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

09/03
21 May 2003

FINAL ASSESSMENT REPORT

APPLICATION A467

ALPHA-AMYLASE AS A PROCESSING AID (ENZYME)

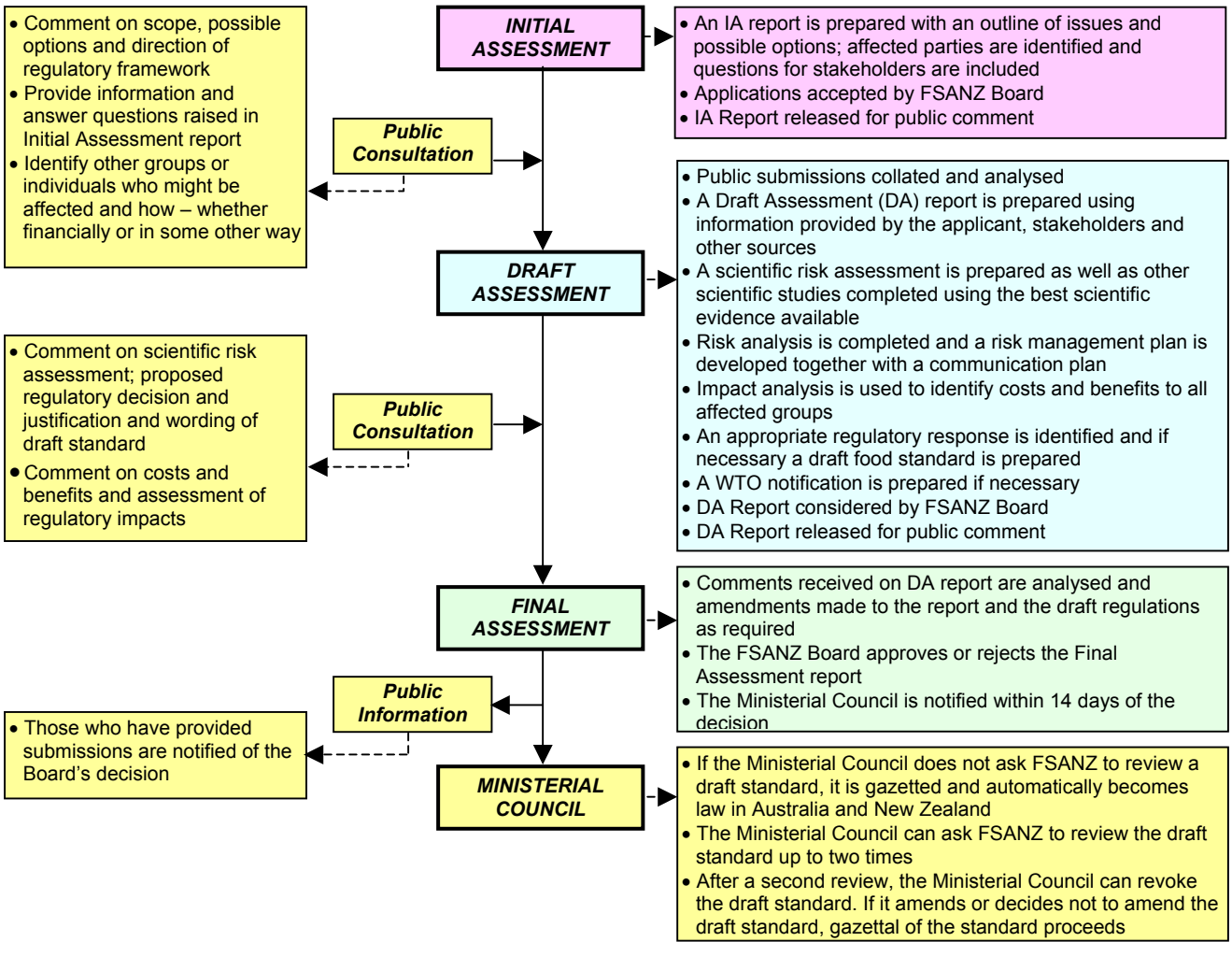
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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, maximum residue limits, 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 and 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 *Australia New Zealand 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 notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council).

If the Ministerial Council does not request FSANZ to review the draft amendments to the *Australia New Zealand Food Standards Code*, an amendment to the 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 Food Safety gazettes the food standard under the *New Zealand Food Act (1981)*. 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

Further information on this Application and the assessment process should be addressed to the FSANZ Standards Liaison Officer 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
Tel (04) 473 9942
www.foodstandards.govt.nz

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.

