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

10-05
7 December 2005

DRAFT ASSESSMENT REPORT

APPLICATION A564

FOOD DERIVED FROM INSECT-PROTECTED CORN LINE MIR604

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 1 February 2006
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

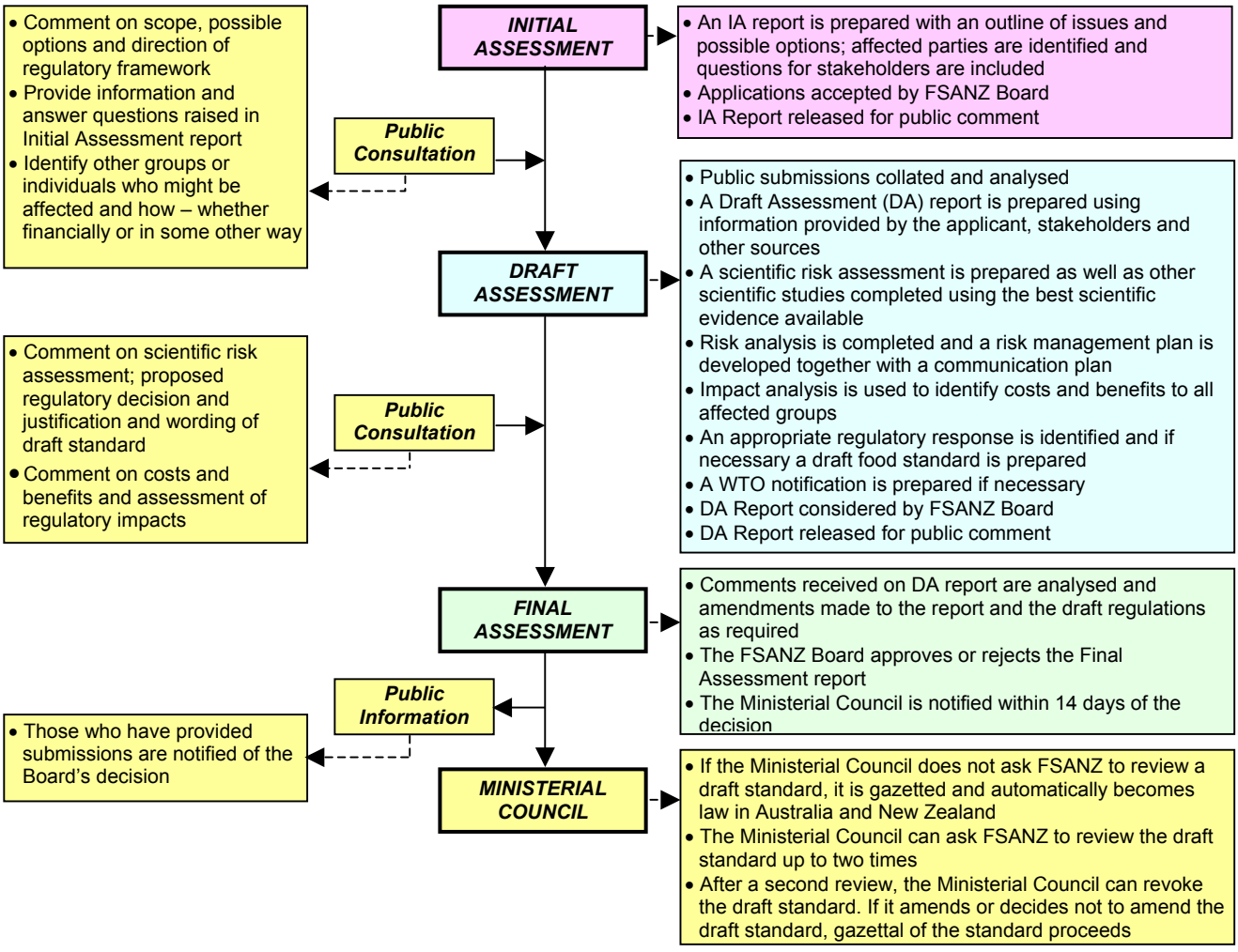
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

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



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A564; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

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

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

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

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

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

Submissions need to be received by FSANZ by 6pm (Canberra time) 1 February 2006.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ Website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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Executive Summary and Statement of Reasons

An Application has been received from Syngenta Seeds Pty Ltd to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from a genetically modified (GM) corn, corn line MIR604. 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 MIR604 has been genetically modified to be resistant to Western corn rootworm, Northern corn rootworm and Mexican corn rootworm. Resistance is conferred by expression of the *mCry3A* gene in the corn plants. A selectable marker gene, *pmi*, encodes phosphomannose isomerase and allows transformed corn cells to utilise carbon from phosphomannose media.

