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

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FINAL ASSESSMENT REPORT

APPLICATION A580

FOOD DERIVED FROM AMYLASE-MODIFIED CORN LINE 3272

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

An Application has been received from Syngenta to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from genetically modified (GM) corn line 3272. Standard 1.5.2 – Food produced using Gene Technology, requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

Corn line 3272 has been genetically modified for use in dry-grind ethanol production in the United States. It produces a heat-stable α-amylase enzyme which retains its activity during the high temperatures required for dry-grind ethanol production. The GM corn is intended primarily for industrial uses, however the ethanol produced may also be used for food applications. In addition, if grain derived from corn line 3272 were inadvertently mixed with corn intended for the food chain it could potentially enter the Australian and New Zealand food supply. If approved, food from amylase-modified corn line 3272 may therefore enter Australia and New Zealand as largely processed food products (corn syrup, corn starch, corn chips, cornflour) and alcohol-fortified products.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from amylase-modified corn line 3272, as required under Standard 1.5.2 in the Code. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel proteins; and (iii) the composition of amylase-modified corn line 3272 compared with that of conventional corn. In addition, due to the unique characteristics of this particular genetic modification, the assessment also considered the human nutritional impact as well as the impact on food technological processes.

The assessment of this application identified no public health and safety concerns. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from amylase-modified corn line 3272 is considered to be as safe and as wholesome as food derived from other commercial corn varieties.

Labelling

Food derived from amylase-modified corn line 3272 will be required to be labelled as genetically modified if novel DNA and/or novel proteins are present in the final food. Studies conducted by the Applicant show that the novel proteins are present in the corn grain, the heat-stable α-amylase in particular, is present at a relatively high level. Labelling addresses the requirement of section 10(1)(b) of the Act; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval; or (2) approval of food derived from amylase-modified corn line 3272 based on the conclusions of the safety assessment.

Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this application is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Purpose

The Applicant seeks amendment to Standard 1.5.2 to include food derived from amylase-modified corn line 3272 in the Table to clause 2.

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from amylase-modified corn line 3272 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from amylase-modified corn line 3272 in Australia and New Zealand is agreed on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce amylase-modified corn line 3272;
- food derived from amylase-modified corn line 3272 is as safe and wholesome as food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- certain food fractions derived from gene technology are required to be labelled 'Genetically Modified' if the final food contains novel DNA and/or novel protein, or it has altered characteristics. An altered characteristic means that a food or food ingredient must be identified on the label as being 'genetically modified' if it is significantly different from its non-genetically modified counterpart with respect to allergenicity, toxicity, nutritional impact or intended use;
- in the case of food that is derived from amylase-modified corn line 3272, labelling as 'Genetically Modified' will be required if novel DNA and/or protein is present in the final food;
- given that the genetic modification relates to the property of the corn grain (that is, the thermal stability of the α-amylase enzyme), the nutritional characteristics of the corn remains unaltered from its non-GM counterpart and no additional labelling will be required; and
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the most appropriate option is Option 2, an amendment to the Code.

The amendment to the Code will come into effect on the date of gazettal.

Consultation

The Initial Assessment was advertised for public comment between 31 May 2006 and 12 July 2006. A total of eight submissions were received during this period. The Draft Assessment was released for public comment between 23 May 2007 and 4 July 2007. A total of nine submissions were received during this period and a summary of these is attached to this report (**Attachment 3**).

FSANZ has taken the submitters' comments into account in preparing the Final Assessment of this Application. Specific issues relating to amylase-modified corn line 3272 have been addressed in the Report.

CONTENTS

INTRODUCTION

An Application was received from Syngenta on 22 March 2006 seeking approval for food derived from amylase-modified corn line 3272, under Standard 1.5.2 – Food produced using Gene Technology, in the *Australia New Zealand Food Standards Code* (the Code).

A Final Assessment of the Application has been completed, including a comprehensive safety assessment, and consideration of issues raised in public consultation.

1. Background

The genetic modification to the corn involved the transfer of two genes:

- The *amy797E* gene, derived from micro-organisms belonging to the archael order *Thermococcales*. The gene encodes a thermostable α-amylase enzyme, which retains its activity during the high temperatures required for starch hydrolysis in dry-grind ethanol production.

- The *pmi* (*manA*) gene, from *Escherichia coli,* which encodes the enzyme phosphomannose isomerase (PMI). This protein was used as a selectable maker during corn transformation.

Amylase-mediated hydrolysis of starch is one of the first steps necessary to produce ethanol from plants. While plants like corn naturally contain their own amylases, these get destroyed when corn is subjected to the high temperatures necessary for ethanol production, making it necessary to add external sources of amylase enzyme during processing. The use of corn line 3272, expressing a thermostable amylase, will make this step unnecessary. Grain from corn line 3272 expressing the thermostable α-amylase enzyme will be mixed with conventional corn at the processing plant.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with a total production of about 700 million tonnes a year. The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used to produce industrial products, such as ethanol by fermentation and highly refined starch by wet milling.

Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. These syrups are used in the manufacture of breakfast cereals, bakery products, confectionery and food coatings. Cornstarch is also imported and used by the food industry for the manufacture of dessert mixes and food coatings. Corn may also be imported in finished products such as corn chips and cornflour.

Corn line 3272 is not being developed for cultivation in Australia or New Zealand. However, if approved, food from this corn line may enter the Australian and New Zealand food supply as alcohol-fortified products and processed food products.

1.1 Current Standard

Standard 1.5.2 requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand. Foods that have been assessed under the Standard, if approved, are listed in the Table to clause 2 of the Standard.

1.2 Overseas approvals

In 2005, Syngenta filed a Pre-market Biotechnology Notification (PBN) with the US Food and Drug Administration (FDA) and a petition for the determination of non-regulated status for corn line 3272 was submitted to the US Department of Agriculture. The consultation with US FDA was recently completed, with the US FDA issuing a letter on 7 August 2007 stating they had no further questions.

During 2006, applications to permit corn line 3272 for food and/or feed use were submitted to relevant authorities in the European Union, Canada, China, Japan, Korea, Philippines, Taiwan, Russia, South Africa and Switzerland.

2. The Issue / Problem

Standard 1.5.2 requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand. The Applicant has developed a GM corn line expressing a thermostable α-amylase enzyme for use in dry-grind fuel ethanol production in the United States. While corn line 3272 has been developed primarily for industrial uses, the ethanol produced may be used for food applications (e.g. fortification of alcoholic products). Although grain from corn line 3272 is intended to be channelled exclusively into dry-grind ethanol production, the potential exists for it to be inadvertently mixed or co-mingled with corn destined for the human food supply. Food from corn line 3272 may therefore potentially enter the Australian and New Zealand food supply.

Before food derived from corn line 3272 can enter the food supply in Australia and New Zealand, it must first be assessed for safety and an amendment to the Code must be approved by the FSANZ Board, and the decision subsequently notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted, once the Ministerial Council process has been finalised.

If approved, food derived from corn line 3272 could enter the Australian and New Zealand food supply as imported and largely processed foods (corn syrup, corn starch, corn chips, cornflour etc.) and alcohol-fortified products.

3. Objectives

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 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.

The objective of this assessment is to determine whether it would be appropriate to amend the code and approve amylase-modified corn line 3272.

therefore the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, FSANZ will also have regard for the need for standards to be based on a risk analysis using the best available scientific evidence, and the benefits of an efficient and internationally competitive food industry.

4. Key Assessment Question

Is food derived from amylase modified corn line 3272 as safe for human consumption as food from conventional corn varieties?

RISK ASSESSMENT

5. Risk Assessment Summary

Amylase-modified corn line 3272 has been assessed for safety according to the guidelines prepared by FSANZ[1]. The summary and conclusions from the full safety assessment report (at **Attachment 2**) are presented below. In addition to information supplied by the Applicant, other available resource material including published scientific literature and general technical information was used for the assessment.

In conducting a safety assessment of food derived from amylase-modified corn line 3272, a number of criteria were addressed including:

(i) characterisation of the transferred genes, their origin, function and stability;
(ii) changes at the level of DNA, protein and in the whole food;
(iii) compositional analyses, including an evaluation of intended and unintended changes; and
(iv) potential for the newly expressed proteins to be either allergenic or toxic in humans.

[1] FSANZ (2005) Guidelines for the Safety Assessment of Genetically Modified Foods.

5.1 Description of the Genetic Modification

Corn line 3272 contains two novel genes. The first, the *amy797E* gene, encodes the thermostable AMY797E α-amylase enzyme. α-Amylase catalyses the hydrolysis of starch by cleaving the internal α-1,4-glucosidic bonds into dextrins, maltose and glucose.

The chimeric *amy797E* gene is derived from three wild-type α-amylase genes from the archael order *Thermococcales*. The *amy797E* gene encoded protein was selected for further development due to its thermostability, catalytic activity and reduced dependency on calcium under acidic conditions. These properties are important for the commercial application of α-amylase in the enzymatic hydrolysis of starch in dry-grind ethanol production from corn.

The second gene, *pmi* (*manA*), derived from *Escherichia coli,* encodes the enzyme phosphomannose isomerase (PMI). The *pmi* gene is used as a selectable marker gene in the corn transformation process.

Molecular and genetic analyses indicate that the transferred genes are stably integrated into the plant genome at one insertion site and are stably inherited from one generation to the next.

5.2 Characteristic of Novel Proteins

Corn line 3272 expresses two novel proteins – the enzymes AMY797E and PMI. AMY797E is expressed at relatively high levels in grain (838 –1627 µg/g fresh weight or 1004–3365 µg/g dry weight) and PMI is expressed at low levels (<0.4– 0.8 µg/g fresh weight or <0.5– 1.8 µg/g dry weight).

A number of studies have been conducted with both PMI and AMY797E to determine their potential toxicity and allergenicity. Neither protein showed any evidence of toxicity in acute oral toxicity studies and also do not exhibit any amino acid sequence similarity with known protein toxins. Neither of the proteins is derived from sources known to be allergenic and both are rapidly digested in simulated digestion studies. AMY797E has no immunologically meaningful amino acid sequence similarity to known allergens. PMI was found to share a short sequence of amino acids (eight residues) with a known allergen, however further testing with human sera from allergic individuals did not demonstrate any cross-reactivity. Overall, the evidence indicates that AMY797E and PMI are non-toxic and have limited potential to be allergenic in humans.

5.3 Compositional Analyses

Compositional analyses were carried out to establish the nutritional adequacy of grain from amylase-modified corn line 3272, in comparison to conventional control lines. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites and anti-nutrients.

No compositional differences of biological significance were observed between the GM corn grain and its non-GM counterpart. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very minor differences and were within the reference range from conventional varieties. It can be concluded that food from amylase-modified corn line 3272 is equivalent in composition to that from other commercial corn varieties.

5.4 Nutritional Impact

The detailed compositional studies indicate that food derived from corn line 3272 is equivalent in composition to food from non-GM corn varieties. Minor differences in composition were consistent with normal variation for corn and would not be expected to have any nutritional impact. A feeding study in broiler chickens supports this finding.

As the α-amylase enzyme expressed in corn line 3272 has the ability to reduce starch molecules into component dextrins and mono/disaccharides at high temperatures, its potential impact on glycaemic index, should corn line 3272 be used as human food, was evaluated. This evaluation concluded that the co-mingling of corn line 3272 with existing corn stocks is unlikely to have any significant effect on population nutrition. It is possible the AMY797E α-amylase may be activated during the cooking or processing of corn line 3272, which could increase the glycaemic index of the final food product. However, even if the final food's glycaemic index was increased, the overall effect on the diet would be minimal given that glycaemic index is heavily influenced by other dietary factors.

5.5 Impacts on Food Technological Processes and Products

FSANZ also assessed the potential impacts of this thermostable α-amylase on different processes of corn refining and on corn products. Mixing of corn varieties in the USA could result in imported processed products containing α-amylase. Food products that could potentially contain the enzyme include corn syrups, corn starches and corn chips and flours. Other food products could contain these products as ingredients. Although the presence of this α-amylase may impact on certain food properties due to the conversion of starch to dextrins and sugars during processing, there is no food safety issue associated with the possible presence of this thermostable enzyme in foods.

5.6 Conclusion

In conclusion, no public health and safety concerns have been identified in the assessment of food produced from corn line 3272. On the basis of the data provided in the present application, and other available information, food derived from corn line 3272 can be considered as safe and as wholesome as food derived from other corn varieties.

RISK MANAGEMENT

6. Risk Management Strategies - labelling

Under Standard 1.5.2, GM food or ingredients must be labelled if novel DNA and/or protein are present in the final food or where the food has altered characteristics.

6.1 Presence of novel DNA and/or novel protein

Foods derived from amylase-modified corn line 3272 will be required to be labelled as genetically modified if novel DNA and/or novel proteins are present in the final food. Studies conducted by the Applicant show that the novel proteins are present in the corn grain, the heat-stable α-amylase in particular, is present at a relatively high level.

6.2 Altered characteristics

The Table to clause 2 of Standard 1.5.2 makes provision for special conditions to be specified in column 2 of that Table, associated with a permission for a particular GM food. Special conditions may be specified in the following circumstances:

- where a particular public health and safety risk is identified for either the general population or a population sub-group; or

- to enable consumers to make an informed choice about the food.

Such special conditions may be referred to as risk management strategies. Strategies may include limiting the use of the food itself or providing information on the label of the food that enables consumers to make an informed choice.

In general, labelling would be the most commonly employed risk management strategy considered for food produced using gene technology.

The intended use of amylase-modified corn line 3272 is in dry-grind ethanol production rather than food use. Division 2 – Labelling etc of food produced using gene technology, of Standard 1.5.2, in clause 7, makes provision for additional labelling/information requirements, where the food has altered characteristics. Where the intended use of the food produced using gene technology is different to the existing counterpart food not produced using gene technology, this is considered an altered characteristic and labelling or other information may be specified in column 2 of the Table to clause 2. However, because there is unlikely to be significant change to the nutritional characteristics of the food and because the intended use of any ingredients derived from this corn is the same there is no need for additional labelling.

7. Options

7.1 Option 1 – prohibit food from amylase-modified corn line 3272

Maintain the *status quo* by not amending the Code to approve the sale and use of food derived from amylase-modified corn line 3272.

7.2 Option 2 – approve food from amylase-modified corn line 3272

Amend the Code to permit the sale and use of food derived from amylase-modified corn line 3272, in the Table to clause 2 of Standard 1.5.2.

8. Impact Analysis

8.1 Affected Parties

1. Consumers, particularly those who have concerns about biotechnology;
2. Food importers and distributors of wholesale ingredients;
3. The manufacturing and retail sectors of the food industry; and

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4. Government generally, where a regulatory decision may impact on trade or World Trade Organization obligations, and enforcement agencies in particular who will need to ensure that any approved products are correctly labelled.

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There is currently no intention to grow amylase-modified corn line 3272 in Australia or New Zealand. Should this be decided in the future, any environmental impact would require assessment by the Office of the Gene Technology Regulator (OGTR) in Australia, and by various New Zealand Government agencies including the Environmental Risk Management Authority (ERMA) and the Ministry of Agriculture and Forestry (MAF) before cultivation in these countries could be permitted. Importation of non-viable corn grain into New Zealand would not require approval by ERMA.

8.2 Benefit Cost Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Following public consultation on the Initial Assessment, FSANZ has identified the following potential costs and benefits of the two regulatory options:

8.2.1 Option 1 – prohibit food derived from amylase-modified corn line 3272

Consumers: Benefit – to consumers who wish to continue to avoid GM products.

Government: No immediate impact.

Cost – high potential for trade disruption if inadvertent co-mingling occurs.

Potential impact – if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Industry: Cost – if inadvertent co-mingling occurs, the quality of certain processed products may be affected by the presence of a heat stable α-amylase.

Potential longer-term impact – any successful WTO challenge has the potential to impact adversely on food industry.

8.2.2 Option 2 – approve food derived from amylase-modified corn line 3272

Consumers: Cost – more concerns for those consumers opposed to GM foods. The amount of amylase-modified corn line 3272 entering the food supply is likely to be low so the cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or increased prices for non-GM food is likely to be low.

Benefit – product safety assessed and assured.

Government: Benefit – this will ensure food imports containing amylase-modified corn line 3272 comply with the Code. This would ensure that there is no potential for trade disruption on regulatory grounds.

Cost – this decision could mean increased monitoring requirements, as foods derived from amylase-modified corn line 3272 will be required to be labelled as genetically modified if novel DNA and/or proteins are present in the final food.

Industry: Benefit – this will ensure food imports containing amylase-modified corn line 3272 comply with the Code. This would ensure that there is no potential for trade disruption on regulatory grounds.

Cost – inadvertent co-mingling may not be ruled out completely, but since the Applicant has agreed to implement a stewardship program, the chances of co-mingling are reduced.

The costs of the two Options to the industry can not be quantified due to limited quantitative information available. This is confirmed by the Business Cost Calculator Report (**Attachment 6**).

8.3 Comparison of Options

As food from amylase-modified corn line 3272 has been found to be as safe as food from other varieties of corn, Option 1 is likely to be inconsistent with Australia's and New Zealand's WTO obligations.

Although Option 1, i.e. non-approval, would offer most benefit to those consumers wishing to avoid GM foods, in reality, the likelihood of corn line 3272 entering the food supply is low.

Option 1 is potentially less costly to governments due to the lower requirement for monitoring and enforcement, however this Option is significantly more costly to the industry and carries with it the potential for trade disruption should inadvertent co-mingling occur.

Overall, Option 2 is the preferred option as food derived from amylase-modified corn line 3272 has not been found to raise any significant safety concerns.

The proposed amendment to Standard 1.5.2, giving approval to food from amylase-modified corn line 3272, is therefore considered appropriate.

COMMUNICATION

9. Communication

FSANZ developed a communication strategy to allay ongoing concerns raised by specific groups and the general public regarding food derived from genetically modified organisms. Part of this strategy was the publication, in June 2000, of an information booklet titled *GM foods and the consumer*.

Due to the success of this booklet in providing information on safety issues, FSANZ recently launched a revised and updated version of this booklet, now titled *GM Foods*[2]. The booklet aims to communicate recent developments in the safety evaluation of GM foods to interested members of the community.