CONTENTS

Executive Summary and Statement of Reasons

FSANZ received a paid application from Genencor International to amend the *Australia New Zealand Food Standards Code* (the Code) to approve the use of an enzyme, *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid. The Application was received on 29 May 2002 and work commenced 9 July 2002.

Alpha-amylase is used as a food enzyme for the hydrolysis of starch in the starch, sugar and alcoholic beverage industries. Genencor's *alpha*-amylase is produced with the use of a non-genetically modified strain of *Bacillus stearothermophilus*.

Alpha-amylases have been approved and used for many years in food manufacture. There are currently a number of approved sources for *alpha*-amylases listed as processing aids in Standard 1.3.3 – Processing Aids. The Applicant contends that this *alpha*-amylase derived from *Bacillus stearothermophilus* has advantages over other approved enzymes in that it has greater thermal stability, produces a different sugar profile and is not derived from genetically modified organisms.

The purpose of this assessment is to determine whether it is appropriate to amend the Code to permit the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid.

The only regulatory options considered were to approve or not approve this application. Approval of the use of this enzyme has advantages for food manufacturers by providing a different source of the *alpha*-amylase enzyme; one, which has greater thermal stability and produces a different sugar profile. There are no significant disadvantages to food manufacturers, consumers or government agencies.

Public comment on the Initial Assessment Report for this application was sought from 21 August till 2 October 2002. Three submissions were received with all supporting approval of the use of the enzyme – subject to an appropriate safety assessment as part of the Draft Assessment.

Public comment was sought on the Draft Assessment Report from 18 December 2002 till 12 February 2003. Two submissions were received, both of which supported the application.

The Final Assessment Report concludes that approval of the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is technologically justified and does not raise any public health and safety concerns.

Statement of Reasons

The draft variation to Standard 1.3.3 – Processing Aids of the Code, thereby giving approval for the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is recommended for the following reasons.

- There are no public health and safety concerns associated with the use of this enzyme.

- The use of the *alpha*-amylase enzyme derived from *Bacillus stearothermophilus* is technologically justified since it has a role in food manufacturing, primarily with starch

hydrolysis. This enzyme has greater thermostability and a different sugar profile.

- The source organism (*Bacillus stearothermophilus*) has a long history of safe use.

- The *alpha*-amylase enzyme has a history of safe use for many years in Australia and New Zealand.

- The enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* complies with the specifications for enzyme preparations in Food Chemicals Codex (4th Edition, 1996) and the Joint Expert Committee on Food Additives (JECFA) Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992, (updated in Addendum 9, 2001).

- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act.

- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.

1. Introduction

FSANZ received a paid application from Genencor International to amend the *Australia New Zealand Food Standards Code* (the Code) to approve the use of an enzyme, *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid. The Application was received on 29 May 2002 and work commenced 9 July 2002.

Alpha-amylase is used as a food enzyme for the hydrolysis of starch in the starch, sugar and alcoholic beverage industries. Genencor's *alpha*-amylase is produced with the use of a non-genetically modified strain of *Bacillus stearothermophilus*.

2. Regulatory Problem

Processing aids are required to undergo a pre-market safety assessment before approval for use. A processing aid is defined in clause 1 of Standard 1.3.3 and is:

(a) the 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; and

(b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.

There are currently six approved sources of *alpha*-amylase listed in Standard 1.3.3 of the Code, however, *Bacillus stearothermophilus* is not an approved source for *alpha*-amylase. The Table to clause 17 of Standard 1.3.3 list of approved sources of *alpha*-amylase includes recombinant *Bacillus licheniformis* and *Bacillus subtilis*, both containing the gene for *alpha*-amylase isolated from *Bacillus stearothermophilus*.

3. Objective

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of another source of *alpha*-amylase. Such an amendment will need to be consistent with the section 10 objectives of the FSANZ Act.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 of the FSANZ Act. These are:

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

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

- 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

Enzymatic processes including the use of *alpha*-amylase have been used for several decades in place of acid hydrolysis in industrial processes for starch conversion. They have been used since they offer advantages including greater yields, better control and specificity of products, and improved economics because of milder conditions with lower energy requirements.

Alpha-amylase degrades both the branched and unbranched forms of starch and related polysaccharides and oligosaccharides by cleaving the internal *alpha*-1,4 bonds connecting the glucose monomers.

