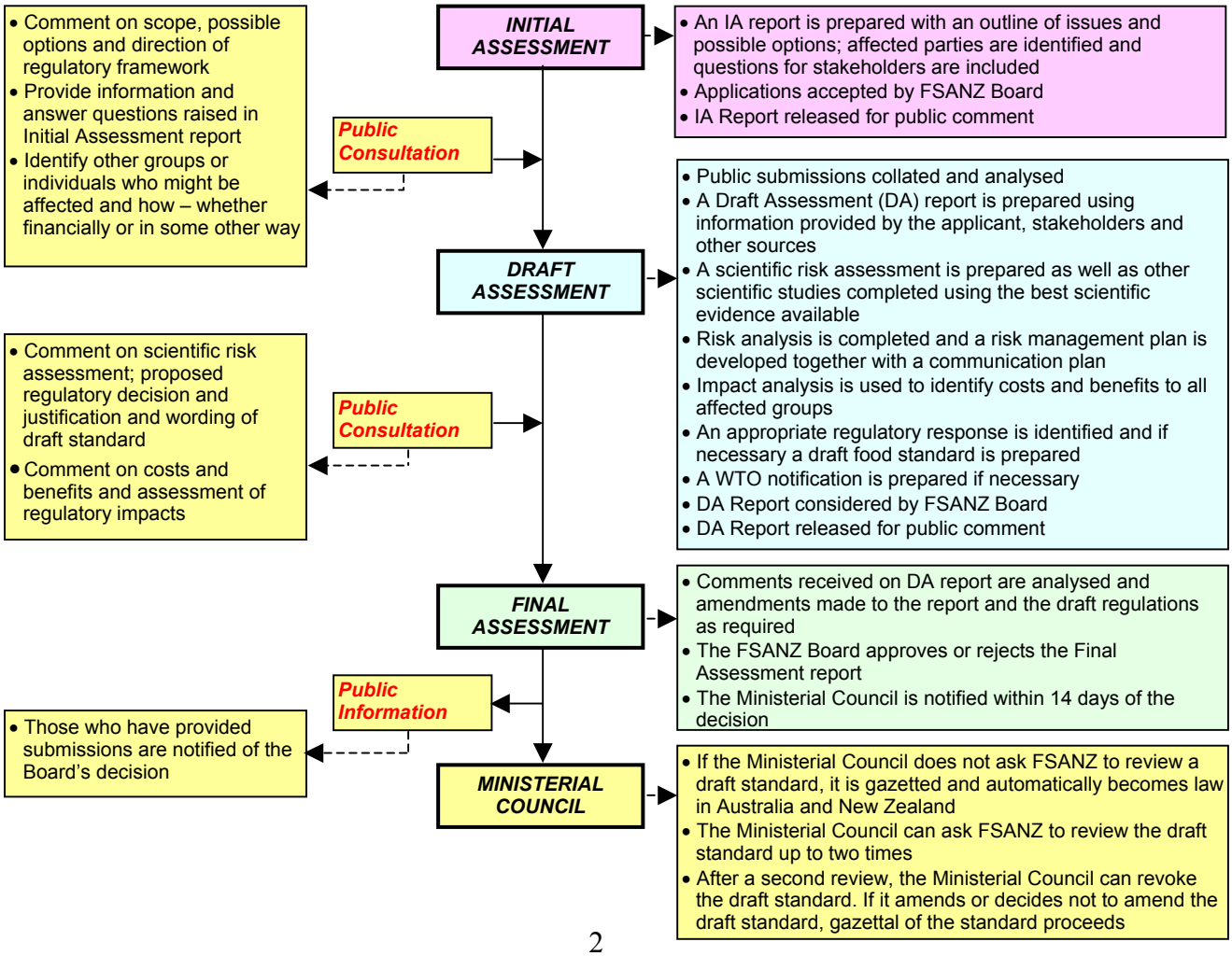
## FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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.



## Final Assessment Stage

The Authority has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this application. This Final Assessment report and its recommendations have been approved by the FSANZ Board and are now being reviewed by the Australia and New Zealand Food Regulation Ministerial Council (ANZFRMC). If accepted by ANZFRMC, a change to the *Food Standards Code* is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand the New Zealand Minister for Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

## Further Information

### Submissions

No submissions on this matter are sought as the Authority has completed its assessment and the matter is now with the Australia and New Zealand Food Regulation Ministerial Council for consideration.