If approved, food from corn line MIR604 may enter Australia and New Zealand as imported products.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from corn line MIR604 as required under the Act. The assessment included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of any new proteins; and (iii) the composition and nutritional adequacy of the food, including whether there had been any unintended changes.

No potential public health and safety concerns were identified. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line MIR604 is as safe and wholesome as food derived from other corn varieties.

Labelling

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

Food products from corn line MIR604 may contain DNA and/or protein. These products would be required to be labelled as GM.

Impact of regulatory options

Two regulatory options were considered in the assessment: either (1) no approval; or (2) approval of food from corn line MIR604 based on the conclusions of the safety assessment. Following an assessment of the potential impact of each of the options on the affected parties (consumers, the food industry and government), Option 2 is the preferred option as it potentially offers significant benefits to all sectors with very little associated cost. The proposed amendment to the Code, giving approval to food from corn line MIR604, is therefore considered of net benefit to both food producers and consumers.

Consultation

In response to the invitation to comment on the Initial Assessment Report, seven submissions were received, of these two were not in favour of approving corn line MIR604. The remaining submitters expressed support for the Application, contingent on a satisfactory safety assessment, or reserved comment for after the draft assessment.

FSANZ Decision

Approval is proposed for food derived from the new GM corn line MIR604. Permission is given by adding this approval into the Table to clause 2 of Standard 1.5.2 – *Foods Produced using Gene Technology* of the Code.

Statement of Reasons

An amendment to the Code to give approval to the sale and use of food derived from corn line MIR604 in Australia and New Zealand is recommended 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 insect-resistance corn line MIR604;
- food derived from corn line MIR604 is equivalent to food from other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from corn line MIR604 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 amendment to the Code is of net benefit to both food producers and consumers; and
- the proposed draft amendment to the Code is consistent with the section 10 objectives of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act) and the regulatory impact assessment.

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

1. Introduction

An Application was received from Syngenta Seeds Pty Ltd on 1 June 2005 seeking approval for food derived from insect-protected corn line MIR604 under Standard 1.5.2 – Food Produced Using Gene Technology, in the Code.

The genetic modification involved the transfer of the following genes into the corn plant:

- the *mcry3A* gene derived from *Bacillus thuringiensis* which encodes the insecticidal protein mCry3A. This protein is selectively toxic to coleopterans including Northern, Western and Mexican corn root worm; and

- the *pmi* gene which encodes phosphomannose isomerase and was used as a selectable marker as plants expressing this gene can utilise mannose as a primary carbon source, whereas cells lacking this gene will fail to proliferate on mannose-based medium.

A Draft Assessment of the Application, including a detailed safety assessment of food from corn line MIR604, has been completed and public comment is now being sought to assist in the Final Assessment of the Application.

2. Regulatory Problem

Standard 1.5.2 requires that a genetically modified (GM) food undergo a pre-market safety assessment before it 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.

Syngenta Seeds Pty Ltd has therefore applied to have Standard 1.5.2 amended to include food derived from corn line MIR604 in the Table to clause 2.

3. Objective

The objective of this assessment is to determine whether it would be appropriate to amend the Code to approve the use of food derived from corn line MIR604 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;

- the provision of adequate information relating to food to enable consumers to make informed choices; and

- the prevention of misleading or deceptive conduct.

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

- the need for standards to be based on risk analysis using the best available scientific evidence;

- the promotion of consistency between domestic and international food standards;

- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

The Applicant has developed corn plants that are resistant to insect attack. These corn plants are referred to as corn line MIR604. The purpose of the modification is to provide growers with an effective method for controlling certain insect pests of corn.

Corn line MIR604 contains one insecticidal gene (*mcry3A*), derived from the common soil bacterium *Bacillus thuringiensis* (*Bt*). This gene expresses the insecticidal protein Cry3A, which is toxic to coleopteran insects, including three significant pests of corn: Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberi*) and Mexican corn rootworm (*Diabrotica vigifera zeae*).

In addition, corn line MIR604 contains the *pmi* gene from *Escherichia coli*, which produces an enzyme (phosphomannose isomerase) that allows the plants to utilise mannose as a source of carbon.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAOSTAT Database 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used in industrial products, such as ethyl alcohol 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. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other corn products such as cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

Applications to permit the use of corn line MIR604 for food and feed use in Canada, Japan, the European Union and South Africa have been made. A notification has been made in the United States. No approvals have been granted to date.

5. Relevant Issues

5.1 Safety assessment of food from corn line MIR604

Food from corn line MIR604 has been evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The safety assessment included the following:

- a characterisation of the genetic modification to the plant;

[1] FSANZ (2003) Information for Applicants – Format for applying to amend the Australian New Zealand Food Standards Code – Food Produced Using Gene Technology.