Alpha-amylase is used in the starch, sugar and alcoholic beverage industries. It is used to liquefy starch to produce soluble dextrins, which can be converted further with other enzymes to produce a range of liquid syrups. Such sugar syrups can then be used in a range of foods. *Alpha*-amylase can also be used in baking to supplement natural sources of the enzyme coming from the grain. The enzyme can also be used in the brewing industry to supplement the natural sources of the enzyme during various steps in beer production and also for different specialty beers (such as low-carbohydrate beers).

5. Relevant Issues

Nature of the enzyme

The common name of the enzyme is *alpha*-amylase. The chemical name is 1,4 *alpha*-D-glucan glucanohydrolase with the Enzyme Commission number EC [3.2.1.1] and CAS number 9000-90-2.

The *alpha*-amylase enzyme catalyses the endohydrolysis of 1-4-*alpha*-D-glucosidic linkages in polysaccharides containing three or more 1,4-*alpha*-linked D-glucose units.

5.2 Efficacy and technological justification

Alpha-amylase derived from *Bacillus stearothermophilus* has advantages over other sources of *alpha*-amylase currently approved in the Code. It offers greater thermostability (enzyme activity maintained at higher temperatures) compared to enzymes derived from *Aspergillus oryzae* and *Bacillus subtilis.* It also produces different sugar profiles to those produced by the enzyme from *Bacillus licheniformis*.

Penford Australia, the producers of a range of glucose syrups from wheat starch, supported this application on the grounds of needing a high temperature *alpha*-amylase, which is involved in one of the stages of producing glucose syrups and maltodextrins. Penford states that it is an enzyme derived from a non-genetically modified source organism and will provide price competition for enzyme suppliers.

Sugar syrups and maltodextrin powders are used in a wide variety of food industries including confectionery, dairy foods, ice cream, beverages and health foods. The enzyme may also be used in the alcoholic beverage industry.

A Food Technology Report (Attachment 4) concluded that the use of the enzyme is technologically justified.

5.3 Safety assessment

Alpha-amylases have been used safely as a component of enzyme preparations in food processing for many decades.

A Safety Assessment Report for this Application is at Attachment 3. This safety assessment of the *Bacillus stearothermophilus* derived *alpha*-amylase found that:

- the source organism *Bacillus stearothermophilus* has a long history of safe use as a production strain for food-grade enzyme preparations;
- the enzyme complies with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications;
- the enzyme produced no evidence of genotoxic potential in *in vitro* assays; and
- there was no evidence of toxic effects of *Bacillus stearothermophilus* derived *alpha*-amylase in the acute and sub-chronic dosing studies in animals.

From the information available, it is concluded that the use of *Bacillus stearothermophilus* derived *alpha*-amylase as a processing aid in food would not raise any public health and safety concerns.

There are currently two approved sources of *alpha*-amylase from *Bacillus stearothermophilus* within the Table to clause 17 in Standard 1.3.3 of the Code. In these cases the gene for *alpha*-amylase is isolated from *Bacillus stearothermophilus* and added into two other hosts

(*Bacillus licheniformis* and *Bacillus subtilis*).

As with most enzymes there are not expected to be any dietary considerations since *alpha*-amylase is used as a processing aid in the initial stage of production of sugar syrups. The heating steps inactivate the enzyme and the subsequent purification steps remove most, if not all, of the enzyme (or protein).

5.4 Other international regulatory standards

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI of 'not specified' for *alpha*-amylase from *Bacillus stearothermophilus* at its thirty-seventh session (1990). This was concluded from JECFA Toxicity monograph (WHO Food Additives Series 28, 37th meeting, page 63), from acute and 13 week feeding studies in dogs and rats indicating very low risk to public health.

The Food and Drug Administration of the United States (FDA) affirmed the generally recognised as safe (GRAS) status of the *alpha*-amylase derived from *Bacillus stearothermophilus* (1995).

The Applicant states that the *alpha*-amylase enzyme preparations comply with the specifications for food enzyme preparations in Food Chemicals Codex (FCC), 4th Edition, 1996, and also the JECFA specifications in the Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO (1992), and relevant updates in addenda 1 to 9 (2001).

6. 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, food industries and governments in Australia and New Zealand. The benefits and costs associated with the proposed amendment to the Code will be analysed using regulatory impact principles.

The following two regulatory options are available for this Application:

Option 1. Not approve the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid.

Option 2. Approve the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid.