As normally applies to all GM food assessments, the Draft Assessment Report for Application A580 was available to the public on the FSANZ website and distributed to major stakeholders. Public comment on the Draft Assessment was sought prior to preparation of this Final Assessment of the Application.

10. Consultation

10.1 Public Consultation

The Initial Assessment was advertised for public comment between 31 May 2006 and 12 July 2006. A total of eight submissions were received during this period. The Draft Assessment was released for public comments between 23 May 2007 and 4 July 2007. A total of nine submissions were received and a summary of all the submissions is attached to this report (**Attachment 3**).

FSANZ has taken the submitters' comments into account in preparing the Final Assessment of this application. Specific issues relating to amylase-modified corn line 3272 have been addressed in the report. The major issues raised are discussed here.

10.1.1 Enforcement costs

Queensland Health and NSW Food Authority indicated a concern about the impact on monitoring resources for jurisdictions if this Application is approved. They also suggest that the costs of monitoring and enforcing GM food legislation should not be left solely to jurisdictions and a national enforcement strategy for GM food, including education, also be considered.

10.1.1.1 Response

The costs associated with detecting GM from non-GM sources depends on the level of detail required for the investigation, as the number of introduced genetic traits is relatively small compared to the number of individually approved GM lines. Routine detection methods can distinguish a GM from a non-GM source when genetic material is present, however other analyses could be required for event-specific detection.

Labelling requirements under Standard 1.5.2 call for food manufacturers to seek and maintain documentation relating to the GM status of individual ingredients used in their products. In approving the expanded labelling requirements for GM foods in 2000, Health Ministers indicated that the purpose of the paper trail was to reduce the reliance on laboratory testing of foods as the sole enforcement tool.

[2] http://www.foodstandards.gov.au/_srcfiles/GM%20Foods_text_pp_final.pdf

Costs associated with the enforcement by jurisdictions of any new food regulatory measure are considered by FSANZ in the Regulatory Impact Statement (RIS) and are not unique to GM foods. Inevitably, enforcement costs would be expected to rise over time as a result of the need to regulate an ever-increasing number of new food additives, processing aids and novel foods in the Code. Australia and New Zealand's current system of food regulation provides for the discussion of such issues by the Implementation Sub-Committee (ISC).

FSANZ will work with the jurisdictions in developing a national enforcement strategy for GM food.

10.1.2 Impacts on food functionality, product stability and shelf life

Food Technology Association of Australia expressed concerns that the abundant presence of the thermostable α-amylase may alter food functionality. The Australian Food and Grocery Council (AFGC) stated that for manufacturing processes that rely on heat to inactivate enzymes, the adventitious presence of a thermostable amylase may reduce the shelf-life and stability of the product. They argue this may have a greater impact on products normally considered to have a long shelf life at room temperature, such as canned soup or UHT packaged soup.

10.1.2.1 Response

Some of the issues have been addressed in Sections 6.1 and 7 of the safety assessment report (**Attachment 2**). It is possible that the presence of a thermostable α-amylase in corn grain, when activated during food processing, may potentially impact on food functionality and glycaemic index. This however does not represent a safety or nutritional concern. The overall effect on the diet would be minimal given that glycaemic index is heavily influenced by other dietary factors.

If approved, food and food ingredient imports containing amylase-modified corn line 3272 will need to comply with the Code. The labelling requirements and technical specifications (such as the α-amylase content) will help food manufacturers and distributors make informed choices.

FSANZ acknowledges that the inadvertent presence of corn containing a thermostable α-amylase may, in certain circumstances, affect the shelf life and quality of some finished food products.

In many situations the point at which the thermostable amylase will become active will be close to the point of consumption therefore there is unlikely to be any significant impact on food properties. However, where the thermostable enzyme has been active during processing, there is potential for shelf life and stability of products to be affected. In the case of most canned soup and UHT products, these are heated above a temperature of 120ºC, which is likely to inactivate most enzymes, and is also unlikely to represent an optimum temperature for activity of the thermostable amylase. Larger pack sizes and high acid products can be subjected to less severe temperature processing and the addition of other ingredients such as acid, salt, sugar and chemical preservatives may be used to extend shelf life. The presence of such ingredients will also have an effect on the activity and/or inactivation of the enzyme.

In order to minimise the chances of this corn line inadvertently mixing with other corn destined for the food supply once commercialised, Syngenta will have in place appropriate stewardship measures including grower production agreements or contracts that will facilitate the delivery of grain from corn line 3272 to its intended end use (**Attachment 4).**

10.1.3 Food vs. intended use for ethanol production

A number of submitters, including Food Technology Association of Australia and Greenpeace Australia Pacific Ltd, questioned the rationale that FSANZ assesses this corn line as food even though it is intended for ethanol production.

10.1.3.1 Response

FSANZ has responsibility for the safety of food sold in Australia and New Zealand. Although amylase-modified corn line 3272 is intended for ethanol production, it is possible that the ethanol produced may be used for food applications (e.g. fortification of alcoholic products) and that it may also enter the food chain inadvertently. FSANZ has therefore assessed corn line 3272 as if it were intended to be consumed by humans. To be approved, corn line 3272 must be shown to be as safe as other varieties of corn currently available.

The approach adopted by FSANZ to GM crops not intended primarily for food use provides a number of advantages to consumers, food industries and government. It minimises the risk of unapproved and unassessed food products entering the food supply as a result of inadvertent co-mingling of grains/seeds during transport and storage and ensures food imports containing amylase-modified corn line 3272 comply with the Code.

10.1.4 External expert reviewers

Greenpeace Australia Pacific Ltd seeks the names, qualifications and affiliations of the two external experts who reviewed FSANZ' safety assessment report at the Draft Assessment stage.

10.1.4.1 Response

The identity of the external experts consulted for the safety assessment is provided in the acknowledgements for the safety assessment report (**Attachment 2**). The reviewer's written opinions are available from FSANZ on request.

10.1.5 International regulatory status

Queensland Health and Greenpeace Australia Pacific Ltd queried the current international regulatory status of this corn line.

10.1.5.1 Response

The Applicant has provided the following update on the current international regulatory status of corn line 3272:

Country	Type of submission	Date	Approval
USA	FDA Consultation USDA Deregulation petition	August 2005 October 2005	7 August 2007
China	Food, Feed, Processing including Environment	Will be submitted after US approvals	
EU	Food, Feed, Import under Regulation (EC) No 1829/2003 was submitted to EFSA	February 2006	
Australia/New Zealand	Food	March 2006	
Taiwan	Food	March 2006	
Korea	Environment Food	April 2006 December 2006	
Canada	Food Feed Environment	May 2006 May 2006 May 2006	
Japan	Food Feed Environment	May 2006 May 2006 June 2006	
Switzerland	Food Feed	June 2006 June 2006	
Russia	Food	June 2006	

The following new submissions and resubmissions will still be made:

- Mexico – August 07
- Philippines, China and South Africa – after US approval
- Russia – feed

10.1.6 Other technical issues raised in submissions

An individual submitter from New Zealand provided an in depth critique of the single dose oral toxicity study in the mouse submitted as part of the application. FSANZ has considered this submission and provides a response in **Attachment 5**.

Another individual submitter from New Zealand and Greenpeace Australia Pacific Ltd made a series of critical technical comments in relation to a number of the submitted studies. FSANZ has considered all the comments and provided responses in **Attachment 5**.

10.2 External Review

External review on the safety assessment report was completed at the Draft Assessment stage. As this Application represents a new GM trait that FSANZ has not assessed before, it is standard practice for FSANZ to seek the opinion of external scientific experts. In general, the reviewers agreed with the conclusions of the safety assessment of corn line 3272. Specific comments have been addressed in the safety assessment report (**Attachment 2**).

10.3 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.

Guidelines for assessing the safety of GM foods have been developed by the Codex Alimentarius Commission and have the status of standards for WTO purposes. The proposed amendment to the Code to allow food derived from amylase-modified corn line 3272 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade.

For these reasons, notification was made to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Sanitary and Phytosanitary Measure (SPS) Agreement. No comments were received from WTO members.

CONCLUSION

11. Conclusion and Decision

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from amylase-modified corn line 3272 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from amylase-modified corn line 3272 in Australia and New Zealand is agreed on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce amylase-modified corn line 3272;
- food derived from amylase-modified corn line 3272 is as safe and wholesome as food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from amylase-modified corn line 3272, will be required if novel DNA and/or protein is present in the final food;
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the most appropriate option is Option 2, an amendment to the Code.

12. Implementation

It is proposed that the draft variation come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Summary of public submissions
4. Syngenta stewardship program for event 3272 corn
5. Response to critical technical comments raised in submissions
6. Business cost calculator report

ATTACHMENT 1

DRAFT VARIATION TO *THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE*

Section 94 of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence: on gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting into the* Table to clause 2 –

food derived from amylase-modified corn line 3272	

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

APPLICATION A580 – FOOD DERIVED FROM AMYLASE-MODIFIED CORN LINE 3272

SUMMARY AND CONCLUSIONS

Background

Food derived from α-amylase modified corn line 3272 has been assessed for its safety for human consumption. This corn line has been genetically modified to express a thermostable α-amylase enzyme (AMY797E) for use in dry-grind fuel ethanol production in the United States.

Although α-amylase corn has not been developed primarily for human food uses, if approved, food derived from corn line 3272 may be imported into Australia and New Zealand as alcohol-fortified products and processed food products.

A number of criteria have been addressed in the safety assessment including: a characterisation of the transferred genes, their origin, function and stability; changes at the DNA, protein and whole food levels; compositional analyses; evaluation of the intended and unintended changes, and the potential for the newly expressed proteins to be either allergenic or toxic to humans.

History of Use

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Corn products may include corn syrup, breakfast cereals, corn chips, canned corn, and dry milled goods such as cornflour.

Description of the Genetic Modification

Corn line 3272 contains two novel genes. The first, the *amy797E* gene, encodes the thermostable AMY797E α-amylase enzyme. α-Amylase catalyses the hydrolysis of starch by cleaving the internal α-1,4-glucosidic bonds into dextrins, maltose and glucose.

The chimeric *amy797E* gene is derived from three wild-type α-amylase genes from the archael order *Thermococcales*. The *amy797E* gene encoded protein was selected for further development due to its thermostability, catalytic activity and reduced dependency on calcium under acidic conditions. These properties are important for the commercial application of α-amylase in the enzymatic hydrolysis of starch in dry-grind ethanol production from corn.

The second gene, *pmi* (*manA*), is present as a selectable marker and encodes the enzyme phosphomannose isomerase (PMI) derived from *Escherichia coli*.

Molecular and genetic analyses indicate that the transferred genes are stably integrated into the plant genome at one insertion site and are stably inherited from one generation to the next.

Characterisation of Novel Protein

Corn line 3272 expresses two novel proteins – the enzymes AMY797E and PMI. AMY797E is expressed at relatively high levels in grain (838 –1627µg/g fresh weight or 1004–3365µg/g dry weight) and PMI is expressed at low levels (<0.4– 0.8µg/g fresh weight or <0.5– 1.8µg/g dry weight).

A number of studies have been conducted with both PMI and AMY797E to determine their potential toxicity and allergenicity. Neither protein showed any evidence of toxicity in acute oral toxicity studies and also do not exhibit any amino acid sequence similarity with known protein toxins. Neither of the proteins is derived from sources known to be allergenic and both are rapidly digested in simulated digestion studies. AMY797E has no immunologically meaningful amino acid sequence similarity to known allergens. PMI was found to share a short sequence of amino acids (eight residues) with a known allergen, however further testing with human sera from allergic individuals did not demonstrate any cross-reactivity. Overall, the evidence indicates that AMY797E and PMI are non-toxic and have limited potential to be allergenic in humans.

Compositional Analyses

Compositional analyses were carried out to establish the nutritional adequacy of grain from corn line 3272, in comparison to conventional control lines. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites and anti-nutrients.

No compositional differences of biological significance were observed between the GM corn grain and its non-GM counterpart. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very minor differences and were within the reference range from conventional varieties. It can be concluded that food from corn line 3272 is equivalent in composition to that from other commercial corn varieties.

Nutritional Impact

The detailed compositional studies indicate that food derived from corn line 3272 is equivalent in composition to food from non-GM corn varieties. Minor differences in composition were consistent with normal variation for corn and would not be expected to have any nutritional impact. A feeding study in broiler chickens supports this finding.

As the α-amylase enzyme expressed in corn line 3272 has the ability to reduce starch molecules into component dextrins and mono/disaccharides at high temperatures, its potential impact on glycaemic index, should corn line 3272 be used as human food, was evaluated.

This evaluation concluded that the co-mingling of corn line 3272 with existing corn stocks is unlikely to have any significant effect on population nutrition. It is possible the AMY797E α-amylase may be activated during the cooking or processing of corn line 3272, which could increase the glycaemic index of the final food product.

However, even if the final food's glycaemic index was increased, the overall effect on the diet would be minimal given that glycaemic index is heavily influenced by other dietary factors.

Impacts on Food Technological Processes and Products

Mixing of corn varieties in the USA could result in imported processed products containing amylase. Food products that could potentially contain the enzyme include corn syrups, corn starches and corn chips and flours. Other food products could contain these products as ingredients. Although the presence of this amylase may impact on certain food properties due to the conversion of starch to dextrins and sugars, there is no food safety issue associated with the possible presence of this thermostable enzyme in foods.

Conclusion

No public health and safety concerns have been identified in the assessment of food produced from corn line 3272. On the basis of the data provided in the present application, and other available information, food derived from corn line 3272 can be considered as safe and as wholesome as food derived from other corn varieties.

1. INTRODUCTION

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide . Worldwide production of maize is about 700 million tons a year, with the United States and China being the major producers.

The majority of grain and forage derived from maize is used as animal feed, however maize also has a long history of safe use as food for human consumption. The grain can be processed into industrial products such as ethanol (by fermentation), and highly refined starch (by wet-milling) and sweetener products. In addition to milling, the maize germ can be processed to obtain corn oil and numerous other products.

Corn line 3272 has been genetically modified to express a thermostable α-amylase enzyme (AMY797E) for use in dry-grind fuel ethanol production in the United States. Amylase is used to hydrolyse starch into smaller sugar subunits, which is the first step in producing ethanol from plants. Amylases used for ethanol production need to be able to work at high temperatures and low calcium concentrations. Plants such as corn naturally contain amylases however these are destroyed when corn is subjected to the high processing temperatures necessary for ethanol production, making it necessary to add microbially-produced amylase preparations. The use of corn line 3272, expressing a highly thermostable amylase, bypasses this step.

2. HISTORY OF USE

2.1 Donor Organisms

Thermococcus / Pyrococcus spp.

The three parental α-amylases used to develop AMY797E came from three different archaeal sources of the order *Thermococcales*. The Thermococcales is currently composed of three genera, *Pyrococcus, Thermococcus*, and *Palaeococcus* (Huber and Stetter, 2001: Itoh, 2003). They represent a large and diverse group of organisms which are increasingly being used as model systems for biochemical and physiological studies of hyperthermophilic archaea. Members of the three genera are metabolically similar; they are obligate heterotrophs, growing on organic substrates, usually in the presence of elemental sulphur (S°), which is reduced to hydrogen sulphide (Amend and Shock, 2001). This group of organisms have gained particular attention because of the potential biotechnological applications of the enzymes that have been isolated from them. Because archaeal enzymes are adapted to extreme environments, they are unusually stable which makes them suitable candidates for industrial processes performed under harsh conditions (Schiraldi et al 2002). Many archael enzymes involved in carbohydrate metabolism, particularly those from the glycosyl hydrolase family (such as α-amylase), are of special interest to the industrial biotechnology sector because of their potential value in starch processing where high temperatures are required to liquefy starch and make it accessible to enzymatic degradation.

Two of the parental α-amylase genes used to generate AMY797E, termed BD5031 and BD5064, were isolated from DNA libraries constructed from pure cultures of *Thermococcus* organisms isolated from samples taken from shallow marine hydrothermal systems at 95°C, pH 7.0 and 85°C, pH 6.0, respectively. The third parental α-amylase gene, BD5063, originated from a DNA library constructed from a primary enrichment culture containing an undetermined number of high temperature organisms isolated from the Deep Sea Pacific Ocean hydrothermal system at 90°C, pH 6.5. Based on sequence comparisons, BD5063 is most likely derived from either a *Pyrococcus* or *Thermococcu*s species.

The three wild-type -amylase enzymes were selected for their superior activity under either high temperature, low Ca^{2+} or low pH conditions, all relevant to the starch liquefaction step of corn processing. In order to combine the best features of all three enzymes, a "gene reassembly" recombinant technique was performed in which fragments from the three parental genes were combined (in the same relative position) to create a library of recombinant -amylase enzymes. The chimeric AMY797E -amylase enzyme was identified by screening these recombined enzymes.

The synthesised AMY797E α-amylase enzyme is closely related to the BD5088 α-amylase developed by Innovase LLC for use as an enzyme in the hydrolysis of edible starch to produce various food products and for ethanol production for use in alcoholic beverages. BD5088 was produced using the same technique and from the same three parental α-amylase genes as AMY797E (Richardson *et al.*, 2002). The AMY797E α-amylase protein has 93% amino acid identity to BD5088. BD5088 was the subject of GRAS Notice No. GRN 000126 and a peer-reviewed publication on its safety (Landry et al., 2003). The FDA has reviewed the notice and in its response letter did not question the use of the substance as "generally recognized as safe" (US FDA, 2003).

Escherichia coli

The bacterium *Escherichia coli*, strain K-12, is the source of the *pmi* gene in corn line 3272. *E. coli* belongs to the Enterobacteriaceae, a relatively homogeneous group of rod-shaped, Gram-negative, facultative aerobic bacteria.

Members of the genus *Escherichia* are ubiquitous in the environment and found in the digestive tracts of vertebrates, including humans. The vast majority of *E. coli* strains are harmless to humans, although some strains can cause diarrhoea and *E. coli* is also the most common cause of urinary tract infections. More recently, a particularly virulent strain of *E. coli*, belonging to the enterohaemorrhagic *E. coli* group, known as 0157:H7, has come to prominence as a food-borne pathogen responsible for causing serious illness.