- characterisation of any novel proteins, including their potential toxicity and allergenicity;

- a comparative analysis of the key constituents of corn line MIR604.

No potential public health and safety concerns were identified in the assessment of food from corn line MIR604. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line MIR604 is as safe and wholesome as food derived from other corn varieties. The safety assessment report is at **Attachment 2** to this document.

5.2 Labelling

Under Standard 1.5.2, GM food must be labelled if novel DNA and/or protein is present in the final food and also where the food has altered characteristics. Food products from corn line MIR604 may contain DNA and/or protein. These products would be required to be labelled as GM.

5.3 Issues arising from public submissions

In addition to the specific issues addressed below, FSANZ has also developed a Fact Sheet: *Frequently Asked Questions on Genetically Modified Foods – August 2002*, which responds to many of the general issues raised in connection with GM foods. The Fact Sheet may be obtained from the FSANZ website[2].

5.3.1 Safety of Bt proteins

One submitter (Mr Elwell-Sutton) was concerned about the safety of the novel protein Cry3A and its effects on health. Mr Elwell-Sutton also expressed concern about new research that indicates the potential for increased toxicity of Bt proteins on insect pests when they are used in conjunction with the compound zwittermicin A.

5.3.1.1 FSANZ Response

Strains of *B. thuringiensis* have been used safely as commercial microbial pesticides for over 40 years. There is a history of safe use of *Bt* proteins in microbial *Bt*-based products[3,4]. Both the US EPA and the World Health Organisation (through IPCS) have recognized the potential for dietary exposure to *Bt* proteins from the use of microbial spray on food crops: 'The use patterns for *B. thuringiensis* may result in dietary exposure with possible residues of the bacterial spores on raw agricultural commodities. However, in the absence of any toxicological concerns, risk from the consumption of treated commodities is not expected for both the general population and infants and children' [5] and '*Bt* has not been reported to cause adverse effects on human health when present in drinking-water or food'.[6]

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[2] www.foodstandards.gov.au/mediareleasespublications/factsheets/factsheets2002/index.cfm
[3] U.S. EPA, 1998 R.E.D. Facts: *Bacillus thuringiensis*. EPA 738-F-98-001
[4] IPCS, 2000, *Bacillus thuringiensis*. Environmental Health Criteria of the International Programme on Chemical Safety, No217. http://www.who.int/ipcs/publications/ehc/en/EHC217.PDF
[5] U.S. EPA, 1998 R.E.D. Facts: *Bacillus thuringiensis*. EPA 738-F-98-001
[6] IPCS, 2000, *Bacillus thuringiensis*. Environmental Health Criteria of the International Programme on Chemical Safety, No217. http://www.who.int/ipcs/publications/ehc/en/EHC217.PDF

A thorough literature review of papers published on the safety of Bt proteins, including information on the safety of *Bt* proteins for human consumption, determined that Bt proteins are not toxic to mammals[7].

The specific novel protein in MIR604, mcry3A, was also assessed for potential toxicity. This is discussed in Attachment 2, Safety Assessment Report.

In regard to the recent research indicating increased insecticidal toxicity noted when Bt proteins were used in conjunction with the novel antibiotic zwittermicin A (http://www.cals.wisc.edu/media/news/05_01/zwitter_Bt.html), the combination of these two compounds is a research project only and would need to be evaluated for effects on human health and safety if it were ever proposed to be used in pest management on food crops.

5.3.2 Labelling

Mr Ellwell-Sutton believes that the current GM labelling requirements are not sufficiently strict, in particular, that products (meat, milk, eggs, etc) from animals feed GM feed is not required to be labelled as genetically modified.

5.3.2.1 FSANZ Response

The labelling requirements for GM food in Australia and New Zealand are among the most comprehensive in the world. Food products from animals fed GM stockfeed are not required to be labelled as GM as they are not genetically modified. However, many animal feeds are derived from the same GM food crops that are used for human consumption. Concerns are occasionally expressed that this practice may pose an indirect risk to humans, through consumption of the meat, milk and eggs derived from such animals.

Scientific evidence published so far, including the OECD document entitled *Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants*[8], indicates that feeding GM plant material to livestock and poultry does not affect the nutritional value or safety of the meat, milk and eggs derived from those animals.

5.3.3 Issue of WTO

Mr Ellwell-Sutton requests that this Application be considered without regard to the WTO and its directives.

5.3.3.1 FSANZ response

FSANZ is required to assess this application on its merits and to determine whether food derived from corn line MIR604 is as safe for consumption as conventional varieties of corn. However, as noted in Section 8 of this report, FSANZ will be recommending that the WTO be notified under the Sanitary and Phytosanitary Measure (SPS) Agreement, in order to enable other member nations to comment on the proposed change to Standard 1.5.2.