7. Impact Analysis

The affected parties to this Application include those listed below:

1. those sectors of the food industry wishing to produce and market food products produced using *alpha*-amylase as a processing aid;

2. consumers; and

3. Commonwealth, State, Territory and New Zealand Government regulatory agencies that enforce food regulations.

7.1 Option 1

There are no perceived benefits to industry, government regulators or consumers if this option is taken.

There are disadvantages to those food industries that wish to use this source of the *alpha*-amylase enzyme. Especially those that wish to use its advantage of higher inactivation temperature and different sugar profile.

7.2 Option 2

There are advantages to food manufacturers to be able to use this different source of *alpha*-amylase enzyme. It is in competition to other approved sources so possibly providing price and supplier competition. This different enzyme source also has advantages to the other approved sources in having greater thermal stability and being able to produce a different sugar profile. There are no adverse public health impacts for this option.

There should be no added costs to government regulators or consumers.

Option 2, which supports the approval of *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid is the preferred option, since it has advantages for the food industry and consumers but has no significant cost for government regulators, consumers or manufacturers.

8. Consultation

8.1 Public consultation

The Initial Assessment Report for this Application was circulated for a round of public comment from 21 August till 2 October. Three submissions were received. All supported option 2 – to approve the use of the enzyme – subject to an appropriate safety assessment as part of the Draft Assessment Report.

The Draft Assessment Report was circulated for another round of public comment from 18 December 2002 till 12 February 2003. Two submissions were received which supported the application.

Attachment 2 summarises the submissions received during the first and second rounds of public comment.

8.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Amending the Code to approve the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is unlikely to have a significant effect on trade. The enzyme preparations are also consistent with the international specifications for food enzymes of Food Chemicals Codex (4th Edition, 1996) and JECFA so FSANZ considers there is no need to notify the WTO.

9. Conclusion and Approval

The Final Assessment Report concludes that approval of the use of the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* as a food processing aid is technologically justified and does not pose a risk to public health and safety.

The draft variation to Standard 1.3.3 – Processing Aids, thereby giving approval for the use of *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid is approved for the following reasons.

- There are no public health and safety concerns associated with the use of this enzyme.
- The use of the *alpha*-amylase enzyme derived from *Bacillus stearothermophilus* is technologically justified since it has a role in food manufacturing, primarily with starch hydrolysis. This enzyme has greater thermostability and a different sugar profile.
- The source organism (*Bacillus stearothermophilus*) has a long history of safe use.
- The *alpha*-amylase enzyme has a history of safe use for many years in Australia and New Zealand.
- The enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* complies with the specifications for enzyme preparations in Food Chemicals Codex (4th Edition, 1996) and the Joint Expert Committee on Food Additives (JECFA) Compendium of Food Additives Specifications, Vol. 1, Annex 1, FAO 1992, (updated in Addendum 9, 2001).
- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act.
- The regulation impact assessment has concluded that the benefits of permitting use of the enzyme outweigh any costs associated with its use.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Summary of Public Submissions.
3. Safety Assessment Report
4. Food Technology Report

ATTACHMENT 1

Draft variations to the *Australia New Zealand Food Standards Code*

To commence: on gazettal

[1] ***Standard 1.3.3*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 17*, for the enzyme* α-Amylase EC [3.2.1.1] *the Source –*

Bacillus stearothermophilus

ATTACHMENT 2

Summary of Public Submissions

Round One

Submitters

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	Tony Downer
2	Food Technology Association of Victoria	David Gill
3	Western Australian Food Advisory Committee	Virginia McLaughlin

Submitter	Comments
Australian Food and Grocery Council	Supports the application subject to an appropriate safety assessment. It supports the statement that *Bacillus stearothermophilus* is considered non-pathogenic and non-toxigenic. It is the donor organism for a number of currently approved enzymes. It states there are technological reasons why the enzyme use is justified even though there are currently other approved *alpha*-amylase enzymes. These include: • price – the enzyme could be cheaper; • temperature sensitivity – different to other sources, important to have different optimum temperatures ranging through cold to hot; • rapidity of operation – this can vary from slow to fast and is critical to the appropriate processing; • efficiency – the mass of product produced per unit of enzyme can often vary. Manufacturers will make a decision on which enzyme preparation to use based on a combination of these factors.
Food Technology Association of Victoria	Supports option 2, to approve the use of the enzyme as a food processing aid.
Western Australian Food Advisory Committee	Supports option 2, to approve the use of the enzyme. It accepts there is substantial evidence that the enzyme is safe but will review the Safety Assessment Report that will be part of the Draft Assessment when it is written.