This particular group of pathogenic *E. coli* are distinct from the strains of *E. coli* (the K-12 strains) that are used routinely in laboratory manipulations. The K-12 strains of *E. coli* have a long history of safe use and are commonly used as protein production systems in many commercial, including pharmaceutical and food ingredient, applications.

Agrobacterium tumefaciens

The species *Agrobacterium tumefaciens* is a Gram-negative, non-spore forming, rod-shaped bacterium commonly found in the soil. It is closely related to other soil bacteria involved in nitrogen fixation by certain plants.

Agrobacterium naturally contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its plasmid (transfer DNA, T-DNA) into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing crown gall disease of a wide range of dicotyledonous (broad-leaved) plants, including sugar beets, pome fruit and viniculture crops. However, adaptation of this natural process has now resulted in the ability to transform a broad range of plant species without causing adverse effects in the host plant. *A. tumefaciens* has no known pathogenicity to humans.

2.2 Host Organism

The recipient corn line in this application was a proprietary line A188 from the University of Minnesota.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the genetic modification

Corn line 3272 was produced by *Agrobacterium*-mediated transformation of immature embryos derived from the proprietary line A188 of *Zea mays* (Negrotto *et al*., 2000) using the transformation vector pNOV7013. Between the right and left borders, this plasmid contains the *amy797E* and *pmi* genes and regulatory elements as shown in Figure 1 and Table 1. Transformed cells were grown on cell culture media containing mannose and tested by PCR for the presence of both the *amy797E* and *pmi* genes and the absence of the *spec* gene (an antibiotic resistant marker in the plasmid backbone). Regenerated plants meeting these criteria were transferred to the greenhouse for propagation.

Table 1: Genetic elements in the plasmid pNOV7013

Genetic element	Size (bp)	Function
Right border	25	T-DNA right border region
GZein promoter	677	Promoter region from the *Zea mays* 27-kDa storage protein (*zein*) gene (GenBank Accession Number X56117; NCBI, 2005a). Provides endosperm-specific expression in *Zea mays* (Das *et al.*, 1991).
amy797E	1383	Chimeric, thermostable α-amylase gene, derived from α-amylase genes from three hyperthermophilic micro-organisms of the archael order *Thermococcales.*
PEPC9	108	Intron 9 from the phosphoenolpyruvate carboxylase gene (GenBank Accession Number X15239) from *Zea mays* (Matsuoka and Minami, 1989).
35S terminator	70	Terminator sequence from the cauliflower mosaic virus genome (Similar to GenBank Accession Number AF140604) (Franck *et al*., 1980).
ZmUbiInt	1993	Promoter region and intron from the *Zea mays* polyubiquitin gene. Provides constitutive expression .
pmi	1176	Phosphomannose isomerase gene from *E. coli*. Selectable marker gene .
NOS terminator	253	Terminator sequence from nopaline synthase gene from *A. tumefaciens* .
Left Border	25	T-DNA left border region

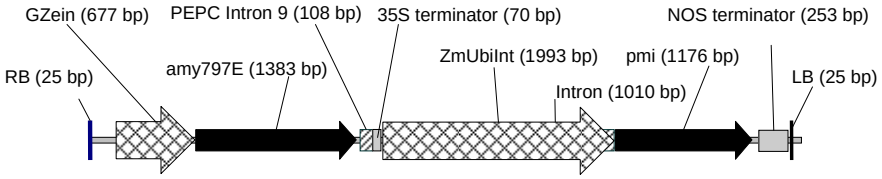


Figure 1: Genes and regulatory elements inserted in corn line 3272

3.2 Function and regulation of novel genes

amy797E

The chimeric *amy797E* gene was generated using Gene Reassembly™ (Diversa Corporation, San Diego, CA) to combine the best features of all three enzymes (Richardson *et al.*, 2002). Fragments from the three parental genes were combined (in the same relative position) based on the natural sequence homology to create a library of recombinant α-amylase genes. The chimeric AMY797E α-amylase enzyme (alternatively known as "797GL3") was identified by screening these recombined enzymes and is composed of four fragments from BD5031, two fragments from BD5064 and three fragments from BD5063.

The *amy797E* gene includes fusions of a 19 amino acid N-terminal maize gamma-zein signal sequence (GZein ss) and a C-terminal SEKDEL endoplasmic reticulum retention signal (ER rs) (Lanahan *et al*., 2003).

The maize gamma-zein signal sequence and the ER retention signal provide for protein targeting to and retention in the endoplasmic reticulum of the cell, respectively. The α-amylase coding region of the *amy797E* gene was synthesized to accommodate the preferred codon usage for corn.

The *amy797E* gene is regulated by the GZein promoter from *Zea mays* and the 35S terminator from the cauliflower mosaic virus.

pmi gene

The *pmi* gene represents the *manA* gene from *E. coli* and encodes the enzyme phosphomannose isomerase (PMI). It was used as a selectable marker gene during the transformation process. Mannose, a hexose sugar, is taken up by plants and converted to mannose-6-phosphate by hexokinase. This product cannot be further utilised in plants as they lack the PMI enzyme. The accumulation of mannose-6-phosphate inhibits phosphoglucose isomerase, causing a block in glycolysis. It also depletes cells of orthophosphate required for the production of ATP. Therefore, while mannose has no direct toxicity on plant cells, it causes growth inhibition. This does not occur in plants transformed with the *pmi* gene as they can utilise mannose as a source of carbon.

The *pmi* gene is regulated by the polyubiquitin promoter (ZmUbilnt) from *Zea mays* and the NOS terminator from *A. tumefaciens*.

3.3 Characterisation of the genes in the plant

Studies submitted:
Molecular Characterization of Event 3272 Maize (Corn) Expressing an AMY797E -Amylase Protein for USDA Petition for Non-Regulated Status, by Chalk, T. and Rabe, S. 2005. Syngenta Seeds Biotechnology Report No. SSB-107-05.

Blast and ORF analysis of maize genomic sequences flanking the event 3272 T-DNA insert, by Markham, T and Hart, H. 2005. Unpublished and confidential commercial information. Syngenta Biotechnology Inc, Report No SSB-119-05.

Breeding pedigree

The original 3272 transformation event (T_0 generation) was crossed with maize inbred lines NP2222 and NP911 creating the F_1 generation. A single plant from each F1 was then backcrossed to the recurrent inbred parent to yield the BC1 generation. Backcrossing to the recurrent inbred parent was repeated until obtaining the BC3 generation for the NP911 backcross and the BC4 generation for the NP2222 backcross (see Figure 2). The NP911 backcross plants were used for the generational stability Southern analyses and the NP2222 backcross plants were used for the Mendelian inheritance studies, functional element Southern analyses and DNA sequencing. Negative segregants from each backcross were used as controls.

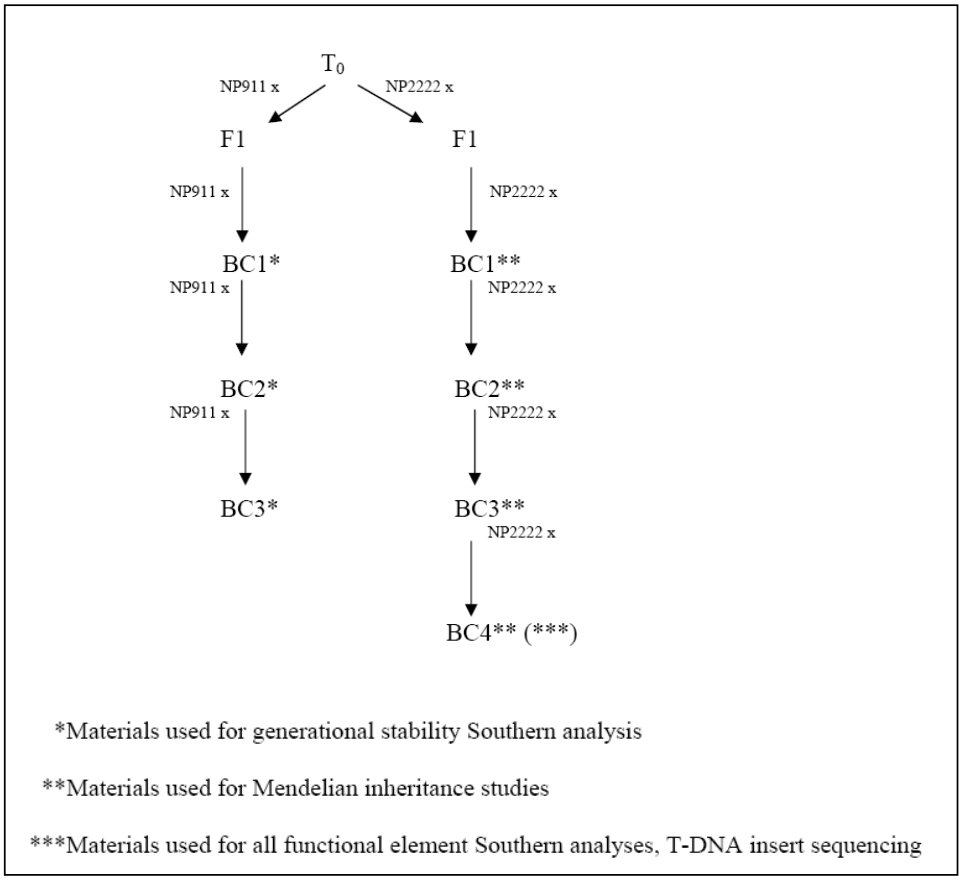


Figure 2: Breeding pedigree indicating the generations tested in the molecular analysis of Event 3272.

Insert and copy number

Southern blot analysis was used to determine the insert and copy number of the *amy797E* and *pmi* genes and to confirm the absence of DNA sequence from outside the T-DNA borders of the transformation vector.

Genomic DNA used for Southern blot analysis was isolated from pooled leaf tissue from ten plants representing the backcross four (BC4) generation of line 3272 and 10 plants representing negative segregants. All plants used for genomic DNA isolation were confirmed for the presence and absence of *amy797E* gene and *pmi* gene using PCR. The Southern analyses demonstrate that event 3272 contains a single copy of the *amy797E* and *pmi* genes inserted as a single piece of T-DNA. The Southern analyses also confirmed the absence of DNA from the plasmid backbone.

Insert sequence analysis

To further characterise the integrity of the inserted T-DNA, the nucleotide sequence of the entire T-DNA insert was determined and compared to the DNA sequence of the transforming plasmid (pNOV7013). The final consensus sequence was determined by combining the sequence data from six individual clones to generate one consensus sequence for event 3272.

In total, 6100 bp of T-DNA was inserted into the corn genome. The consensus sequence data for line 3272 T-DNA demonstrated the overall integrity of the insert and confirmed that the functional elements within the insert had been maintained. Sequence analysis revealed that some truncation occurred at the right and left borders of the T-DNA insert, most likely during the transformation process. The right border portion of the T-DNA insert was truncated by 23 bp and the left border portion by 7 bp. These deletions do not affect the normal function of the *amy797E* and *pmi* gene expression cassettes.

Flanking sequence analysis

One thousand bp of genomic sequence flanking both 5' and 3' of the T-DNA insert was analysed using BLAST and ORF analyses. Short regions of homology were found between the flanking sequence and maize genomic sequence, but this short homology unlikely represents meaningful homology to any known functional sequences. It is therefore concluded that the insertion does not interrupt any maize functional sequences. No novel ORFs were identified spanning either junctions between the T-DNA insert and maize genomic DNA.

Conclusion

Molecular analyses have been performed on corn line 3272 to characterise the novel genes present in the genome. Results indicate that there is one insertion site consisting of the entire T-DNA from plasmid pNOV7013. Sequence analysis of the entire T-DNA insert present in line 3272 confirms that the two novel genes are intact, and that no rearrangements or mutations to either coding or regulatory sequences have taken place. No interruptions to maize functional genomic sequences and no novel ORFs spanning the junctions were found.

3.4 Stability of the genetic changes

Studies submitted:
Molecular Characterization of Event 3272 Maize (Corn) Expressing an AMY797E -Amylase Protein for USDA Petition for Non-Regulated Status, by Chalk, T. and Rabe, S. 2005. Syngenta Seeds Biotechnology Report No. SSB-107-05.

Stability of AMY797E α-amylase and Phosphomannose Isomerase (PMI) Expression over Multiple Generation in Maize (Corn) Derived from Event 3272, by Hill, K. 2005. Syngenta Seeds Biotechnology Report No SSB-005-05.

Generational stability

Southern analyses were done to demonstrate that the insert in corn line 3272 is stable over a number of generations. Genomic DNA for Southern analysis was isolated from pooled leaf tissue of ten plants each from three generations – BC1, BC2 and BC3 – and probed with an *amy797E*-specific probe. Negative segregants from the BC3 generation were used as controls. The hybridization pattern obtained with the *amy797*-specific probe was found to be identical over the three generations examined.

Segregation analysis

The inheritance pattern of the T-DNA insert in line 3272 was investigated. Individual plants from the BC1, BC2, BC3 and BC4 generations were analysed for the presence of the *amy797E* gene by Taqman PCR (Table 2). The expected Mendelian inheritance ratio of positive and negative plants for a hemizygous trait in these populations is 1:1. The goodness-of-fit of the observed genotypic ratio to the expected genotypic ratio was tested using Chi Square (X^2) analysis with Yates correction factor (Strickberger, 1976).

Table 2: Observed vs. Expected* Genotype for Multiple 3272 Generations as determined by Taqman® PCR Analysis. * **O** = Observed values and **E** = Expected values.

	BC1		**BC2**		**BC3**		**BC4**	
	O*	**E***	**O***	**E***	**O***	**E***	**O***	**E***
Trait Positive	95	90.5	15	17.5	122	119	105	102.5
Trait Negative	86	90.5	20	17.5	116	119	100	102.5
Total	181	181	35	35	238	238	205	205
X^2 value	0.354		0.457		0.105		0.078	

This analysis tested the hypothesis that the genetic trait is segregating in a Mendelian fashion. The critical value to reject the hypothesis at the 5% level is 3.84 (Strickberger 1976). Since the Chi squared value is less than 3.84 for all generations tested, the hypothesis that the genetic trait is behaving in a Mendelian fashion is accepted for all generations.

Stability of the expressed protein

The stability of AMY797E and PMI protein expression was evaluated over multiple generations of corn line 3272. Plants from four backcross generations (BC1, BC2, BC4 and BC5) derived from NP2052 (the recurrent parent)/ (NP911/(3272)4)1 were grown under field conditions in Hawaii. Leaf tissue from 5-8 plants that were sampled at the anthesis stage for analysis for PMI was used. Plants were self-pollinated to produce the grain used in this study. At the grain maturity stage, grain was sampled from the same plants from which leaves were sampled for AMY797E analysis. Identical plant tissues from two control plants (negative segregants) per backcross generation were sampled in the same way.

Mean AMY797E concentrations measured in grain from the maturity stage across the four backcross generations were *ca.* 1044 - 1264 µg/g fresh weight (fw; 1147 - 1389 µg/g dry weight; dw). Mean PMI concentrations in leaves from the anthesis stage across the four backcross generations were *ca.* 6.6 - 9.3 µg/g fw (25.6 - 35.7 µg/g dw). Overall, concentrations of AMY797E and PMI protein were similar across the four generations analyzed, demonstrating that the expression of the proteins across multiple generations is stable. Therefore, AMY797E and PMI appear to be stably expressed in corn line 3272 across multiple generations.

Conclusion

The studies indicate that the T-DNA insert is stably integrated into the genome in corn line 3272 and segregates according to a Mendelian pattern of inheritance over four generations. The novel proteins, AMY797E and PMI, are stably expressed from one generation to the next.

3.5 Antibiotic resistance genes

No antibiotic resistance marker genes are present in corn line 3272.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Biochemical function and phenotypic effects

Corn line 3272 contains two novel proteins: AMY797E and PMI.

AMY797E

The precursor protein of AMY797E contains targeting signals at both the N and C-termini. At the N- terminus there is the maize gamma-zein signal peptide for targeting the precursor protein to the endoplasmic reticulum. At the C-terminus there is the SEKDEL signal for retention of the protein in the endoplasmic reticulum. Cleavage of the N-terminal signal from the precursor protein yields the mature AMY797E protein consisting of 441 amino acids with a molecular weight of 50.2 kDa.

The α-amylase activity of the AMY797E protein has been compared to that of generic commercial Bacillus α-amylase using studies such as dose-equivalents, HPLC analysis of the size distribution of starch hydrolysis products, HPLC analysis of residual sugars and organic compounds post-fermentation liquefacts, and ethanol production yield. These studies demonstrate that the AMY797E α-amylase is functionally equivalent in starch hydrolysis to other commercial α-amylases. (Kramer 2005) demonstrated that purified AMY797E protein from grain of corn line 3272 had a specific α-amylase activity of 33,000 U/g test substance using the Ceralpha amylase assay kit from Megazyme (Wicklow, Ireland).

Phosphomannose isomerase

Phosphomannose isomerase (PMI) catalyses the reversible inter-conversion of mannose-6-phosphate and fructose-6-phosphate, and requires zinc for activity. The PMI reaction is specific for mannose-6-phosphate and fructose-6-phosphate and no other natural substrates for PMI are known (Freeze, 2002). The PMI protein produced in line 3272-derived plants is encoded by the native *pmi* gene from *E. coli* (strain K-12). The protein consists of 391 amino acids and has a molecular weight of *ca*. 45 kDa. Plant cells expressing the *pmi* gene are capable of survival and growth in the presence of mannose as the only or primary carbon source. Under the same conditions, plant cells lacking PMI accumulate mannose-6-phosphate fail to grow.

4.2 Protein expression analysis

Studies submitted:
Characterization of Lyophilized Amylase Test Substance (AMY797E-0104) and Certificate of Analysis, by C. Kramer, 2005. Syngenta Seeds Biotechnology Study No. AMY-04-01.