Further information on this application and the assessment process should be addressed to the Standards Liaison Officer at Food Standards Australia New Zealand at 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**
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Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from the Authority's Information Officer at info@foodstandards.gov.au including other general enquiries and requests for information.

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## Executive Summary

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 – *Food Standards Code*) and Standard 1.3.3 (Volume 2 – Australia New Zealand *Food Standards Code*). The enzyme was produced from a new source 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. The Australia New Zealand Food Authority (ANZFA) to FSANZ transitional requirements for an application at Full Assessment (Draft) stage have been followed. The Authority has not been notified of any ministerial Council policy guidelines relevant to this application.

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. One late submission was received from New Zealand Ministry of Health.

Three submissions were received during the second round of public consultation at Draft Assessment– all three were supportive.

Only two regulatory options were considered: to approve or not approve the new source of the enzyme, glucose oxidase. The option to approve the new source of the enzyme was considered appropriate because the donor and host organisms, and the new enzyme were demonstrated to be safe, and the enzyme is technologically justified.

**Statement of Reasons**

The Statement of Reasons forms the basis for the Authority's decision to adopt draft variations to 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 Authority agreed to adopt the draft variation to the *Food Standards Code* 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 donor and the source organisms have 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 change to Volume 2 of the *Food Standards Code* is 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.

## 1. Introduction

Food Standards Australia New Zealand (FSANZ) is a bi-national statutory body responsible for the development and approval of food standards and variations to standards in accordance with policy parameters set by the Australia New Zealand Food Regulation Ministerial Council (Ministerial Council). FSANZ is also responsible for notification of the approval of a food standard or variation to a food standard to the Ministerial Council, as well as the review of a proposed or existing standard or variation at the request of the Council.

The role of the Ministerial Council is to set policy guidelines for the development of food standards. The Council may also request that FSANZ review a proposed or existing standard.

On 24 November 2000, the Australia New Zealand Food Standards Council adopted the *Australia New Zealand Food Standards Code* (known as Volume 2 of the *Food Standards Code*) that applies in both 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 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.

## 2. Regulatory Problem

Standard 1.3.3 (Volume 2) of the *Food Standards Code* 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. 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 application is to determine whether the food regulatory measures should be changed to approve the use of another source of the enzyme glucose oxidase. Any such an amendment to the *Food Standards Code* would need to be consistent with the section 10 objectives of FSANZ Act. FSANZ's primary objectives in developing and varying food standards (in descending priority order) are:

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

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

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

# 4. Background

FSANZ received an Application (A458) on 26 October 2001, from Novozymes A/S seeking to amend Standard 1.3.3 of the *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*.

This Application reached Preliminary (Initial) Assessment stage under the operation of the *Australia New Zealand Food Authority Act 1991* and has been finalised in accordance with the provisions of the FSANZ Act. Volume 1 drafting is therefore excluded from this Report.

## 4.1 Relevant Provisions

Standard 1.3.3 - Processing Aids, Table to clause 17 – Permitted Enzymes of Microbial Origin (Volume 2) of the *Food Standards Code* does 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 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.

# 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.

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.*

# 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.

## 6.1 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 of glucose oxidase with no additional costs to government, industry or consumers, is the preferred option.

## 6.2 Option 1

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.

### 6.3 Option 2

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.

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

Two rounds of public consultations have 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). A second round of public consultation during Draft Assessment was held from 18 August 2002 until 21 September 2002. Three submissions received were positive (Attachment 2).

### 7.2 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).

It was considered that this change to the *Food Standards Code* is a liberalising measure under the Sanitary/Phytosanitary Agreement and therefore was not 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.

[1] Prepared at the 25th JECFA (1981), published in FNP 19 (1981), FNP 52 (1992) - FAO (1992) and FNP 52 Addendum 9 2001 (with amendments to the Appendix B to Annex 1), and General Specifications for Enzyme Preparations. Compendium of Food Additives Specifications, Vol. 1, Annex 1.