Round Two

#	Submitter Organisation	Name
1	Australian Food and Grocery Council	Tony Downer
2	Food Technology Association of Victoria	David Gill

Submitter	Comments
Australian Food and Grocery Council	Supports the Application. The AFGC believes approving the application is consistent with FSANZ's statutory objectives; that is it is consistent with the protection of public health and safety, consistent with international standards and consistent with improving efficiency and international competitiveness of the food industry.
Food Technology Association of Victoria	Supports option 2, to approve the Application.

ATTACHMENT 3

Safety Assessment Report

Application A467 – *ALPHA*-AMYLASE AS A PROCESSING AID (ENZYME)

1. Introduction

Application A467 seeks approval for the use of *alpha*-amylase from a non-genetically modified *Bacillus stearothermophilus* as a processing aid.

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.

2. The source (production) organism - *Bacillus stearothermophilus*

The safety of the production organism is an important consideration in the safety assessment for enzymes used as processing aids. *Bacillus stearothermophilus* is considered to be non-pathogenic and non-toxic, and has a long history of safe use as a production strain for food-grade enzyme preparations[1]. It is the source for a protease (AMFEP – Association of Manufacturers and Formulators of Enzyme Products enzyme list)as well as for the gene coding for maltogenic amylase approved in Standard 1.3.3. In particular this strain and its derivatives have been used for the production of food grade *alpha*-amylase for two decades.

3. 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. The detailed specifications to which the preparation was found to conform are shown in Table 1. They meet and exceed JECFA specifications[2].

Table 1. Complete specification of *alpha*-amylase preparation

Criteria	Applicant Specification	JECFA Specification[2]
Heavy Metals	not more than 30 ppm	not more than 40 ppm
Potassium sorbate	0.25-0.4 % w/w	
Sodium benzoate	1.0- 1.7 % w/w	
Sodium chloride	>10%	
Arsenic	not more than 3 ppm	not more than 3 ppm
Lead	not more than 5 ppm	not more than 10 ppm
Total viable count (cfu/g)	not more than $5x10^4$	not more than $5x10^4$
Total coliforms (cfu/g)	not more than 30	not more than 30

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

[2] Annex 1 of the Compendium of Food Additive Specifications, Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1992.

Production organism (cfu/ml)	<1	
Mycotoxins	negative by test	negative by test
Antibacterial activity	negative by test	negative by test
Total carbohydrate	18-25% w/w	
pH	5.5.- 6.0	
Salmonella	negative by test	negative by test

Alpha-amylase from the source organism, *B. stearothermophilus* complies with the recommended purity specifications for food-grade enzymes[2,3].

4. Evaluation of the submitted studies

Five toxicological studies were submitted in support of this application. These were: a) acute oral toxicity study in rats, b) a 90-day sub-chronic oral toxicity study in dogs, c) a 90-day sub-chronic oral toxicity study in weanling rats, d) a bacterial mutagenicity assay and e) a human lymphocyte cytogenetic assay. The test material was produced in the same manner as the commercial preparations. The enzyme activity is measured as the amount of enzyme required to hydrolyse 10 mg starch per minute under the conditions of assay. The catalytic activity was measured to be 3900- 6540 U/g with an amount of 10% Total Organic Solids.

4.1 Acute oral toxicity study with *Bacillus stearothermophilus* α-amylase in rats.

Study number 81213 by G.W. Thompson, F. E. Reno and T.E. Palmer, Hazelton Raltech, Inc. Hazelton Laboratories America, Inc, USA. *March 30, 1982.*

Test material:	*Bacillus stearothermophilus* α-amylase
Test Species:	Fischer 344 albino rats, 10 males and 10 females per test dose, administration via gavage.
Dose:	Acute doses at 0, 2.5, 5.0 and 10.0 g/kg bw.

Test article

The test material *Bacillus stearothermophilus* α-amylase, as a lyophilised powder had an activity of 6540 units/g and a total protein value of 71.8%.

Study conduct
Rats (10 male and 10 females) were administered test article via gavage at 0, 2.5, 5.0 and 10.0 g/kg bw/day. They were observed for clinical signs at 1-hour intervals for 8 hours post dosing; and daily for 14 days for any mortality and clinical signs. Rats were provided with rodent diet *ad libitum* except for an overnight fasting period prior to dosing. Body weights were recorded before and after fasting on day 0 and on days 7 and 14 post-dosing. Animals were necropsied on day 14 post-dose.

---

[2] Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. General specifications and considerations for enzyme preparations used in food processing. FAO Food and Nutrition Paper 52, Add. 9, pp. 37-39.