Characterization of Phosphomannose Isomerase (PMI) Produced in Maize (Corn) Plants Derived from Event 3272 and Comparison to PMI as Contained in Test Substance PMI-0198, by G. Graser, 2005. Syngenta Seeds Biotechnology Report No. SSB-022-05.

Quantification of AMY797E and PMI Proteins in Transgenic Maize (Corn) Tissues and Whole Plants Derived from Event 3272, by de Fontes, J. and Kramer, C. 2005. Syngenta Seeds Biotechnology Report No SSB-028-04 A1.

Protein characterisation

The biochemical and functional properties of corn-expressed AMY797E and PMI were characterised using a number of different studies. These studies were also used to determine the equivalence of *E. coli*-expressed PMI to the plant expressed protein, in order that it may be used as a surrogate for plant-expressed PMI in the safety studies.

AMY797E

AMY797E protein was purified from grain of corn line 3272 using a high temperature extraction process. Ultraviolet absorbance at 280 nm indicated that the purified test substance contained *ca*. 460 mg total protein/g test substance. The purity of the test substance determined by densitometric analysis of a coomassie blue-stained SDS-PAGE gel showed that AMY797E represented 91% of the total protein in the test substance. The overall purity was therefore determined to be 42% AMY797E protein in the test substance. The specific activity was determined to be 33,000 U/g test substance.

Following separation of the test substance by SDS-PAGE, coomassie blue staining demonstrated it contained a major band of an approximate molecular mass of 50.2 kDa (consistent with the predicted molecular mass of the mature AMY797E) and a minor low molecular mass band of <14 kDa.

Western blot analysis of the test substance revealed a single immunoreactive band corresponding to the predicted molecular mass of AMY797E of 50.2 kDa for the mature protein. The rabbit polyclonal antiserum used in the Western blot analysis was raised against the 797GL1 protein expressed in bacteria. The 797GL1 protein is the same as AMY797E except that it lacks the C-terminal ER retention sequence. The N-terminal amino acid sequence was determined to be AKYLELEEGG, corresponding to the expected N-terminal sequence of AMY797E after cleavage of the maize gamma-zein peptide signal sequence.

The AMY797E protein in the test substance was analysed for glycosylation and no bands representing glycosylated AMY797E were visible.

PMI

The PMI protein was extracted from leaf tissue from line 3272 and its size, immunoreactivity, and specific enzymatic activity were compared to *E. coli* PMI. The PMI protein expressed in *E. coli* is identical in amino acid sequence to that encoded by the vector used to transform corn producing line 3272, with the exception of 16 amino acids added to the N-terminus as a result of the *E. coli* expression vector.

Samples of leaf extracts from line 3272 and *E. coli* produced PMI were subjected to SDS-PAGE, followed by immunoblotting. A single immunoreactive band corresponding to a molecular mass of 42.8 kDa was detected in four separate leaf extracts. The *E. coli*-expressed PMI protein showed one major band with a slightly lower mobility compared to the plant expressed PMI, consistent with a slightly higher predicted molecular mass of 44.4 kDa (resulted from the additional 16 amino acids at the N-terminus).

The mean enzymatic activity of PMI in leaf extracts ranged from 81.6 – 112.6 U/mg PMI, corresponding to an overall mean enzymatic activity of 96.9 ± 14.2 U/mg PMI. *E. coli*-produced PMI had a slightly lower mean enzymatic activity of 52.9 ± 0.0 U/mg PMI. The two enzyme activities are comparable.

Protein expression levels

The expression levels of AMY797E and PMI in corn line 3272 in several plant tissues (roots, leaves, kernel, pollen) and whole plants at five growth stages were determined by enzyme-linked immuno-sorbent assay (ELISA) The analyses were done in two field grown hybrids, hybrid A and hybrid B, which were hemizygous for both novel genes. For each hybrid, non-transgenic isogenic plants were grown and sampled as controls.

Ten plants per hybrid, plus two plants from each of the corresponding controls were harvested at each of five growth stages: whorl (6-7 weeks), anthesis (10-11 weeks), kernel dough (14-15 weeks), kernel maturity (18-19 weeks) and senescence (21-23 weeks). Protein was extracted from samples of leaves, roots, kernel, pollen, and whole plants and analysed quantitatively for AMY797E and PMI by ELISA. AMY797E and PMI extraction efficiencies were measured and ranged from 62.2 to 100% for various tissues. ELISA values provided were not corrected for extraction efficiency. Limit of quantitation (LOQ) and limit of detection (LOD) values were determined for all plant samples.

AMY797E

Expression of the AMY797E α-amylase in line 3272 plants is driven by the maize gamma-zein promoter for endosperm-specific expression in the kernel. As expected, relatively high levels of AMY797E protein were measured in kernels of both maize hybrids A and B derived from line 3272 (Table 3). Mean AMY797E levels in kernels of both hybrids at all growth stages ranged from *ca*. 838 - 1627 µg/g fw and 1004 - 3365 µg/g dw.

In addition to kernels, AMY797E levels were assessed in roots, leaves, pollen and whole plants at five developmental stages. The expression was essentially undetectable in leaves and pollens and it was detected at low levels in the roots of some whorl stage plants (< 0.1 µg/g fw). Samples from whole plants showed varied expression levels ranging from not detectable (ND) to <327 µg/g fw.

In whole plants at kernel dough, maturity and senescence stages, the presence of a proportion of grain in these samples accounts for the AMY797E protein detected. Control sample levels were either not detectable or below the limit of quantitation (<LOQ) for all stages and tissues, except the whole plants of the kernel dough stage (*ca*. 0.02 - 0.1 µg/g fw, 0.1 - 0.4 µg/g dw), which were slightly above LOQ.

Table 3: AMY797E levels in corn kernels (µg/g)

Genotype	Developmental stage					
	Kernel dough		Kernel maturity		Senescence	
	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight
Hybrid-A	1627 ± 338 (1177–1951)	3365 ± 780 (2286–4151)	905 ± 208 (659 – 1147)	1259 ± 303 (908 – 1562)	955 ± 225 (679 – 1294)	1153 ± 268 (850 – 1573)
Hybrid-B	874 ± 160 (638–1057)	1994 ± 228 (1841–2316)	924 ± 201 (686 – 1130)	1335 ± 358 (893 – 1730)	838 ± 268 (512 – 1168)	1004 ± 322 (624 – 1380)

Data is expressed as mean ± standard deviation (range)

PMI

PMI protein expression in line 3272 plants is driven by the constitutive maize polyubiquitin promoter. PMI was detected at low levels in most of the plant tissues analyzed, except for some senescence-stage samples. Mean PMI levels measured in kernels at all developmental stages (shown in Table 4) ranged from *ca*. <0.4 - 0.8 µg/g fw (<0.5 - 1.8 µg/g dw). PMI levels were generally similar for each of the hybrids at each time point for each tissue type, with the highest levels detected in pollen: *ca*. 8.0 - 8.5 µg/g fw (17.0 - 18.2 µg/g dw). PMI levels in control samples were either ND or <LOQ for all stages and tissues.

Table 4: Phosphomannose isomerase levels in corn kernels (µg/g)

Genotype	Developmental stage					
	Kernel dough		Kernel maturity		Senescence	
	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight
Hybrid-A	0.4 ±0.02 (0.3–0.4)	0.7± 0.1 (0.6–0.8)	<0.4 (LOQ– 0.5)	<0.5 (<LOQ–0.7)	<0.4 (LOQ– 0.4)	<0.5 (<LOQ–0.5)
Hybrid-B	0.8 ± 0.1 (0.6–0.9)	1.8 ± 0.4 (1.3–2.2)	0.5 ± 0.2 (0.4– 0.7)	0.7 ± 0.2 (0.5 – 0.9)	0.4± 0.1 (0.3 – 0.6)	0.5 ±0.2 (0.3 – 0.7)

Data is expressed as mean ± standard deviation (range)

Conclusion

Corn line 3272 expresses two novel proteins – AMY797E and PMI. AMY797E is expressed at high levels in grain, with expression levels ranging from 838 - 1627 µg/g fresh weight (fw) (1004 - 3365 µg/g dry weight (dw)). In comparison, PMI is expressed at low levels in grain, with expression levels ranging from *ca*. <0.4 - 0.8 µg/g fw (<0.5 - 1.8 µg/g dw).

A number of studies were also done on AMY797E and PMI expressed in corn line 3272 to confirm their identity and functional properties. These studies have shown that the two novel proteins conform in size and identity to that expected and also exhibit the expected enzymatic activity.

4.3 Potential toxicity of novel proteins

AMY797E α-amylase

History of use

Currently, there is limited human experience with preparations of α-amylases from the *Thermococcales*, although this is likely to change in the future as a wide range of archaeal enzymes are currently being investigated for their potential use in industrial applications, including in food processing.

While there is currently no history of use of α-amylases from the *Thermococcales*, α-amylases from both fungal and bacterial sources have a long history of safe use in food processing. -amylase enzymes occur widely in nature, including in the gastrointestinal tract of humans, and amylase activity is not known to be associated with toxicity.

Acute oral toxicity study

Studies submitted:
AMY797E-0104: Single Dose Oral Toxicity Study in the Mouse, by E. Barnes, 2005. Syngenta Seeds Inc, Study No. AM7506.

Characterization of Lyophilized Amylase Test Substance (AMY797E-0104) and Certificate of Analysis, by C. Kramer, 2005. Syngenta Seeds Biotechnology Study No. AMY-04-01.

Test substance	AMY797E protein preparation purified from grain of transgenic corn line 3272 (42% AMY797E protein w/w in test substance)
Vehicle	0.5% w/v aqueous carboxymethyl cellulose
Test species	AP_fCD-1 mice (five males and five females per group)
Dose	1511 mg/kg bw test substance, equivalent to 635 mg/kg body weight AMY797E by gavage
Control	Vehicle only

Groups of five male and five female mice were dosed orally by gavage with 0 (control) or 635 mg AMY797E /kg body weight (bw) and were observed for two weeks. Clinical observations, body weight and food consumption were measured throughout the study. At the end of the study, all animals were killed and subjected to necropsy. Blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were processed and examined for histopathological changes.

No treatment-related effects were observed in mice following the oral administration of 635 mg AMY797E /kg bw.

Similarities with known protein toxins

Studies submitted:
AMY797E Protein as Expressed in Transgenic Event 3272: Assessment of Amino Acid Homology with Known Toxins, by Hart, H. 2005. Syngenta Seeds Biotechnology Report No SSB-108-05.

To determine whether the AMY797E α-amylase has any significant amino acid similarity with known protein toxins, the precursor protein sequence, including the gamma-zein signal sequence and endoplasmic reticulum signal as expressed in corn line 3272, was systematically compared to the latest posting of the National Centre for Biotechnology Information Entrez Protein Database (NCBI, 2005) containing all publicly available protein sequences. The cut-off expectation (E) value was determined to be 0.035 and amino acid sequences with E values lower than this were considered to be significant.

Seven hundred and eleven entries in GenBank were returned with E values below 0.035. Most of these (649) were identified as enzymes involved in carbohydrate metabolism. The remaining 62 entries were identified as hypothetical or unnamed proteins or proteins with unknown function. No significant sequence homology to any proteins identified as, or known to be, toxins was identified.

Phosphomannose isomerase

History of use

PMI is an enzyme commonly found in nature. The gene encoding PMI has been found in several bacteria and yeast species, some plant species such as soybeans and other legumes, and is also present in mammals, including humans (Privalle et al 2000). PMI has also been detected in human intestinal flora. The ubiquitous nature of PMI indicates that humans are likely to have been continually exposed to small amounts of PMI from various sources through the diet.

Acute oral toxicity study

Study submitted:
Phosphomannose isomerase: Acute Oral Toxicity Study in Mice, by J. Kuhn, 1999. Stillmeadow Inc. Sugar Land TX. Study No. 4708-98.

Characterization of Phosphomannose Isomerase (PMI) Produced in Maize (Corn) Plants Derived from Event 3272 and Comparison to PMI as Contained in Test Substance PMI-0198, by G. Graser, 2005. Syngenta Seeds Biotechnology Report No.SSB-022-05.

As it is difficult to extract and purify sufficient quantities of PMI protein from corn line 3272 for the acute oral toxicity studies given its low expression levels, the study was done using the equivalent protein produced in the laboratory using a bacterial expression system. Prior to use, the bacterially produced PMI was compared to PMI produced *in planta* in order to establish their equivalence.

The molecular identity and biochemical characteristics of PMI expressed *in planta* and in the bacterial-expression system was examined by analysis of biochemical and functional parameters, including molecular weight, immunoreactivity, and specific enzyme activity. The PMI proteins from both sources were confirmed to have the predicted molecular weights and both immunologically cross-reacted with the same anti-PMI antibodies. The PMI proteins from the bacterial-expression system and corn line 3272 were therefore determined to be equivalent and the microbial test substance PMI-0198 was considered a suitable surrogate for PMI protein produced in corn line 3272.

Test material	Phosphomannose isomerase preparation from *E. coli* (60% phosphomannose isomerise protein) (PMI-0198)
Vehicle	0.5% w/v aqueous carboxymethyl cellulose
Test Species	HSD:ICR albino mice (seven males and six females per group)
Dose	5050 mg/kg bw (equivalent to 3080 mg/kg bw PMI protein) in two gavage doses, 1 hour apart.
Control	Vehicle only

Seven male and six female mice received a gavage dose of 3080 mg PMI /kg bw (administered in two parts, 1 hour apart) and were observed for two weeks for any signs of acute oral toxicity. Parameters evaluated included body weights and detailed clinical observations. At the end of the study all animals were killed and subject to necropsy. Brain, liver, kidneys and spleen were weighed.

One male in the control group and two in the test group died shortly after dosing or were in distress after dosing and subsequently died. Necropsy revealed perforated oesophagi in these animals, a sign of gavage error and not test-substance related. One replacement animal was available for each group and dosed in the same manner on day 0. There was no test article-related mortality during the study.

No clinical signs of toxicity were observed in either group. There were no test-substance related effects on body weight, organ weights or gross pathology.

Under the conditions of this study, the acute oral LD_{50} of the PMI protein in mice was determined to be greater than 3080 mg PMI /kg bw.

Similarity to known protein toxins

Studies submitted:
Phosphomannose Isomerase Protein: Assessment of Amino Acid Sequence Homology with Known Toxins, by Hart, H. 2005. Syngenta Seeds Biotechnology Report No SSB-120-05.

To determine whether the PMI protein has any significant similarity with known protein toxins, its amino acid sequence was systematically compared to the latest posting of the NCBI Entrez Protein Database (NCBI, 2005) containing all the publicly available protein sequences. The cut-off expectation (E) value was determined to be 0.14 and amino acid sequences with E values lower than this were considered to be significant.

One hundred and forty one protein entries in GenBank were returned with E values below 0.14. One hundred and eighteen of these were identified as known or putative PMI proteins. The remaining 23 entries were identified as hypothetical proteins or unnamed proteins or proteins with unknown function. No significant sequence homology to any proteins identified as, or known to be, toxins was identified.

Other relevant studies

Study submitted:
Corn Amylase Event 3272: 90 Day Whole Food Safety Study in Rats, by E. Barnes, 2005. Syngenta Central Toxicology Laboratory Document No CTL/PR1307.

Test substance	Amylase corn line 3272 positive grain
Diet	CTI
Test species	AP_fSD rats (12 males and 12 females per group)
Dose	10% and 41.5% corn grain in the diet
Control	Amylase corn line 3272 negative isoline grain

To investigate any adverse effects of food from corn line 3272, groups of male and female rats were fed diets incorporating the corn line 3272 grain at 10% and 41.5% for at least 90 consecutive days. The low dose (10%) represents a level that is at least equivalent to the human chronic dietary intake of maize (3 g/kg bw/day) and the high dose (41.5%) was selected as the highest achievable without causing nutritional imbalances in the rats. The study was designed in accordance with OECD, US EPA and US FDA guidelines for 90 day oral toxicity studies in rodents.

Clinical observation, bodyweights and food consumption were measured throughout the study; a functional observation battery of tests and monitoring of locomotor activity were performed during week 13. An ophthalmoscopic examination was performed on all animals pre-study and in week 13. At the end of the scheduled period, the animals were killed and subjected to necropsy. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specific tissues were taken for subsequent histopathological examination.

No differences were observed in bodyweight, food consumption, clinical condition (including ophthalmoscopy and functional observation battery), clinical pathology, organ weights or histopathology between the animals receiving corn line 3272 and controls. The inclusion of grain from corn line 3272 in the diet of rats at 10 and 41.5% for 90 consecutive days therefore was not associated with any treatment-related adverse effects.

Conclusion

The results from the acute oral toxicity studies and bioinformatics analyses of the novel proteins indicates that neither of the proteins is toxic at high levels in mice, nor do they show any similarity with known protein toxins. The lack of adverse findings in a 90 day feeding study in rats supports this conclusion.

4.4 Potential allergenicity of novel proteins

A possible concern is that new proteins introduced into food will cause allergic reactions in some individuals. The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid similarity between the novel protein and that of known allergens, and the structural properties of the novel protein, including susceptibility to degradation in simulated digestion models.

Applying such criteria systematically provides reasonable evidence about the potential of the newly introduced proteins to act as an allergen .

AMY797E α-amylase

Studies submitted:
In vitro Digestibility of AMY797E α-Amylase (Test Substance AMY797E-0104) Under Simulated Mammalian Gastric Conditions, by de Fontes, J. and Kramer, C. 2005. Syngenta Seeds Biotechnology Report No SSB-034-04 A1.

Amylase Protein as Expressed in Transgenic Maize Event 3272: Assessment of Amino Acid Sequence Homology with Known Allergens, by Hart, H. 2005. Syngenta Seeds Biotechnology Report No SSB-136-05.

Source of the protein

AMY797E is a chimeric protein derived from three different α-amylases from the archaeal order *Thermococcales*. The *Thermococcales* are not known to be allergenic to humans, although currently there is limited human experience with these organisms, either in occupational exposure situations or via food.

Similarity to known allergens

To determine whether AMY797E has any significant amino acid sequence homology with allergenic proteins, the protein sequence was systematically compared to the Syngenta Biotechnology Inc (SBI) allergen database version 4.