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:

(c) the source organism has a long history of safe use;
(d) the glucose oxidase gene is stably integrated into the host genome;
(e) the enzyme preparation complies with the JECFA specifications;
(f) 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.

The Applicant has stated that glucose oxidase complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC) 4$^{th}$ 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.

Some submissions to FSANZ have questioned the need for alternative sources of enzymes, in this case whether a further source of glucose oxidase from a genetically modified bacteria is technologically justified. The primary objective for FSANZ under the section 10 objectives of the FSANZ Act is the protection of public health and safety. Glucose oxidase from the new source has been shown to be safe. Under the Section 10 objectives FSANZ must also have regard to "the desirability of an efficient and internationally competitive food industry; and the promotion of fair trading in food". In this regard, the approval of an alternative production system for glucose oxidase provides opportunities for greater flexibility and efficiency for industry. Rejection of the application based on the argument that a new source is not justified would mitigate against the requirements under the section 10 obligations.

### 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. Should it be present in the final food, a labelling requirement will apply.

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. 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. Other Relevant Matters

### 9.1 Work Plan Classification

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

## 10. Conclusions and Recommendations

The Final 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.

The draft variation, giving approval for the use glucose oxidase as a processing aid in Australia and New Zealand, is adopted 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 donor and the source organisms have 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 change to Volume 2 of the *Food Standards Code* is 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 VARIATION TO *FOOD STANDARDS CODE*

**To commence: On gazettal**

[1] ***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 EC [1.1.3.4] –

| |
|---|
| *Aspergillus oryzae,* containing the gene for glucose oxidase isolated from *Aspergillus niger* |

## 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.<br><br>Considers that this application is not technologically justifiable as other non-GE glucose oxidase is available.<br><br>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. |
| 5 | **NZ Ministry of Health** | No comment on option supported | Commented that the most recent version of the JECFA specifications should be reference. This issue has now been addressed in the Final Assessment. Also commented that the report should make clear that the benefits of the new enzyme accrue to the applicant. This was made clear in the draft Assessment report. |

**Round Two**

| No. | Organisation | Position | Comments |
|---|---|---|---|
| 1 | **AFGC** | Supports Option 2 | Supports this application; Believes that enzyme from new source is technologically justified |
| 2 | **Department of Health, Western Australia** | Supports Option 2 | Supports this application |
| 3 | **Food Technology Association, Victoria Inc.** | Supports option 2. | Supports this application. |

## Attachment 3

### 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 – *Aspergillus. 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 *A. niger*. Glucose oxidase from a non-genetically modified *A. 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 cytoxic 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 – $5x10^5$ cells per well) was added to 96-well microplates and incubated for 24 hours at $37^oC$ 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 ($NRU_{50}$). The $NRU_{50}$ 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<br>300<br>1000<br>3000<br>10,000<br>30,000 | 100<br>82<br>75<br>75<br>75<br>71 | >30 |
| | Concentration µg/ml | Viability % | $NRU_{50}$ mg/ml |
| SDS | 0<br>80<br>100<br>120 | 100<br>92<br>39<br>1 | 96 |

**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 Krogshojvej 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$^{o}$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:

(g) the source organism has a long history of safe use;
(h) the glucose oxidase gene is stably integrated into the host genome;
(i) the enzyme preparation complies with the JECFA specifications;
(j) 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 poses no public health and safety risk.

**Attachment 4**

## 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 sulphydryl 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) 4$^{th}$ 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<br>2. None |
| SOURCES | Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus niger* var. |
| ACTIVE PRINCIPLES | 1. Glucose oxidase<br>2. Catalase |
| SYSTEMATIC NAMES AND NUMBERS | 1. ß-D-glucose: oxygen 1-oxidoreductase (EC 1.1.3.4)<br>2. Hydrogen-peroxide: hydrogen-peroxide oxidoreductase (EC 1.11.1.6) |
| REACTIONS CATALYZED | 1. ß-D-glucose + $O_2$ --> D-glucono-delta-lactone + $H_2O_2$<br>2. $H_2O_2$ + $H_2O_2$ --> $2H_2O$ + $O_2$ |
| 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<br>Used in the preparation of and/or use in milk, cheese, eggs, beverages and salads |
| GENERAL SPECIFICATIONS | Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* |

**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.