[3] National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemical Codex. 1996. *Food Chemical Codex*, 4th edition, National Academy Press, Washington DC.

Results & Conclusion

There were no deaths, clinical signs, effects on bodyweights or gross necroscopy findings related to treatment.

4.2 Ninety-day subchronic oral toxicity study of *Bacillus stearothermophilus* α-amylase in dogs.

Study No. 81170 by P.S. Mac and W.N. Hauck. Hazelton Raltech, Inc. Hazelton Laboratories America, Inc, USA. *February 24, 1983.*

Test material:	*Bacillus stearothermophilus* α-amylase
Test Species:	Beagle dogs 4 males and 4 females per test dose, administration in diet
Dose:	0, 0.56 or 1.11% in diet for 13 weeks.
Guidelines:	USFDA, non-clinical Laboratory Studies, GLP Regulations (21 CFR 58)

Test article

The test material *Bacillus stearothermophilus* α-amylase, as a lyophilised powder had an activity of 6540 units/g and a total protein value of 71.8%.

Study conduct

Three groups of dogs (4/sex/group) were treated with 0, 0.56, or 1.11% of the test article in the diet equivalent to 0, 36 or 72 units α-amylase /g. During the acclimation period, all animals were fed the basal diet.

Clinical observations, bodyweight and food consumption were recorded weekly; haematology, clinical chemistry and urinalysis every month; and ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necroscopy performed (gross examination and organ weights tissue sampling). Macroscopic and microscopic examination of terminally sacrificed males and females were conducted and histopathological parameters were measured.

Results

No deaths were associated with treatment. There were no treatment related clinical signs, adverse effects on food consumption and body weights or bodyweight gains. The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material.

Analysis of the blood chemistry parameters revealed a slight but statistically significant decrease in the direct bilirubin values of the Group 3 males after 1 month but returned to normal afterwards. Total protein and globulin concentrations decreased after the completion of 2 months in the Group 2 and Group 3 female dogs. After the completion of 3 months the total protein concentration in Group 3 males was also reduced ($p<0.05$) compared to the controls.

Urinalysis showed an increase in the urine specific gravity of Groups 2 and 3 females were increased (≤ 0.05).

Organ weights were generally unaffected by treatment up to the highest dose. The absolute weight and relative adrenal weights of the Group 2 females were lower than the control animals (≤ 0.01) but the variation is isolated and not considered to be treatment-related.

Macroscopic observations revealed redness on the mucosal surfaces of the caecum, or duodenum or jejunum of some animals. Microscopic examination of these events was unremarkable. Other findings were inflammation and mononuclear cell infiltration of the liver and tube mineralisation in the kidney. These occurrences were isolated and random and therefore were not considered to be treatment-related.

Conclusion

The observed decreases in the total protein and globulin concentrations are not toxicologically significant as the changes were small and the values were within limits of those previously observed for Beagle dogs of similar age and sex. Further, the changes were not correlated with clinical observations or with differences in body weight and feed consumption data. The dogs were all healthy right through the experiment. In conclusion, no evidence of toxicity was noted following treatment with *Bacillus stearothermophilus* α-amylase at levels up to 1.11% in the diet.

4.3 Subchronic oral toxicity study of *Bacillus stearothermophilus* α–amylase in *in utero* exposed F1 rats.

Study No. 81168 by J Mielenz & K.M. MacKenzie, Hazelton Raltech, Inc., Hazelton Laboratories America, Inc, USA. *March 18, 1983*.

Test material:	*Bacillus stearothermophilus* α-amylase
Test Species:	CDF (F-344)/CrlBR rats 12 males and 24 females per P_1 dose group; 20 F_1 animals /sex/group
Dose:	0, 0.56, or 1.11% (w/w) in diet for 13 weeks.
Guidelines:	USFDA, non-clinical Laboratory Studies, GLP Regulations (21 CFR 58)

Test article

The test material *Bacillus stearothermophilus* α-amylase, as a lyophilised powder had an activity of 6540 units/g and a total protein value of 71.8%.

Study Conduct

Groups of 12 male and 24 female Fischer 344 rats were treated with *Bacillus stearothermophilus* α-amylase in the diet at 0, 0.56, or 1.11% (w/w) (equivalent to 0, 36 or 72 units α-amylase /g). P_1 animals were treated 13 weeks before mating. The animals were sacrificed after 22 weeks. At 4 weeks of age selected F_1 males and females (20/sex/group) were dosed as above for 13 weeks prior to terminal sacrifice.