This database was compiled from entries identified as allergens or putative allergens in public protein databases, and was supplemented with additional amino acid sequences identified from the scientific literature. The latest version of the SBI allergen database (version 4.0) contains a total of 1414 non-redundant entries and was last updated in March 2005.

Overall similarity was examined by comparing sequential 80-amino acid sequences covering the entire AMY797E protein sequence (such that each 80-amino acid window was offset from the previous one by one residue and overlapped by 79 residues) to the allergen sequences using the FASTA search algorithm. Any 80-amino acid peptide having greater than 35% amino acid identity was defined as having significant similarity to the allergen sequence .

The AMY797E sequence was also screened for matches of eight or more contiguous amino acids. The purpose of this is to identify any short local regions of identity that might indicate the presence of common IgE binding epitopes.

No significant similarity was found between any of the sequential AMY797E α-amylase 80-amino acid peptides and any entries in the SBI allergen database.

There was one region of sequence homology of eight contiguous identical amino acids between AMY797E α-amylase and a known species-specific allergen, Per a 3, from the American cockroach. The IgE-binding epitopes of Per a 3 have been identified (Wu *et al*., 2003) and there is no overlap between these binding epitopes and the region of sequence homology with AMY797E α-amylase.

Therefore, the observed sequence identity between AMY797E α-amylase and Per a 3 is not biologically relevant and has no implication for the allergenic potential of the AMY797E α-amylase.

In relation to similarity to fungal α-amylases, which are know to cause allergic reactions in some individuals, AMY797E was found to have less than 17% sequence identity to an α-amylase from *A. oryzae* (CAA31218), with no more than four contiguous identical amino acids.

In vitro digestibility

AMY797E from corn line 3272 was digested in simulated gastric fluid, at pH 1.2 containing 10 units pepsin/µg protein. Samples were examined by SDS-PAGE and Western blot analysis. No intact AMY797E (approximately 50.2 kDa) or immunoreactive fragments were detected following digestion for five minutes. This indicates that AMY797E is readily digested in the peptic and acidic conditions of the digestive system.

Glycosylation

As indicated in section 4.2, AMY797E purified from corn line 3272 was assessed for glycosylation by DIG glycan detection analysis. No evidence of glycosylation of corn-expressed AMY797E was found.

Heat stability

The AMY797E α-amylase is a highly thermostable protein, being derived from organisms inhabiting high temperature environments. The enzyme therefore would be expected to remain stable and active at very high temperatures, e.g. 85-95ºC.

Phosphomanno isomerase

Studies submitted:
Phosphomannose Isomerase Protein: Assessment of Amino Acid Sequence Homology with Known Allergens, by Hart, H. 2005. Syngenta Seeds Biotechnology Report No SSB-140-05.

Effects of Temperature on the Stability of Phosphomannose Isomerase (PMI), by Hill, K. 2003. Syngenta Seeds Biotechnology Report No SSB-013-03.

In vitro Digestibility of PMI Protein under Simulated Mammalian Gastric and Intestinal Conditions, by Privalle, L, 1999. Novartis Seeds Biotechnology Report No. NSB-002-99.

Source of the protein

The *pmi* gene was cloned from *E. coli*, which is not known to be source of allergens.

Similarity to known allergens

To determine whether PMI as expressed in corn line 3272 has any significant homology with known allergenic proteins, the PMI amino acid sequence was systematically compared to the SBI allergen database version 4.

Overall similarity was examined by comparing sequential 80-amino acid sequences covering the entire PMI protein sequence to the allergen sequences using the FASTA search algorithm. Any 80-amino acid peptide having greater than 35% amino acid identity was defined as having significant similarity to the allergen sequence .

The PMI sequence was also screened for matches of eight or more contiguous amino acids. The purpose of this is to identify any short local regions of identity that might indicate the presence of common IgE binding epitopes.

There was no significant similarity between any of the sequential 80-amino acid peptides and any entries in the SBI allergen database.

There was one region of sequence homology of eight contiguous identical amino acids between PMI and a known allergen, α-parvalbumin from *Rana species* CH2001 (unidentified edible frog) (Hilger *et al*., 2002). Further investigation using sensitive serum screening methodology (Codex Alimentarius Commission, 2003) demonstrated no cross-reactivity between PMI and the serum from the single individual known to have demonstrated IgE-mediated allergy to this specific α-parvalbumin. The patient's serum did not recognize any portion of the PMI protein as an allergenic epitope, indicating the sequence identity between the two proteins is not biologically relevant.

In vitro digestibility

PMI was digested in simulated gastric fluid containing pepsin and in simulated intestinal fluid containing pancreatin. Samples were examined by SDS-PAGE. PMI was degraded rapidly by pepsin: no PMI was detected by SDS-PAGE upon immediate sampling of the reaction mix (0 seconds). When the pepsin was diluted to 0.0001 times the standard concentration, no PMI remained after 10 minutes of incubation. Similarly, no PMI enzymatic activity was detectable after 10 minutes under these conditions. PMI was degraded by pancreatin in simulated intestinal fluid after two minutes.

Glycosylation

The PMI protein, as expressed in corn line 3272, was not directly analysed to determine if it had been glycosylated. There is low likelihood that PMI would be glycosylated because it contains no consensus sequences for N-glycosylation (although O-glycosylation could theoretically occur at the serine or threonine residues present in the protein) and the protein itself is not targeted to a cellular glycosylation pathway.

Heat stability

The stability of PMI to heat was evaluated. Loss of enzyme activity was used to determine the instability of the protein after exposure to various temperatures (25, 37, 55, 65 and 95ºC) for 30 minutes. Incubation at ambient temperature (25ºC), 37ºC or 55ºC for 30 minutes had little effect on enzyme activity. Incubation at 65ºC and 95ºC essentially inactivated the enzyme.

Conclusion

A number of studies have been done with AMY797E and PMI to determine their potential allergenicity. Neither of the proteins is derived from sources known to be allergenic, although, in the case of AMY797E there is limited human experience with the source organisms. AMY797E was not found to exhibit any meaningful amino acid sequence homology with known allergens, including fungal sources of α-amylase which are known to be human allergens. PMI was found to share 8 contiguous amino acids with a known allergen (α-parvalbumin) but further screening with human serum did not demonstrate any cross-reactivity. Neither of the proteins are considered likely to be glycosylated and both are rapidly digested in simulated digestion studies, with AMY797E exhibiting slightly more resistance to pepsin than PMI. PMI is inactivated at high temperatures, whereas AMY797E is heat stable. Given the digestive lability of AMY797E, the stability of the enzyme to heat treatment does not raise any concerns regarding potential allergenicity. Taken together, the evidence indicates that AMY797E and PMI have limited potential to be allergenic to humans.

5. COMPOSITIONAL ANALYSES

Study submitted:
Compositional Analysis of Grain and Forage from Transgenic Maize Event 3272 with an Introduced -Amylase (AMY797E) Enzyme, by Kramer, C. and de Fontes, J. 2005. Syngenta Seeds Biotechnology Report No SSB-101-05.

A comparative approach focussing on the determination of similarities and differences between the GM food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods. A critical part of the comparative assessment is the compositional analysis. The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question.

The key nutrients and toxicants/anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased). The key components of corn that should be considered in the analysis include protein, fat, carbohydrates, amino acids, fatty acids, vitamins, minerals, phytic acid and various secondary plant metabolites.

It is worth noting that most crops, including corn, exhibit considerable variability in their nutrient composition. Environmental factors and the genotype of the plant can have a significant impact on composition of the food derived from the plant. Thus, variation in nutrient content is to be expected and is regarded as normal.

5.1 Study design

To determine whether unexpected changes had occurred in the composition of grain from corn line 3272 as a result of the genetic modification, and to assess the nutritional adequacy of food from this line, compositional analysis was done on grain from hybrid pairs (a pair consisting of the transgenic and non-transgenic near isogenic control plants).

The hybrid pairs were designated as follows

In 2003: Pair 1: A1= 3272 positive grain; A2 = control grain
Pair 2: B1 = 3272 positive grain; B2 = control grain

In 2004: Pair 3: B3 = 3272 positive grain; B4 = control grain

The transgenic corn and control lines were grown at three replicate plots at 6 locations in 2003 and 7 locations in 2004. Grain samples were from pooled ears harvested from 15 plants from each genotype from each replicate plot at each location. Analyses performed are listed in Table 5.

For each analyte, data for each year were considered separately, and were subjected to analysis of variance where effects of genome, location and block within location as well as effects of location and genotype interactions were analysed. The statistical significance of the genotype effect was determined using a standard F-test. An F-test probability of <5% indicates that the difference between the genotypes was statistically significant. An F-test was also used to assess the significance of the location and genotype interaction and a significant outcome (F-test probability <5%) indicates that the effect of genotype was not consistent across all locations.

Table 5: Analytes Measured in Grain

Starch
Acid Detergent Fiber (ADF)
Neutral Detergent Fiber (NDF)
Total Dietary Fiber (TDF)
Minerals: Ca, Cu, Fe, Mg, Mn
P, K, Na, Zn, Se
Amino Acid Composition
Fatty Acid Profile (5 abundant)
Beta Carotene
Cryptoxanthin
Folic Acid
Vitamin B_1 (Thiamine)
Vitamin B_2 (Riboflavin)
Vitamin B_3 (Niacin)
Vitamin B_6
Vitamin C
Vitamin E (Tocopherols)

Proximates:
ash
fat
moisture
protein
carbohydrate
Antinutrients:
ferulic and p-coumaric acids
furfural
inositol
phytic acid
raffinose
trypsin inhibitor

5.2 Key nutrients

Proximates

Results of the proximate analysis for grain are shown in Table 6 with literature ranges presented in Table 7.

Sporadic significant differences are noted in grain but are not consistently associated with the presence of the inserted DNA (Table 6). Mean TDF levels were lower in grain from the transgenic hybrid B1 and B3 grown in 2003 and 2004. However, a degree of variability in genotype and location interactions was identified, which reduces the significance of this result. All proximate levels (including TDF and protein) for all hybrids were within levels reported in the literature (Table 7), except for starch, which was lower than reported values for both hybrids in 2004. The published starch data consist of a relatively small number of data points and may not be a complete reflection of the natural variation of this analyte in maize grain.

Minerals

Although not essential for the compositional analysis of corn, the mineral composition of grain from line 3272 hybrids and corresponding non-transgenic hybrids is shown in Tables 8. No consistent statistically significant differences were observed. Sodium and selenium levels were <LOQ in many samples. All values were within the ranges reported in the literature (Tables 9).

Vitamins

Vitamin analyses are shown in Table 10. For some of the vitamins the genotype and location interaction effect was too variable to allow for statistical analysis of genotype effect, so only the average values are shown. Where statistical analysis was possible, no consistent significant differences were observed. All analytes measured were within natural variation, where sufficient data to establish those ranges is reported (Table 11).

Amino acids

Amino acid composition of grain from line 3272 hybrids and the control hybrids is shown in Table 12. Amino acid levels in line 3272 hybrid B1 were significantly higher than in the control B2. This difference was consistently observed at most locations in 2003 and correlates with the small but statistically significantly increased protein levels observed for that same hybrid in 2003. The magnitude of the difference was small and there were no significant differences observed for hybrid B3 grown in 2004, or for hybrid A1, except for tryptophan. Tryptophan was higher in the grain from line 3272 for both years and for both hybrids. All levels of amino acids were within published ranges (Table 13), therefore it is unlikely that there is any biological significance associated with this difference.

Fatty acids

Levels of the five most abundant fatty acids in grain from line 3272 hybrids and the control grain are shown in Table 14. No statistically significant differences were observed and all values are within the ranges reported in the literature (Table 15).

5.2 Key anti-nutrients and secondary plant metabolites

A number of key anti-nutrients and secondary plant metabolites are recognised for maize (OECD 2002). The anti-nutrients are phytic acid, DIMBOA and raffinose; and the secondary plant metabolites are: furfural, ferulic acid and *p*-coumaric acid.

Maize or corn also contains low levels of the anti-nutrients trypsin and chymotrypsin inhibitor, neither of which is considered nutritionally significant. There are no known toxicants in corn.

The secondary plant metabolites and anti-nutrients that were analysed for the compositional analysis are shown in Table 16.

Furfural level was below the LOQ in all grain samples and was therefore not presented in Table 16. Statistically significant differences were noted for ferulic acid in one hybrid pair with lower levels in the line 3272 samples compared to the control samples, but this difference was not observed in other hybrid pairs. All other substances, while quantifiable, did not show significant differences and the levels were within reported values (Table 17).

5.2 Conclusion

Detailed compositional analyses of key nutrients, anti-nutrients and secondary plant metabolites were done on grain from hybrids derived from corn line 3272 compared to conventional counterpart hybrids. Several minor differences in key nutrients and other constituents were noted, however, the levels observed were generally within the range of natural variation for commercial corn lines and do not indicate an overall pattern of change that would warrant further investigation. Overall, food from corn line 3272 is equivalent in composition to food from conventional corn.

Table 6: Proximate Composition of Grain from Corn Line 3272 (all values % dry weight except moisture)

2003	Moisture (% fw)	Protein	Fat	Ash	Carbohydrates	ADF	NDF	TDF	Starch
A1 (Positive)	10.27	10.88	3.14	1.46	84.52	4.20	10.96	11.83	63.3
A2 (Negative)	10.04	10.54	3.23	1.37	84.86	4.18	11.22	12.44	60.8
F-test Probability for Genotype		6.0%	41.9%	4.9%	11.6%	95.0%	57.4%	5.7%	*
B1 (Positive)	10.36	10.74	3.68	1.35	84.22	4.73	11.24	12.29	63.3
B2 (Negative)	10.17	10.05	3.66	1.29	85.02	4.79	12.00	13.94	60.3
F-test Probability for Genotype		**0.2%**	82.7%	6.0%	**0.6%**	83.9%	8.0%	**0.4%**	**0.2%**

2004	Moisture (% fw)	Protein	Fat	Ash	Carbohydrates	ADF	NDF[1]	TDF	Starch
B3 (Positive)	10.00	9.57	3.78	1.54	85.06	4.69	11.40	13.85	50.5
B4 (Negative)	10.28	9.43	3.83	1.54	85.20	5.32	12.61	16.24	47.2
F-test Probability for Genotype		34.1%	73.7%	100.0%	45.3%	4.3%	**2.3%**	**<0.1%**	47.9%

* genotype x location interaction too variable for statistical analysis of genotype effect.

[1] NDF value for hybrid B3, replicate 2, at Brookings location, excluded (outlier)

Table 7: Proximate Composition of Maize Grain Reported in the Literature (all values % dw except moisture)

Source		Moisture %fw	Protein	Fat	Ash	Carbohydrate	ADF	NDF	TDF	Starch
OECD (2002)	**Range**	7.0 - 23	6 - 12.7	3.1 - 5.8	1.1 - 3.9	82.2 - 82.9	3.0 - 4.3	8.3 - 11.9	11.1	
ILSI (2004)	**Range** **Average** **N**	6.1 - 26.2 11.2 773	6.15 - 15.01 10.25 773	1.742 - 5.564 3.554 719	0.616 - 6.282 1.44 749	77.4 - 89.5 84.7 749	1.82 - 11.34 3.79 725	5.59 - 22.64 11.01 725	11.8 - 25.63 16.22 80	67.8 - 73.8 71.8 24
USDA (2004a)[a]	**Average** **N**	10.37 10	9.42 7	4.74 5	1.2 4	74.26				
Watson (1987)	**Range** **Average**	7 - 23 16	6 - 12 9.5	3.1 - 5.7 4.3	1.1 - 3.9 1.42		3.3 - 4.3 3.3	8.3 - 11.9 9.5		61 - 78 71.7
Souci (1994)	**Range** **Average**	12 - 13.2 12.5	7.61 - 9.84 8.54	3.20 - 4.30 3.8						60.98 - 63.80 61.45

[a] USDA values supplied as g/100g, shown here as %

Table 8: Mineral Composition[1] of Grain from Corn Line 3272 (mg/100g dry weight)

2003	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorus	Potassium	Zinc	Selenium[2]
A1 (Positive)	4.26	0.168	2.42	112	0.650	296	363	2.29	<0.011
A2 (Negative)	4.42	0.176	2.42	111	0.619	293	365	2.27	<0.010
F-test Probability for Genotype	30.6%	38.8%	97.0%	79.8%	14.6%	74.2%	87.7%	77.6%	
B1 (Positive)	4.16	0.180	2.25	110	0.568	279	349	2.04	<0.0108
B2 (Negative)	4.11	0.179	2.17	106	0.515	273	356	2.02	<0.0105
F-test Probability for Genotype	86.6%	92.8%	9.3%	13.2%	**0.2%**	45.8%	35.3%	58.1%	

2004	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorus	Potassium	Zinc	Selenium
B3 (Positive)	4.51	0.192	2.20	125	0.566	319	394	2.09	<0.0240
B4 (Negative)	4.56	0.187	2.06	125	0.542	315	390	2.04	<0.0206
F-test Probability for Genotype	51.4%	25.4%	24.4%	90.5%	11.4%	48.5%	48.7%	9.7%	

[1] Sodium levels in all samples <Limit of Quantitation (LOQ)

[2] Where some of the sample values were <LOQ (selenium), an estimated average is calculated using the known LOQ for that analyte

Table 9: Mineral Composition of Maize Grain Reported in the Literature (dry weight)

Source		Calcium	Copper	Iron	Magnesium	Manganese
OECD (2002)	**Range**	3 - 100 mg/100g	0.09 - 1.0 mg/100g	0.1 - 10 mg/100g	82 - 1000 mg/100g	
ILSI (2004)	**Range** **Average** **N**	21.6 - 208.4 ppm 47.3 ppm* 720	0.73 - 5.01 ppm 1.72 ppm 625	10.42 - 49.07 ppm 21.78 ppm 632	788.3 - 1605.5 ppm 1199.4 ppm 633	2.61 - 11.25 ppm 6.25 ppm 632
USDA (2004a)	**Average** **N**	7 mg/100g 4	0.314 mg/100g 6	2.71 mg/100g 6	127 mg/100g 1	0.485 mg/100g 3
Watson (1987)	**Range** **Average**	0.01 - 0.1 % 0.03 %	0.9 - 10 mg/kg 4.0 mg/kg	1 – 100 mg/kg 30.0 mg/kg	0.09 - 1.0 % 0.14 %	0.7 - 54 mg/kg 5.0 mg/kg
Souci (1994)	**Range** **Average**	10.0 - 19.0 mg/g 15.0 mg/g	70.0 - 250.0 ug/g	0.5 - 2.40 mg/g	 120.0 mg/g	150 – 800 ug/100g 480 ug/100g