A clinical examination was performed twice daily, food consumption weekly (except during pairing) and bodyweights measured weekly (pre-mating, gestation and lactation for females).

Detailed necropsy was performed on adult P_1 and F_1 animals. Histopathology was carried out on all F_1 animals in the control and high dose groups.

Results

P_1 generation-There were no deaths or abnormal or dose related clinical observations during the study that was attributed to treatment. Ophthalmic examination revealed ocular lesions or irritations as the most common occurrences. These were diagnosed to have been caused by non-specific coronal virus. Variations in mean bodyweight were observed only in females during weeks 14 through 22 due to gestation and lactation periods. Mean body weight gains were lower for both sexes treated with 0.56% and 1.11% test article at various time periods. Food consumption was lower in females during weeks 2,4,5,10 and 11; whereas, for males, there were no significant differences in mean food consumption between test and control groups throughout the treatment period.

No significant differences between the mean values of the control groups and the treated groups were observed in the haematology, clinical chemistry or urinalysis carried out after 6 and 12 weeks of treatment.

F_1 generation- Clinical observations revealed no abnormalities. All animals appeared healthy till the end of the experiment. There were no differences in mean body weights between test groups and control groups. In general, mean body weight gain was lower for treated animals. The mean food consumption was higher for the treated male rats, but not for female rats, when compared to control group. Only three animals had eye lesions and none were treatment related.

A few minor differences in serum enzyme activities were noted but they were not treatment related. There was no significant difference in haematology or urinalysis values detected between the treated animals and the control animals.

There was no observable difference in the absolute organ weights of F_1 rats sacrificed at 13-weeks post-weaning; however relative weights of some organs (spleen, kidneys and brain) showed statistically significant variations. These changes are due to increased terminal body weights of the treated animals accounting for the decreases in the relative weights of kidneys and brain. The changes are not considered biologically important as confirmed by macroscopic and microscopic observations at necropsy.

Conclusions

Administration of *Bacillus stearothermophilus* α-amylase to P_1 rats for 13 weeks followed by post weaning administration of F_1 rats for a further 13 weeks did not produce treatment-related effects in the haematology, urinalysis or clinical pathology data. Microscopic and macroscopic examinations did not reveal any abnormalities.

In conclusion daily treatment with test substance at concentrations of up to 1.11% (72 units α-amylase/g) g for 13 weeks resulted in no treatment related effects.

4.4 Test for Mutagenic Activity of *Bacillus stearothermophilus* α–amylase using *Salmonella typhimurium* strains.

Report No. 7899-M-04800 by S. Cinelli and J. Brightwell, Research Toxicology Centre, Roma. *Dec 04 2000*.

Test article

The test item, *Bacillus stearothermophilus* α–amylase, was a brown liquid, with an activity of 6540 units/g.

Study conduct

Bacillus stearothermophilus α–amylase was examined for mutagenic activity in five strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537). Experiments were performed with or without metabolic action using liver S9 fraction from chemically pre-treated rats. The study design is in accordance with OECD Guidelines[4].

The study comprised of negative and positive controls with or without S9 metabolising system. Experiments for survival determination and estimation of mutant numbers were carried out in triplicates at each test point.

The study comprising was conducted using the direct plate incorporation assay. Five doses of the test substance were applied with 5 mg/plate as the highest dose level followed by successive bi-sections between doses. The test was carried out both in the presence and absence of metabolic activation (in the form of a liver preparation, S-9, and co-factors). 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, cumene hydroperoxide, dimethyl sulfoxide sodium azide and 2-Nitrofluorene).

Results and conclusion

No dose-related or reproducible increases in mutation frequency were obtained with any of the bacterial strains exposed to α-amylase either in the presence or absence of metabolic activation. It was concluded that the test material *Bacillus stearothermophilus* α–amylase did not exhibit any mutagenic activity under the conditions of the test.

4.5 Chromosome aberration assay in human lymphocytes cultured *in vitro*.

Report No. 7900-M-05100 by S. Cinelli and J. Brightwell, Research Toxicology Centre, Roma. *Dec 04 2000*.

Test article

The test item, *Bacillus stearothermophilus* α–amylase, was a brown liquid, with an activity of 6540 units/g.

[4] OECD Guideline for the testing of chemicals No. 471 (Adopted July 1997).

Study design

The potential of *stearothermophilus* α–amylase to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using duplicate human lymphocyte cultures from a healthy male donor. Tests were carried out in the presence and absence of S-9 metabolic activation, over a broad range of doses. The highest dose for chromosome analysis from cultures sampled at 20 hours should be one at which at least 50% mitotic inhibition has occurred or should be the highest dose tested.

Results and conclusion

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation. Positive control, cyclophosphamide, gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

5. Conclusions

This safety assessment of the *Bacillus stearothermophilus* α–amylase found that:

- The source organism *Bacillus stearothermophilus* has a long history of safe use as a production strain for food-grade enzyme preparations;
- The enzyme preparation complies with JECFA specifications;
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;
- There was no evidence of toxic effects of *Bacillus stearothermophilus* α-amylase in the acute and sub-chronic dosing studies in animals.
-

From the information available, it is concluded that the use of *Bacillus stearothermophilus* α–amylase as a processing aid in food would pose no public health and safety risk.

ATTACHMENT 4

Food Technology Report

Application A467 – *ALPHA*-AMYLASE AS A PROCESSING AID (ENZYME)

Introduction

FSANZ received an application from Genencor International to amend the Food Standards Code to approve the use of the enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* as a processing aid.

Alpha-amylase

The enzyme class called amylases have had a long history of use in the food industry. They are primarily involved in the hydrolysis of starch. Amylases catalyse the hydrolysis of 1,4-*alpha*-D-glucosidic linkages of polysaccharides such as starch, glycogen, or their degradation products. The specific enzyme, *alpha*-amylase, is termed an endoamylase. It catalyses the endohydrolysis of 1,4-*alpha*-D-glucosidic linkages in polysaccharides, containing three or more 1,4-*alpha*-linked D-glucose units, in a random manner. The term *alpha* (α) relates to the optical configuration of the released sugar group, and not to the configuration of the linkage that is hydrolysed (which is random).

In the common usage of *alpha*-amylase the enzyme attacks the random *alpha*-1,4 linkages of amylose and amylopectin of starch, converting them to dextrin so reducing the viscosity and increasing the dextrose equivalence (DE). The *alpha*-amylase enzyme is used to liquefy and dextrinise starch.

Advantages of use of *alpha*-amylase

Enzymes (in this case *alpha*-amylase) are used to perform specific chemical reactions more easily, cheaply and specifically compared to older chemical reactions relying on inorganic acids and heat to perform reactions. In this case use of *alpha*-amylase is preferred to the use of inorganic acids to perform starch hydrolysis since it has advantages with increased yields and more economic production.

The advantages of using *alpha*-amylase hydrolysis include;

- better specificity of reaction and therefore fewer reaction products;
- greater control over amylolysis;
- milder reaction conditions (lower temperature and pH conditions) so producing less unwanted side reactions and by-products responsible for unwanted off-flavours and odours;
- milder reaction conditions being responsible for more economic production, with lower energy requirements; and
- less neutralisation of acids is required.

The advantages of the *alpha*-amylase derived from *Bacillus stearothermophilus* over other sources (such as *Aspergillus oryzae* and *Bacillus subtilis*) are that it has greater thermostability (enzymic activity maintained at higher temperature) and it produces a different sugar profile.

Enzymes sourced from alternative organisms have slightly different properties. Advantages or disadvantages depending on the source organism include;

- cost;
- temperature sensitivity (temperature dependence on enzymatic activity);
- efficiency of reactions catalysed;
- speed of reaction; and
- reaction products produced (profile).

Production of the enzyme

The *alpha*-amylase is produced using a submerged fed-batch fermentation using *Bacillus stearothermophilus.* The production is standard for many commercially used food enzymes. Good Manufacturing Practice (GMP) is used throughout the production process, meeting the requirements and specifications for food enzymes within Food Chemicals Codex (4th Edition, 1996) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the Compendium of Food Additives Specifications, Vol 1, Annex 1 Addendum 9 (2001) (and earlier relevant Addenda).

Conclusion

The use of the food enzyme *alpha*-amylase derived from *Bacillus stearothermophilus* is technologically justified as a food processing aid.

References

Enzymes in Food Processing, third edition, edited by Tilak Nagodawithana, Academic Press, California (1993).

Food Enzymes, Structure and Mechanism, Dominic Wong, Chapman & Hall, New York (1995).

IUBMB (International Union of Biochemistry and Molecular Biology) Enzyme Nomenclature internet site, http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/1.html.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) Compendium Addendum 2, FAO Food and Nutrition Paper 52, Addendum 2, page 9 (1993).

Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO Food Additives Series 28, 37th meeting, page 63 (1991).