*ppm = mg/kg

Source		Phosphorus	Potassium	Sodium	Zinc	Selenium
OECD (2002)	**Range**	234 - 750 mg/100 g	320 - 720 mg/100g	0 - 150 mg/100 g	1.2 - 3.0 mg/100g	0.001 - 0.1 mg/100g
ILSI (2004)	**Range** **Average** **N**	2080.5 - 4341.8 ppm 3314.8 ppm 725	2710.0 - 5275.6 ppm 3808.4 ppm 633	5.08 - 440.18 ppm 111.86 ppm 43	6.5 - 37.2 ppm 21.4 ppm 633	0.07 - 0.36 ppm 0.18 ppm 7
USDA (2004a)	**Average** **N**	210 mg/100g 5	287 mg/100 g 1	35 mg/100g 1	2.21 mg/100g 5	15.5 ug/100g 5
Watson (1987)	**Range** **Average**	0.26 - 0.75% 0.29%	0.32 - 0.72% 0.37%	0.0 - 0.15% 0.03%	12 - 30 mg/kg 14.0 mg/kg	0.01 - 1.00 mg/kg 0.08 mg/kg
Souci (1994)	**Range** **Average**	 256 mg/100g	310.0 - 350.0 330.0 mg/100g	1.0 - 10.0 6.0 mg/100g	 2.5 mg/100g	 16 ug/100g

Table 10: Vitamin Analysis[1] of Grain from Corn Line 3272 (mg/kg dry weight except where indicated)

2003	Beta Carotene (RE/g)[a]	Cryptoxanthin (RE/g)	Folic Acid	Vitamin B1 Thiamine	Vitamin B2 Riboflavin	Vitamin B3 Niacin	Vitamin B6	Vitamin C	Tocopherol (mg/100g)	Gamma Tocopherol (mg/100g)
A1 (Positive)	0.131	0.044	0.638	4.03	1.27	23.30	6.05	< LOQ	<0.797	3.43
A2 (Negative)	0.159	0.051	0.629	4.08	1.23	23.13	6.38	< LOQ	0.761	3.44
F-test Probability for Genotype	13.1%	*	79.8%	31.0%	12.0%	72.6%	4.4%			93.7%
B1 (Positive)	0.246	0.048	0.678	4.38	1.15	20.70	6.10	< LOQ	<0.799	3.62
B2 (Negative)	0.262	0.049	0.592	4.08	1.16	21.82	6.18	< LOQ	<0.954	3.63
F-test Probability for Genotype	40.0%	58.7%	5.9%	**1.0%**	84.0%	18.8%	72.7%			94.9%

2004	Beta Carotene (RE/g)	Cryptoxanthin (RE/g)	Folic Acid	Vitamin B1 Thiamine	Vitamin B2 Riboflavin	Vitamin B3 Niacin	Vitamin B6	Vitamin C	Tocopherol (mg/100g)	Gamma Tocopherol (mg/100g)
B3 (Positive)	0.159	0.042	0.671	4.52	1.77	25.30	5.00	<10.79	<0.717	3.94
B4 (Negative)	0.178	0.045	0.694	4.66	1.76	25.04	5.30	<11.36	<0.701	3.93
F-test Probability for Genotype	*	*	68.2%	*	90.1%	54.4%	**4.2%**			87.4%

* genotype x location interaction too variable for statistical analysis of genotype effect.

[1] Beta tocopherol and delta tocopherol <Limit of Quantitation (LOQ) in all samples

[a] 1 RE of beta-carotene = 6 ug (example calculation: 0.131 RE x 6 ug/g = 0.786 ug/g = 0.786 mg/kg = 0.0786 mg/100g

Where some of the sample values were <LOQ (vitamin C, tocopherol) an estimated average is calculated using the known LOQ for that analyte and the quantifiable values, and expressed as < the estimated average.

Table 11: Vitamin Analysis of Maize Grain Reported in the Literature (dry weight).

		Beta Carotene	Cryptoxanthin	Folic Acid	Vitamin B1 Thiamin	Vitamin B2 Riboflavin	Vitamin B3 Niacin
OECD (2002)	**Range**	mg/kg 0.49 - 2.18 RE			mg/kg 2.3 - 8.6	mg/kg 0.25 - 5.6	mg/kg 9.3 - 70
ILSI (2004)	**Range** **Average** **N**	0.053 - 1.640 0.67 mg/100g 28		0.0147 - 0.1209 0.0576 mg/100g 341	0.126 - 0.854 0.371 mg/100g 342	0.070 - 0.193 0.112 mg/100g 326	1.411 - 3.628 2.021 mg/100g 80
USDA (2004a)	**Average** **N**				0.385 mg/100g 1	0.201 mg/100g 1	3.627 mg/100g 1
Watson (1987)	**Range** **Average**	2.5 mg/kg		0.3 mg/kg	3.0 - 8.6 3.8 mg/kg	0.25 - 5.6 1.4 mg/kg	9.3 - 70 28 mg/kg
Souci (1994)	**Range** **Average**	74 - 960 923 ug/100g	370 ug/100g	20.0 - 40.0 26.0 ug/100g	200.0 - 600.0 360 ug/100g	100 - 240 200 ug/100g	

		Vitamin B6 Pyridoxine	Vitamin C	Vitamin E	Beta Tocopherol	Gamma Tocopherol	Delta Tocopherol	Total Tocopherol
OECD (2002)	**Range**	4.6 - 9.6 mg/kg	0 mg/100g					
ILSI (2004)	**Range** **Average** **N**	0.457 - 0.732 0.625 mg/100g 80	0 mg/100g	0.0015 - 0.0687 0.01 mg/g 609	0.081 - 2.280 0.849 mg/100g 30	1.920 - 6.100 3.565 mg/100g 30	0.196 - 1.610 0.679 mg/100g 28	2.630 -13.300 6.628 mg/100g 30
USDA (2004a)	**Average** **N**	0.622 mg/100g 2						
Watson (1987)	**Range** **Average**	5.3 mg/kg		17 - 47 30 IU/kg[a]				
Souci (1994)	**Range** **Average**	400 ug/100g						

[a] IU x 0.67 = mg -tocopherol (Linus Pauling Institute's Micronutrient Information Center 2004)

Table 12: Amino Acid Composition of Grain from Corn Line 3272 (% dry weight)

2003	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
A1 (Positive)	0.717	0.343	0.546	2.058	0.908	0.394	0.809	0.201	0.488
A2 (Negative)	0.711	0.331	0.554	2.047	0.896	0.395	0.807	0.205	0.487
F-test Probability for Genotype	60.7%	12.5%	35.9%	77.1%	39.5%	86.8%	84.5%	13.0%	83.1%
B1 (Positive)	0.729	0.334	0.559	2.094	0.893	0.387	0.827	0.204	0.489
B2 (Negative)	0.663	0.311	0.520	1.927	0.840	0.367	0.762	0.196	0.453
F-test Probability for Genotype	**<0.1%**	**<0.1%**	**<0.1%**	**<0.1%**	**<0.1%**	**<0.1%**	**<0.1%**	**1.9%**	**<0.1%**

2004	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
B3 (Positive)	0.666	0.323	0.496	1.851	0.808	0.365	0.734	0.184	0.449
B4 (Negative)	0.651	0.322	0.488	1.826	0.813	0.361	0.720	0.186	0.437
F-test Probability for Genotype	18.2%	81.9%	39.4%	53.0%	70.1%	53.7%	35.5%	51.6%	21.3%

2003	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Trp
A1 (Positive)	0.199	0.368	1.365	0.407	0.546	0.294	0.319	0.488	0.0683
A2 (Negative)	0.199	0.365	1.355	0.387	0.544	0.296	0.328	0.491	0.0637
F-test Probability for Genotype	98.6%	60.4%	68.6%	13.0%	84.2%	58.9%	11.3%	78.6%	*
B1 (Positive)	0.214	0.371	1.404	0.393	0.554	0.293	0.318	0.471	0.0673
B2 (Negative)	0.202	0.339	1.284	0.364	0.511	0.277	0.301	0.448	0.0604
F-test Probability for Genotype	*	**<0.1%**	**<0.1%**	**9.1%**	**<0.1%**	**<0.1%**	**<0.1%**	**0.7%**	**<0.1%**

2004	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Trp
B3 (Positive)	0.204	0.335	1.227	0.359	0.480	0.275	0.315	0.424	0.0643
B4 (Negative)	0.198	0.326	1.203	0.345	0.469	0.273	0.310	0.423	0.0586
F-test Probability for Genotype	18.5%	18.2%	36.8%	17.9%	22.0%	76.7%	45.4%	87.9%	**3.4%**

Table 13. Amino Acid Composition of Maize Grain Reported in the Literature (dry weight)

		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
OECD (2002)	**Range**	0.48 - 0.85 %	0.27 - 0.58 %	0.35 - 0.91 %	1.25 - 2.58 %	0.63 - 1.36 %	0.26 - 0.49 %	0.56 - 1.04 %	0.08 - 0.32 %	0.21 - 0.85 %
ILSI (2004)	**Range**	4.17 - 9.5	2.24 - 6.5	2.35 - 7.66	10.41 - 30.35	5.76 - 14.57	2.8 - 4.98	4.39 - 12.03	1.48 - 3.16	3.16 - 7.23
	Average	6.82 mg/g	3.53 mg/g	5 mg/g	19.8 mg/g	9.44 mg/g	3.81 mg/g	7.9 mg/g	2.17 mg/g	4.98 mg/g
	N	725	725	725	725	725	725	725	725	725
USDA (2004a)	**Average**	0.655 g/100g	0.354 g/100g	0.447 g/100g	1.768 g/100g	0.822 g/100g	0.386 g/100g	0.705 g/100g	0.170 g/100g	0.477 g/100g
	N	29	35	29	29	28	29.000	29	30	34
Souci (1994)	**Range**	590 - 630	320 - 510	500 - 530	1.74 - 1.88	0.93 - 1.19	430 - 440	770 - 830	70 - 280	430 - 740
	Average	620 mg/100g	390 mg/100g	520 g/100g	1.78 g/100g	1.02 g/100g	430 g/100g	790 mg/100g	140 mg/100g	510 mg/100g

		Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Trp
OECD (2002)	**Range**	0.10 - 0.46 %	0.22 - 0.71 %	0.79 - 2.41 %	0.12 - 0.79 %	0.29 - 0.64 %	0.15 - 0.38 %	0.05 - 0.55 %	0.22 - 0.64 %	0.04 - 0.13 %
ILSI (2004)	**Range**	1.30 - 3.44	2.04 - 5.96	6.42 - 21.74	1.10 - 5.95	2.63 - 8.30	1.97 - 4.18	2.36 - 5.57	2.58 - 6.23	0.355 - 0.900
	Average	2.03 mg/g	3.74 mg/g	13.41 mg/g	3.46 mg/g	5.26 mg/g	2.97 mg/g	3.05 mg/g	4.45 mg/g	0.599 mg/g
	N	725	725	725	725	725	725	725	725	725
USDA (2004a)	**Average**	0.197 g/100g	0.337 g/100g	1.155 g/100g	0.383 g/100g	0.463 g/100g	0.287 g/100g	0.265 g/100g	0.470 g/100g	0.067 g/100g
	N	34	35	35	34	35	19.000	101	31	16
Souci (1994)	**Range**	90 - 400	350 - 620	0.91 - 2.11	190 - 690	320 - 510	130.0 - 330.0	40 - 480	190 - 560	40 - 100
	Average	190 mg/100g	430 mg/100g	1.22 g/100g	380 mg/100g	460 mg/100g	260 mg/100g	290 mg/100g	420 mg/100g	70 mg/100g

Table 14: Fatty Acid Composition[1] of Grain from Corn Line 3272 (% dry weight)

2003	**16:0 Palmitic**	**18:0 Stearic**	**18:1 Oleic**	**18:2 Linoleic**	**18:3 Linolenic**
A1 (Positive)	0.394	0.057	0.652	1.654	0.054
A2 (Negative)	0.411	0.061	0.679	1.693	0.056
F-test Probability for Genotype	20.6%	5.6%	30.6%	50.4%	12.6%
B1 (Positive)	0.447	0.071	0.832	1.907	0.057
B2 (Negative)	0.447	0.070	0.861	1.860	0.057
F-test Probability for Genotype	100.0%	69.7%	41.2%	48.1%	97.2%

2004	16:0 Palmitic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic
B3 (Positive)	0.458	0.066	0.847	1.892	0.063
B4 (Negative)	0.454	0.064	0.841	1.909	0.063
F-test Probability for Genotype	83.6%	46.2%	84.5%	81.4%	94.4%

[1] Five most abundant fatty acids only

Table 15: Fatty Acid Composition of Maize Grain Reported in the Literature (dry weight)

Source		16:0 Palmitic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic
OECD (2002)	**Range**	0.29 - 0.79 %	0.04 - 0.17 %	0.70 - 1.39 %	0.67 - 2.81 %	0.03 - 0.10 %
USDA (2004a)	**Average** **N**	0.569 g/100g 197	0.075 g/100g 197	1.247 g/100g 197	2.097 g/100g 197	0.065 g/100g 197
Souci (1994)	**Range** **Average**	250 - 690 mg/100g 470 mg/100g	36 - 145 mg/100g 90 mg/100g	1.10 g/100g	0.59 - 2.46 g/100g 1.63 g/100g	30 - 70 mg/100g 40.0 mg/100g

Table 16: Secondary Plant Metabolites and Anti-Nutrients in Grain from Corn Line 3272 (dry weight)

2003	**Inositol (ug/g)**	**Phytic Acid (%)**	**Raffinose (%)**	**Trypsin Inhibitor (TIU/mg)**	**Ferulic Acid (ppm)**	***p*-Coumaric Acid (ppm)**
A1 (Positive)	2293	0.588	<0.135	2.76	1939	144
A2 (Negative)	2271	0.571	<0.130	2.79	1999	150
F-test Probability for Genotype	70.8%	60.6%		68.3%	32.6%	49.7%
B1 (Positive)	2212	<0.506	<0.129	2.66	2277	176
B2 (Negative)	2269	<0.430	<0.120	2.60	2404	174
F-test Probability for Genotype	40.6%			31.4%	11.9%	74.9%

2004	**Inositol (ug/g)**	**Phytic Acid (%)**	**Raffinose (%)**	**Trypsin Inhibitor (TIU/mg)**	**Ferulic Acid (ppm)**	***p*-Coumaric Acid (ppm)**
B3 (Positive)	2689	0.804	<0.133	2.79	3034	325
B4 (Negative)	2944	0.769	<0.129	2.82	3153	319
F-test Probability for Genotype	**3.8%**	14.0%		45.6%	**3.0%**	62.3%

Where some of the samples were <LOQ (phytic acid, raffinose), the estimated average is calculated as the LOQ value for those samples and the quantifiable values and expressed as < that estimated average.
Furfural was below the Limit of Quantitation (0.500 ppm) in all samples.

Table 17: Secondary Plant Metabolites and Anti-Nutrients in Maize Grain Reported in the Literature (dry weight)[a]

Source			Phytic Acid	Raffinose		Ferulic Acid	*p*- Coumaric Acid
OECD (2002)	**Range**		0.45 - 1.0 %	0.21 - 0.31 %		0.02 - 0.3 %	0.003 - 0.03 %
ILSI (2004) [b]	**Range** **Average** **N**		0.290 - 1.287 % 0.75 % 609	0.040 - 0.290 % 0.13 % 270		1340 - 3725.5 mg/kg 2454.6 mg/kg 275	90.7 - 576.2 mg/kg 247.5 mg/kg 275
EuropaBio (2003)	**Average**		0.89 %dw				
Naczk (1997)	**Average**			0.21 - 0.31 g/100 gdw			
Souci (1994)	**Range** **Average**		890 – 990 mg/100g 940 mg/100g	190 – 270 mg/100g 230 mg/100g			

[a] ppm=mg/kg=ug/g

[b] below LOQ values are not included

6. NUTRITIONAL IMPACT

Studies submitted:
Evaluation of Event 3272 Transgenic Maize (Corn) in Broiler Chickens, by Brake, J. 2005. North Carolina State University.

Quantification and Characterization of AMY797E α-Amylase and Phosphomannose Isomerase (PMI) Proteins from Event 3272 Maize in Broiler Chicken Diets, by Hill, K. 2005. Syngenta Seeds Biotechnology Report #SSB-004-05.

In assessing the safety of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food.

To date, all approved GM foods from plants that have modified agronomic productions traits (e.g. herbicide tolerance) have been shown to be compositionally equivalent to food from their conventional counterparts. Feedings studies with these foods have shown equivalent animal performance to that observed with the non-GM food. Thus, the evidence to date is that for GM foods shown to be compositionally equivalent to conventional counterpart foods, feeding studies with target livestock species will add little to a safety assessment and generally are not warranted (OECD 2003).

For plants that have been genetically modified for animal feed with the intention of significantly changing their composition or nutrient bioavailability and thus their nutritional characteristics however, suitable comparators may not be available for nutritional assessment based solely on compositional analysis. In such cases, feeding trials with one or more target species may be useful to demonstrate wholesomeness for the animal.

For corn line 3272, additional testing involving a 49-day feeding study using commercial broiler chickens has been undertaken. Broiler chickens are highly sensitive to small nutrient changes within their diets because of their extremely rapid growth. Male and female broiler chickens were fed diets containing line 3272-derived corn grain (line 3272 positive), non-transgenic near-isogenic control grain (line 3272 negative) or commercially available (NC 2004) corn grain.

There were no detected differences between broiler chickens fed on the diet containing corn line 3272 when compared to those consuming diets containing control corn grain. All diets supported the rapid growth of the birds without significant impact on overall carcass yield or quality.

6.1 Human nutrition assessment

In the case of corn line 3272, which has been genetically modified primarily for industrial purposes, the compositional analyses indicate that the derived food is equivalent in composition to food from conventional corn. The genetic modification to this line however has introduced a heat stable α-amylase enzyme which may alter some nutritional parameters such as the glycaemic index (GI) if present in human foods.

The α-amylase enzyme expressed by corn line 3272 (AMY797E) has the ability to reduce starch molecules into component dextrins and mono/di-saccharides. As such, this expression could impact on the nutritional profile of corn line 3272 or foods derived from this corn line if these products are used in a manner that replicates a similar pH and temperature environment.

The Applicant has indicated that the AMY797E enzyme is active at the pH and temperature of the processes for which corn line 3272 has been developed. FSANZ has not been informed of this exact pH and temperature, however the common pH and temperatures used in the cooking stage of dry-grind ethanol production from corn are a pH of 5-6 and a temperature range of 95-105^{o}C (Kohl 2003). It is therefore assumed that the AMY797E α-amylase enzyme will be active during similar environmental conditions.

For the purposes of this assessment it is assumed that corn line 3272 will enter the Australian and New Zealand food supply chains via co-mingling with existing corn stocks; the actual likelihood of this occurring is an issue that is outside the scope of this assessment.

Consumption of Whole Corn Kernels or Milled Corn, Corn Flour and Corn Meal

The activation of the AMY797E α-amylase enzyme could potentially occur during the cooking of foods containing corn line 3272 if the ideal pH and temperature were reached. This is a realistic scenario for the cooking of whole or milled corn, where the corn may be used as a vegetable in its own right or in cooked dishes, as such cooking may involve the boiling or steaming of corn. Also these cooking processes would likely involve temperatures being held at 95-105^{o}C for considerable periods of time, allowing the AMY797E α-amylase enzymes to act upon any available starch.

For whole corn or corn niblets, the AMY797E α-amylase enzymes are likely to remain inside the corn and thus act only on the corn starch. However for milled corn products, the enzymes may also be able to act on both corn starch and any other starch from ingredients being cooked in the same food at that time.

Should conditions be suitable for the AMY797E α-amylase enzymes to act on the starch in a food, then these enzymes would change the final food's nutritional profile to one that contains a greater proportion of dextrins, disaccharides and monosaccharides. This breakdown would depend on the amount of corn line 3272 that has been introduced into the food's recipe, and the ability of the AMY797E α-amylase enzymes to act on non-corn starches. Such a change could be noticeable by consumers, through changes to the taste and texture of the final food.

Should the food be consumed despite any taste and texture changes, then a consumer's net carbohydrate intake would remain the same, but consist of more dextrins, disaccharides and monosaccharides.

The main nutritional effect of this intake would be the consumption of a food with a potentially higher glycaemic index[3], although the impact on the overall glycaemic index of a meal would depend on the final form of the food and any other foods with which it is consumed.

If the AMY797E α-amylase enzymes are not activated during the cooking of foods containing corn line 3272, then these foods are likely to continue having a similar nutritional profile to foods made using typical corn varieties. This similarity is reflected in analytical tests conducted by the Applicant comparing the nutritional profile of corn line 3272 to conventional corn (see Section 5).

Processed Corn-Based Ingredients

The consumption of whole/milled corn kernels is not the only manner in which corn line 3272 could be available in the food supply. Kernels from corn line 3272 could be processed into a number of ingredients that have important applications in food manufacture, including high-fructose corn syrup, corn starch, and corn oil.

It is unlikely that the AMY797E α-amylase enzyme would be present in corn oil or have an impact on this product, as corn line 3272 contains high levels of the enzyme in the endosperm of the kernel, and not in the germ from where corn oil is extracted.

High-fructose corn syrup and corn starch are obtained from the endosperm of corn kernels, and therefore may contain the AMY797E α-amylase enzyme if corn line 3272 was used in their manufacture. In the case of high-fructose corn syrup, the presence of the AMY797E α-amylase enzyme will have little nutritional impact, as the production of this syrup already involves the conversion of starch to monosaccharides. However, corn starch may be affected by the presence of AMY797E α-amylase enzyme should the starch be used in a manner that activates this enzyme. In this instance, the nutritional profile of foods using the corn starch could be affected in the manner outlined above for whole/milled corn.

Conclusion

It is unlikely that the co-mingling of corn line 3272 with existing corn stocks will have any significant effect on population nutrition. It is possible that this corn line's AMY797E α-amylase enzymes may be activated during the cooking or processing of corn line 3272, which could increase the glycaemic index of the final food product. However, even if the final food's glycaemic index was increased, the overall effect on the diet would be minimal given that the glycaemic index is heavily influenced by other dietary factors.

[3] The glycaemic index is a ranking of carbohydrates on a scale from 0 to 100 according to the extent to which they raise blood sugar levels after consumption. Increases in the glycaemic index mean that the carbohydrates in a food will be more readily digested and absorbed, and thus result in more pronounced blood glucose level fluctuations following a meal. Consumption of foods with higher glycaemic index values is undesirable for individuals who need to maintain good blood glucose control (e.g. individuals with diabetes).

7. IMPACTS ON FOOD TECHNOLOGICAL PROCESSES

The unique characteristics of this genetic modification, the thermostability in particular, may affect corn grain processing and lead to altered food properties. FSANZ therefore assessed the potential impacts of this thermostable enzyme on different processes of corn refining and on corn products.

7.1 Corn Refining

Corn grain is refined using different milling processes and the products of milled corn include grits, meal, flour, germ and germ oil, hominy, starch, starch hydrolysates, protein and corn steep liquor (Fig 3). Nearly 5% was used to make high fructose corn syrup, 2.5% was used as starch and about 5% for other food uses.

Corn can be milled using wet or dry processes and in both cases the germ is separated from the kernel to recover most of the maize oil as a separate valuable component. Wet milling separates most of the starch as slurry from the remaining maize components with the floating germ also easily removed. This process therefore provides a cleaner corn starch for further processing with maize protein as a separate stream. Grain for wet milling is steeped in warm water for about 48 hours to soften the kernels and facilitate the separation of the starch from other components. Excess activity of amylase during the wet milling process can cause some problems as the starch can begin to hydrolyse before it is separated from other components. Losses of soluble starch hydrolysates can occur from the starch slurry reducing the yield of starch. The Applicant states that the probability of this corn line 3272 being used in wet milling is very low.

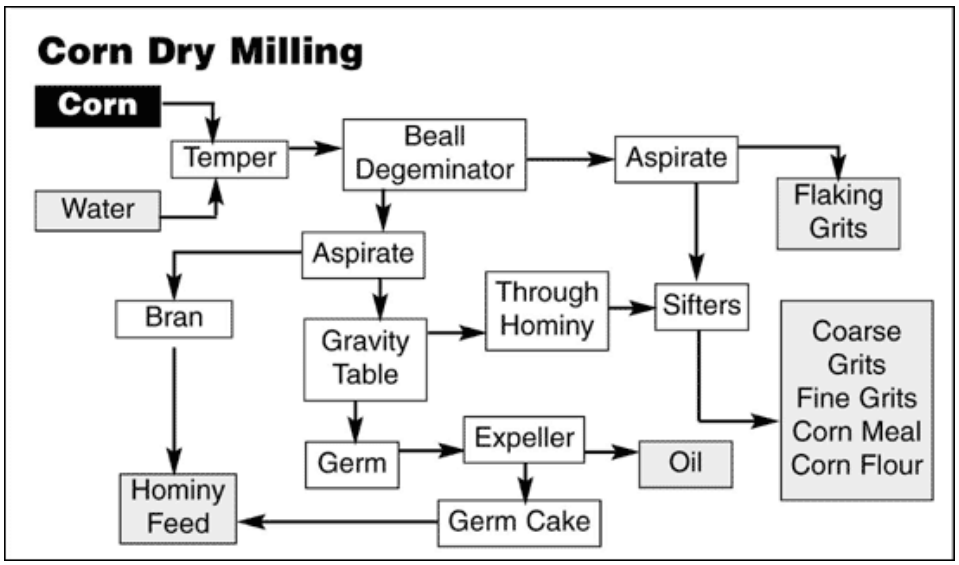
Dry milling employs rollermills and separators similar to wheat milling to reduce particle sizes and separate the streams for various products. Dry milled maize starch therefore contains higher protein and fat levels and greater levels of contamination with other grain components. Dry milled corn grits are used for further processing into flakes. The presence of the amylase has no or little impact on this process.

Other corn products such as tortillas and corn chips are produced from the masa process (Fig. 3), which involves cooking the grain and steeping in alkali before grinding. Small amounts of thermostable amylase surviving the masa process could lead to some quality issues due to conversion of some starch to dextrins and sugars.

As there is no intention to grow line 3272 corn in Australia or New Zealand, Australian grown corn products containing high levels of resistant starch should not be affected by this Application. The product is intended to be used for dry-grinding mainly for the purpose of producing ethanol as a fuel.

7.2 Conclusion

Unintentional mixing of corn varieties in the USA could result in imported processed products containing amylase. Food products that could potentially contain the enzyme include corn syrups, corn starches and corn chips and flours. Other food products could contain these products as ingredients. Although the presence of this amylase may impact on certain food properties due to the conversion of starch to dextrins and sugars, there is no food safety issue associated with the possible presence of this thermostable enzyme in foods.


Corn Dry Milling
Corn
Temper
Beall Degeminator
Aspirate
Flaking Grits
Water
Aspirate
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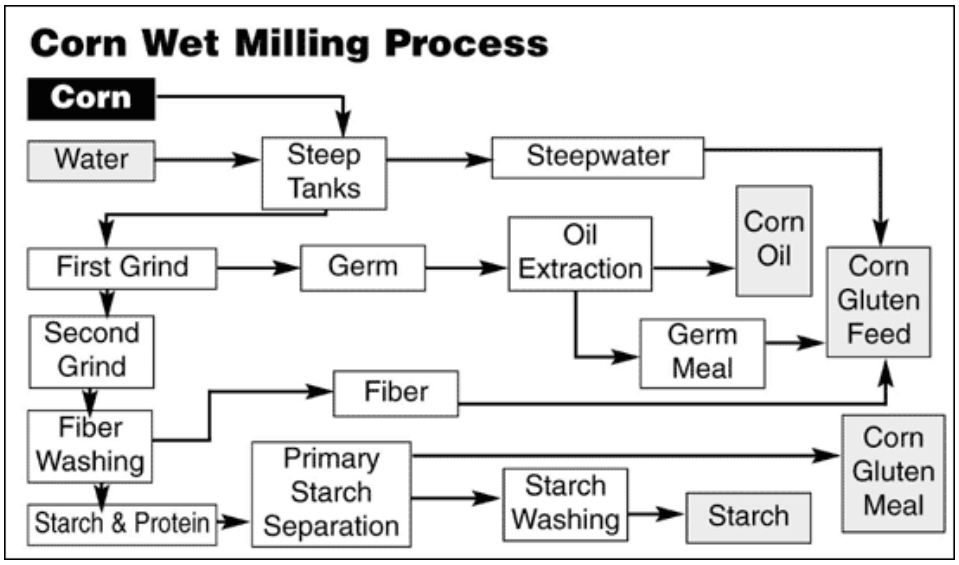
Through Hominy
Sifters
Gravity Table
Coarse Grits
Fine Grits
Corn Meal
Corn Flour
Germ
Expeller
Oil
Hominy Feed
Germ Cake


Corn Wet Milling Process
Corn
Water
Steep Tanks
Steepwater
Corn Oil
First Grind
Germ
Oil Extraction
Corn Gluten Feed
Second Grind
Germ Meal
Fiber
Fiber Washing
Corn Gluten Meal
Primary Starch Separation
Starch Washing
Starch
Starch & Protein

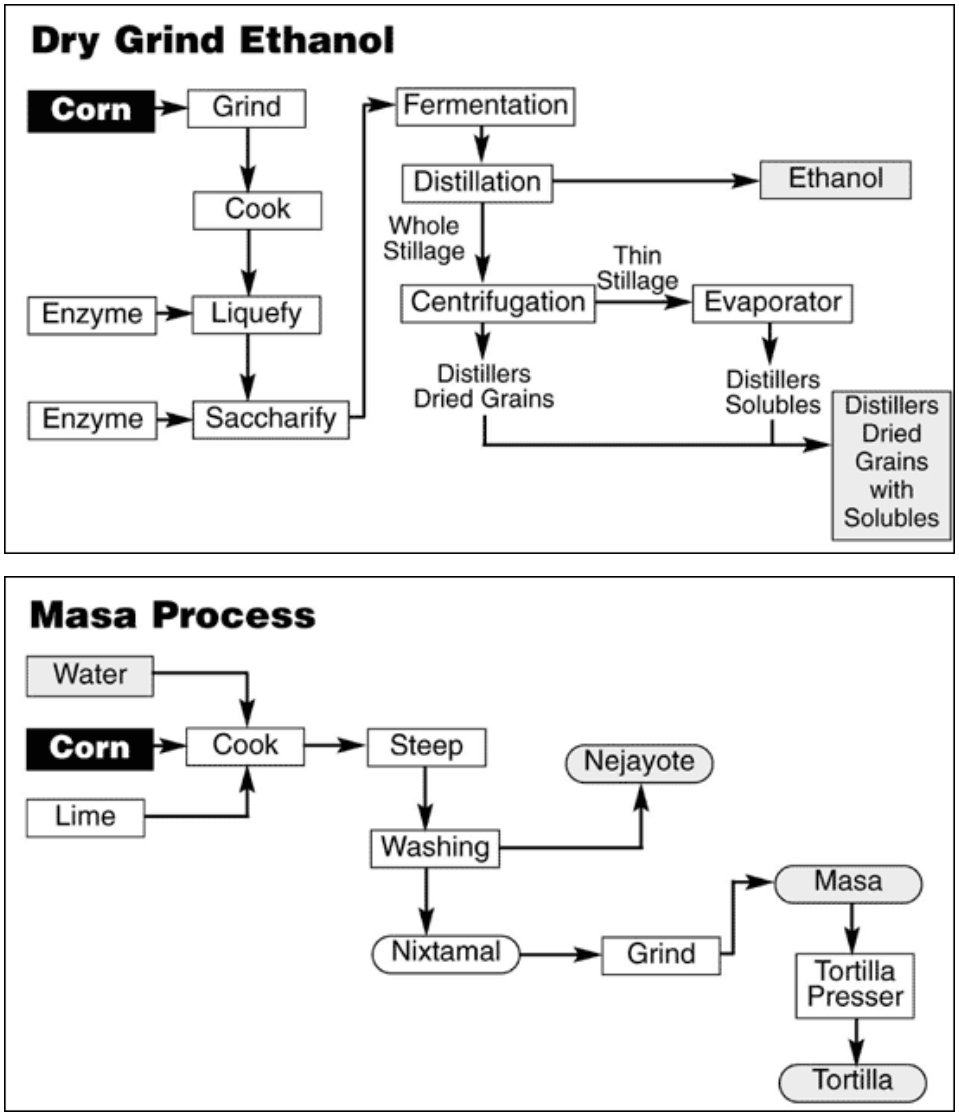


Figure 2: The main corn refining processes and products (Iowacorn [4])

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[4] http://www.iowacorn.org

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ATTACHMENT 3

SUMMARY OF PUBLIC SUBMISSIONS

First Round

A total of 8 submissions were received – 3 from New Zealand and 6 from Australia.

Submissions from New Zealand

1. Paul Elwell-Sutton

- Opposes the Application because of concerns that food labelling laws in New Zealand fail to ensure consumers' rights to make informed decisions regarding GM food.
- Concerned that approval of this Application will open the door for corn grown for ethanol production to enter the human food chain.
- Amylase-modified corn, when combined with other food, may affect overall food quality and glycemic index (GI), and consequently, altered nutritional value.
- Growing GM corn threatens the livelihood of growers of conventional (non-GM) corn due to cross-pollination by the GM corn as corn is wind-pollinated.
- Concerned that GM constructs are unstable and may lead to altered food value and allergenicity over time.

2. Centre for Integrated Research in Biosafety

- Questions how the Applicant established the safety of exposure to the gene product at the concerns that the Amy797E and PMI proteins would be present in human food at normal levels of corn ingestion.
- In the broiler chicken feeding study, one of the negative control corn used were contaminated with AMY797E. Question how the Applicant determined the contamination of the control did not affect the measurement of effects on the broiler chickens.
- Questions how the Applicant determined that the contamination of the Line 3272 Negative control was due to mixing of grain rather than a low level of hybridization between the Positive and Negative Lines.
- Questions whether the applicant conducted feeding, acute oral toxicity, allergenicity and inhalation exposure studies sourcing the protein from the grain of the modified corn line, and using whole corn or whole corn products after cooking and processing, to determine the effects of exposures as humans would be exposed.
- Questions how the negative segregant, used as a control, was derived and whether the negative segregant ever derived from cells exposed to recombinant DNA. Question why was a non-transgenic parental line not used as a control.
- Questions how PMI sourced from corn leaf tissue compared with PMI in grain.
- A region of PMI predicted to be identical to the Per a 3 allergen of cockroach was excluded as a possible allergen because PMI did not have an epitope with strong IgE binding potential. Question how this conclusion was determined, by assay or by folding prediction programs.

- Questions whether the 'stability to degradation in simulated gastric and intestinal fluids' studies were conducted according to FAO/WHO specifications. Question why only PMI was subjected to SIF studies and why AMY797E was not also digested with pepsin at the 0.0001X concentration.

3. New Zealand Food Safety Authority

- Reviews this Application once the Draft Assessment Report is released.

Submissions from Australia

4. Ivan Jeray

- Opposes to approval as GM ingredients have not been proven safe. Mentions example of CSIRO GM field pea causing illness in mice.
- Concerned GM derived food may not be required to be labelled as such.

5. Food Technology Association of Victoria

- Opposes the Application because corn line 3272 is developed for a non-food purpose, i.e. for ethanol production.
- Concerned that unprocessed corn contaminated with this corn line could be imported into Australia. Such importation may subsequently lead to contamination of locally grown corn.
- Concerned that the cross contamination of locally grown corn with corn line 3272 could lead to the loss of functionality of foods through the presence of the high alpha-amylase content.
- Concerned that this corn line is not suitable or meant for food/ food production, therefore there is no rationale or precedent for its inclusion in ANZFSC.
- Suggests that it is best controlled by AQIS whose role is to prevent importation of food and feed contaminates since this is not a food issue.
- Concerned that inclusion of this corn line in ANZFSC is a de facto permission for it to be used in food, for which the Applicant does not seek permission.
- Suggests that the inclusion of this corn line in ANZFSC as a prohibited substance in food, justified by the fact that corn is usually permitted to be consumed as a food.

6. Victorian Department of Human Services (DHS)

- No objection to this Application proceeding to Draft Assessment.
- Requests that the information on source of the amylase is made available in the application.

7. Queensland Health

- Awaits the Draft Assessment Report before stating support or opposition.
- Expresses concerns with the costs of monitoring and enforcing GM food legislation and suggests a national enforcement strategy for GM food, including education, due to the limited availability of resources for such activities from the jurisdiction.

8. Australian Food and Grocery Council (AFGC)

- Supports this Application, contingent upon satisfactory safety assessment by FSANZ.

Second Round

A total of 9 submissions were received – 4 from New Zealand and 5 from Australia.

Submissions from New Zealand

1. Paul Elwell-Sutton

- Opposes the Application because of concerns that food labelling laws in New Zealand fail to ensure consumers' rights to make informed decisions regarding GM food.
- Concerned that no long-term human physiological and biochemical studies carried out, and no human aging and neurological studies untaken.
- Concerned New Zealand is under-represented in the current food regulation structure.

2. Sarah Pilkington

- Raised technical issues, i.e. use of non-transgenic isogenic plants as a control, use of hemizygous hybrids for AMY797E and PMI quantitation, use of ELISA in AMY797E and PMI quantitation, in relation to Syngenta report No. SSB-028-04: Quantitation of AMY797E and PMI proteins in transgenic maize (corn) tissues and whole plants derived from event 3272.
- Questioned the justification of pH used in simulated mammalian gastric juice experiment, the presence of two minor protein products, and the comparative digestibility of PMI related to Syngenta report No.SSB-034-04 A1: In vitro digestibility of AMY797E α-amylase (test substance AMY797E-0104) under simulated mammalian gastric conditions.
- Questioned the evidence base on which the equivalence of E. coli and in-planta produced PMI was established (i.e. Western-blotting method and enzyme specificity assay), and suitability of using a negative segregant as a control in Syngenta report No. SSB-022-05: Characterisation of Phosphomannose isomerase (PMI) produced in maize (corn) plants derived from event 3272 and comparison to PMI as contained in test substance PMI 0198.
- Opposes the approval of this GM corn based on concerns mentioned above and concerns that FSANZ does not have enough evidence to declare the safety and equivalent wholesomeness of amylase-modified corn line 3272 for human consumption.

3. David O'Keefe

- Provided an in depth critique on Syngenta CTL/AM7506/Regulatory/Report AMY 797E-0104: Single dose oral toxicity study in the mouse.
- Alleged flaws in experimental design that would limit the power of the analysis: justification and adequacy of using a dose level of 1511 mg of AMY797E-104 protein/kg, inadequate sample size, appropriateness of using mice, flawed random block design, flawed statistical methods and inadequate length of treatment, incomplete analysis of DNA fragmentation and insufficient blood sample volume.

- Raised other specific issues about analyses and conclusions including: body weight, food consumption, haematology, blood clinical chemistry, organ weights, macroscopic finding, microscopic findings.

4. New Zealand Food Safety Authority (NZFSA)

- NZFSA had the Draft Assessment Report reviewed by the Institute of Environmental Science and Research Limited (ESR) and as a result NZFSA recommends referencing studies or publications to substantiate the functional equivalence of the AMY797E α-amylase to other commercial α-amylase.

Submissions from Australia

5. Greenpeace Australia Pacific Ltd (Greenpeace)

- Strongly objects to the proposed approval of this GM corn.
- Stated it was unnecessary to genetically engineer the gene for the enzyme into the maize when it can be simple be added to the maize during the ethanol processing stage.
- Made general comments on defects in FSANZ standards and requirements.
- Alleged inadequate labelling requirement in relation to altered characteristics: the raised glycaemic index, the intended use and the potential allergenicity.
- Questioned FSANZ conclusion that the potential benefits to all sector outweigh the cost associated with the approval.
- Stated that a potential increase in cost effectiveness (the sole justification for the maize) is a poor justification for releasing new, untested protein into human diet.
- Stated that global acceptance of this GM corn is not forthcoming in the face of a regulatory rejection from South Africa and FSANZ should fully describe its WTO obligation, including legal weight.
- Raised health and safety concerns based on: flawed practice in relying on Syngenta's digestion study which used a single pH (1.2) condition, failed discloser of the two expert reviewers, the scientific reliability of the "functional equivalence" of AMY797E to other commercial α-amylase, the use of a surrogate protein of PMI in acute toxicity study, the lack of sufficient historic data to judge identified compositional differences in some of the measurements, and the limited relevance of the broiler chicken feeding study to human health.

6. Food Technology Association of Australia (FTAA)

- Opposes the proposed approval of this corn line.
- Stated that the reasoning submitted during the Initial Assessment Report was still applicable.
- Emphasized that because of the very high amylase content in this corn line, once the corn was incorporated into food, it would destroy the functionality of the food, causing problems including costs and possible litigation.
- Concerned that due to the very heat-stable property of this α-amylase, certain processing conditions would not inactivate this enzyme with all the consequent effects.

7. Queensland Health

- Remains concerned with the cost of monitoring and enforcing GM food legislation and requests that FSANZ provides detailed advice on what costs associated with the enforcement by jurisdictions and how they were agreed upon.
- Seeks advice as to why no overseas' approvals have been granted to date given that the applications were submitted to many countries globally, including USA, Canada, European Union, Japan, South Africa etc.

8. NSW Food Authority

- Supports option 2 and progression to Final Assessment.
- Expressed concerns with the costs incurred in monitoring for the presence of genetically modified foods and welcomes discussions by the Implementation Sub-Committee ISC) with the aim of developing a national enforcement strategy for GM foods.

9. Australian Food and Grocery Council (AFGC)

- Supports this Application, contingent upon satisfactory safety assessment by FSANZ.
- Commented that FSANZ did not assess the possible consequences of adding a thermostable amylase to manufacturing processes for which it is not intended.
- Commented that as the amylase was present at high concentrations, where adventitious presence to occur at 1% in normal corn supplies the thermostable amylase would be present at concentrations of 10-33 μg/g dry weight.
- Commented that the adventitious presence of such a thermostable enzyme might reduce the shelf life and stability of products where inactivation of enzymes relies on heat treatment.
- Considered the current labelling requirements were adequate and no additional labelling requirements are necessary.
- Considered there was a net benefit to the Australian economy given the improvement offered by this corn in the viability of alternatives to fossil fuels and the potential benefits in reducing green house gases.

ATTACHMENT 4

Syngenta stewardship program for Event 3272 Corn

Most U.S. corn is marketed as commodity corn, primarily yellow dent corn. Commodity corn is used for animal feed and is processed by wet and dry milling industries for numerous and diverse food and industrial products, including ethanol. Specialty corn such as popcorn, sweet corn, white corn, high oil corn for example, is handled differently to commodity corn throughout the supply chain (from seed production, to cultivation, and grain handling) to ensure a sufficient quantity of high quality corn is produced and delivered to the end-user. As with other specialty corn the inherent value of Event 3272 corn will be best realised by ensuring its smooth and uninterrupted delivery to end use markets. In this case, the dry grind ethanol plants. Therefore, upon commercialisation, Syngenta will have in place appropriate stewardship measures including grower production agreements or contracts that will facilitate the delivery of the Event 3272 grain to its intended end use. Syngenta is committed to a robust stewardship program concerning Event 3272 grain.

Event 3272 corn will be commercialised only upon successful completion of the U.S. FDA biotechnology consultation process.

The following highlights some of the key elements of the stewardship program that Syngenta anticipates employing:

- Farmers will receive an economic incentive to grow Event 3272 corn and participate in Event 3272 corn value enhanced programs. However, the amount and nature of the incentive cannot be determined until product development is completed.

- There will be grower grain contracts between growers and the dry grind ethanol plants and/or grain handlers associated with ethanol plants for the production and delivery of Event 3272 grain. Syngenta expects to facilitate development of these contracts which would require a grower grain contract be in place as a condition of sale of Event 3272 seed to the grower. The grower grain contracts will specify a delivery location (either an ethanol plant or a storage site), a delivery date (a window for delivery may be specified), and the acres or bushels per acre to be delivered.

- Each grower will be required to sign a Syngenta Stewardship Agreement prior to planting Event 3272 corn and adherence to the stewardship program will be included in the grower grain contract. Many growers are likely to be already familiar with these types of agreements, especially if they have previously grown specialty corn products.

The stewardship program will be designed to ensure high quality Event 3272 grain is produced and delivered to the dry grind ethanol plant and/or grain handler.

Syngenta will provide a written stewardship guide on cultivation of Event 3272 corn and handling of Event 3272 grain. Periodic updates, if necessary, will be provided to keep growers informed of any new recommendations or requirements.

The Syngenta sales team will develop and implement a communication program for growers consistent with the stewardship approach described above.

As part of the stewardship program, Syngenta recognises that there may be certain sectors of the corn value chain that will want the capability to test for the presence of Event 3272. As such, Syngenta will have available an appropriate detection method upon commercial launch.

Dry grind ethanol plants that have contracted for Event 3272 grain will be required to ensure domestic consumption of all Distillers Grains co-products until regulatory approvals have been obtained in export market countries to allow for import of any Distillers Grains co-products.

The stewardship program and grain contract will specify a procedure for handling any grain produced in excess of the contracted amount.

Syngenta will budget appropriately to implement the stewardship practices outlined above.

ATTACHMENT 5

RESPONSE TO CRITICAL TECHNICAL COMMENTS RAISED IN SUBMISSIONS

David O'Keefe – an in depth critique on Syngenta CTL/AM7506/Regulatory/Report AMY 797E-0104: Single dose oral toxicity study in the mouse. The submission concluded that a more extensive testing regime (over a six months period and with increased sample size) should be implemented.

The assessment of potential toxicity of AMY797E protein is based on an integrated analysis of a number of studies including: sequence similarity with known protein toxins; *in vitro* digestibility; and acute oral toxicity; as well as consideration of the biochemical characteristics and properties of the protein. As the protein has no sequence homology to any known protein toxins and is readily degraded in the peptic and acidic conditions of the digestive system, it is expected to behave like any other dietary protein when ingested. Given the digestibility of this protein, there is unlikely to be any systemic exposure to the protein. In this case, an acute oral toxicity study merely serves as confirmation of its lack of toxicity and additional studies of longer duration are neither necessary nor ethical given the absence of toxicity.

OECD Guidelines on toxicity testing are a collection of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to assess the safety of chemical products. For acute toxicity testing, the OECD Guidelines recommend using a single sex with up to 5 animals per dose group (2001).

NZFSA and Greenpeace – questioned the evidence base on which the "functional equivalence" of AMY797E to other commercial α-amylase was established.

The α-amylase activity of the AMY797E protein has been compared to that of generic commercial Bacillus α-amylase using studies such as dose-equivalents, HPLC analysis of the size distribution of starch hydrolysis products, HPLC analysis of residual sugars and organic compounds post-fermentation liquefacts, and ethanol production yield. These studies demonstrate that the AMY797E α-amylase is functionally equivalent in starch hydrolysis to other commercial α-amylases.

Study No. AMY-04-01 by C. Kramer, 2005: Characterization of Lyophilized Amylase Test Substance (AMY797E-0104) and Certificate of Analysis demonstrated that purified AMY797E protein from grain of corn line 3272 had a specific α-amylase activity of 33,000 U/g test substance using the Ceralpha amylase assay kit from Megazyme (Wicklow, Ireland).

Greenpeace and Sarah Pilkington – questioned the justification of conducting the *in vitro* digestibility study of AMY797E protein under a single pH of 1.2.

Syngenta study No.SSB-034-04 A1: *In vitro* digestibility of AMY797E α-amylase (test substance AMY797E-0104) under simulated mammalian gastric conditions used experimental conditions identified by Thomas et al (2004) as the experimental conditions resulting in the most consistent results across different laboratories.

The Thomas et al study tested digestibility of a range of different proteins in laboratories across the world under pH conditions of 1.2 and 2. The study concluded that overall assay pH did not influence the time to disappearance of the full-length protein or protein fragment. Test results across laboratories were more consistent at pH 1.2 (91%) than pH 2.0 (77%).

The International Life Sciences Institute (ILSI) established the Thomas et al study as a standard protocol for *in vitro* digestibility study for regulatory purpose (Taylor 2006).

Greenpeace and Sarah Pilkington – Questioned the equivalence of *E. coli* and *in planta* produced PMI proteins and the use of surrogate protein of PMI in acute oral toxicity study.

Syngenta study No. SSB-022-05: Characterisation of Phosphomannose isomerase (PMI) produced in maize (corn) plants derived from event 3272 and comparison to PMI as contained in test substance PMI 0198 demonstrated the PMI proteins from both sources have a predicted molecular weight of ca. 42.8 kDa for the plant-expressed PMI and 44.4 kDa for the microbially expressed PMI. Both proteins immunologically cross-reacted with the same anti-PMI antibodies. PMI in the microbial test substance PMI-0198 was found to have a specific enzymatic activity of ca. 53. U/mg PMI. The plant-expressed enzyme showed a specific activity of ca. 97 U/mg PMI. As there are 16 additional amino acids attached to the N-terminus of the microbially expressed PMI protein as also indicated by the molecular weight, it is possible that these additional amino acids contributed to the reduction of the specific enzyme activity. The addition of the 16 amino acids is essential for cloning and expressing the native PMI protein in *E coli* and also essential for the subsequent purification of the expressed protein from *E coli*. In conclusion, the plant-expressed PMI and the microbially expressed PMI proteins are not identical because of the 16 additional amino acids present in the microbially expressed PMI protein, but as both proteins reacted with the same antisera and showed the expected enzyme activity, the microbially expressed PMI protein can be regarded as an appropriate surrogate protein in an acute oral toxicity study.

Sarah Pilkington – Questioned the appropriateness of using ELISA to quantify AMY 797E and PMI protein expression and recommended the use of Bradford assay instead.

The *Sandwich* ELISA is a commonly used method to detect and quantify a specific protein in a crude protein extract. The Bradford assay is widely used to quantify total protein in a crude protein extract but it has no ability to distinguish and quantify specific proteins. The *Sandwich* ELISA is therefore the most appropriate method to quantify the AMY797E and PMI proteins.

Sarah Pilkington – Claims the results of the *in vitro* digestibility of PMI are not provided.

Syngenta provided the *In vitro* Digestibility of PMI Protein under Simulated Mammalian Gastric and Intestinal Conditions, by Privalle, L, 1999. Novartis Seeds Biotechnology Report No. NSB-002-99 as an appendix to the Application. FSANZ has already evaluated this study and provided an assessment summary in section 4.4 of the Safety Assessment Report (Attachment 2).

Sarah Pilkington – Questioned the suitability of using a negative segregant as an experimental control in two protein expression studies.

- A negative segregant is an individual selected from the homozygous negative progeny following self fertilisation of hemizygous individuals, and therefore lacks the introduced transgene. .
- Often when complex breeding is involved in the development of GM plants lines for commercialisation, the genetic background of the line ultimately used for food will be quite different to the original parental line. In these circumstances, negative segregants are often the only lines available that are close enough to the GM line to serve as a suitable control.
- A negative segregant identified in the breeding process represents a near-isogenic control in molecular and compositional studies and leads to more accurate and sensitive comparisons.
- The negative segregant however is not the only comparator used in the assessment; additional non-GM varieties are also used, such as in the broiler chicken feeding study.

References

OECD 2001: OECD Series on testing and assessment Number 24 Guidance document on acute oral toxicity testing. At: http://www.olis.oecd.org/olis/2001doc.nsf/43bb6130e5e86e5fc12569fa005d004c/c1256985004c66e3c1256a92005087fe/$FILE/JT00111082.PDF

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Taylor SL. (2006): Review of the development of methodology for evaluating the human allergenic potential of novel proteins. Mol Nutr Food Res. 50(7):604-609. Review.

ATTACHMENT 6

Business Cost Calculator Report

A580 - Food Derived From Amylase Modified Corn Line 3272

Problem: The applicant has developed a GM corn line for use in dry-grind fuel ethanol production in the United States. Although the grain from GM corn line 3272 has been developed primarily for industrial uses, the ethanol produced may be used for food applications (e.g. fortification of alcoholic products). The applicant is seeking approval for food derived from GM corn line 3272 that could potentially enter the Australian and New Zealand Food Supply through imported and largely processed foods (corn syrup, corn starch, corn chips, corn flour etc).

Objective: To determine whether it would be appropriate to amend the code and approve amylase-modified corn line 3272.

Policy Options

Option Name	Quickscan Result
Prohibit food from amylase-modified corn line 3272	FALSE
Approve food from amylase-modified corn line 3272	FALSE

Compliance Cost Summary

Option Name Prohibit food from amylase-modified corn line 3272

Businesses Affected: N/A

Type	Cost per Business	Total Cost of Regulation
N/A	N/A	N/A

Option Name: Approve food from amylase-modified corn line 3272

Businesses Affected: N/A

Type	Cost per Business	Total Cost of Regulation
N/A	N/A	N/A

Caution should be used comparing options and interpreting results over time. The Business Cost Calculator does not estimate the future values of ongoing costs. Refer to the User Guidelines for further information.

This report contains summaries of compliance costs only. An assessment on the compliance cost in itself does not provide an answer to which policy option is the most effective and efficient one. Rather, it provides information which needs to be considered alongside other relevant factors and issues when deciding between alternative policy options.