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[7] Betz, F.S., Hammond, B.G., Fuchs R.L. (2000). Safety and Advantages of Bacillus thuringiensis-Protected Plants to Control Insect Pests. Regulatory Toxicology and Pharmacology 32, 156-173

[8] http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/env-jm-mono(2003)10

6. Regulatory Options

6.1 Option 1 – prohibit food from insect-protected corn line MIR604

Maintain the *status quo* by not amending the Code to approve the sale and use of food derived from insect-protected corn line MIR604.

6.2 Option 2 – approve food from insect-protected corn line MIR604

Amend the Code to permit the sale and use of food derived from insect-protected corn line MIR604, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

- consumers, particularly those who have concerns about biotechnology;
- food importers and distributors of wholesale ingredients;
- the manufacturing and retail sectors of the food industry; and
- Government generally, where a regulatory decision may impact on trade or WTO obligations and enforcement agencies in particular who will need to ensure that any approved products are correctly labelled.

7.2 Impact of regulatory options

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. The following is a draft assessment by FSANZ of the costs and benefits of the two regulatory options.

This is based on information supplied by the applicant, issues raised in the first round of public comment on the Application and experience FSANZ has gained from consideration of previous applications relating to GM foods. Your further comments are invited on the costs and benefits identified for the options below.

7.2.1 Option 1

Consumers: Cost in terms of a possible reduction in the availability of certain food products.

Cost associated with higher retail prices for segregated foods.

No impact on consumers wishing to avoid GM foods, as food from corn line MIR604 is not currently permitted in the food supply.

Government: No immediate impact.

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

Industry: Cost in terms of restricting innovation in food production for some sectors of the food industry. Cost to the food industry to source non-GM supplies.

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

7.2.2 Option 2

Consumers: Possible benefit if production efficiencies result in savings to producers, to the extent that savings are passed on.

Benefit of access to a greater range of products including imported food products containing ingredients derived from corn line MIR604.

Cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or increased prices for non-GM food.

Government: No direct impact.

This decision may impact on monitoring resources as food derived from corn line MIR604 will be required to be labelled as GM.

Industry: Benefit to importers and distributors of overseas food products as the product range is extended.

Benefit for food manufacturers in that the choice of raw ingredients is extended.

Benefit to food retailers in an increased product range.

Possible cost to food industry as food derived from corn line MIR604 will be required to be labelled as genetically modified.

7.2.3 Discussion

Option 1 would impose costs, particularly on consumers and the food industry sector, without offering any commensurate health benefit. This option is also likely to be inconsistent with Australia and New Zealand's obligations under the WTO. This option would also offer very little benefit to those consumers wishing to avoid GM foods, as food from other GM corn varieties is already permitted in the food supply.

Option 2 is the preferred option as it potentially offers significant benefits to all sectors with very little associated negative impact.

The proposed amendment to the Code, giving approval to food from corn line MIR604, is therefore considered necessary, cost effective and of net benefit to both food producers and consumers.

8. Consultation

8.1 Public submissions

The Initial Assessment of this Application was advertised for public comment between 3 August 2005 and 14 September 2005. A total of seven submissions were received during this period and a summary of these is included in **Attachment 3** to this Report.

FSANZ has taken the submitters comments into account in preparing the draft assessment of this application. Specific issues relating to corn line MIR604 have been addressed in the report.

8.2 WTO notification

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 corn line MIR604 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, FSANZ will be recommending to the agencies responsible that the WTO be notified under the Sanitary and Phytosanitary Measure (SPS) Agreement, in order to enable other member nations to comment on the proposed changes to standards that may have a significant impact on them.

9. The Decision

Approval is proposed for food derived from corn line MIR604. Permission is given by adding this approval into the Table to clause 2 of Standard 1.5.2 – *Foods Produced using Gene Technology* of the Code.

9.1 Statement of Reasons

An amendment to the Code to give approval to the sale and use of food derived from corn line MIR604 in Australia and New Zealand is recommended 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 insect-resistant corn line MIR604;

- food derived from corn line MIR604 is equivalent to food from other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from corn line MIR604 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 amendment to the Code is of net benefit to both food producers and consumers; and

- the proposed draft amendment to the Code is consistent with the section 10 objectives of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act) and the regulatory impact assessment.

ATTACHMENTS

1. Draft variation to standard 1.5.2 of the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Summary of issues raised in public submissions

Attachment 1

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

To commence: on gazettal

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

Food derived from insect-protected corn line MIR604

Attachment 2

DRAFT SAFETY ASSESSMENT REPORT

APPLICATION A564 – FOOD DERIVED FROM INSECT-PROTECTED CORN LINE MIR604.

SUMMARY AND CONCLUSIONS

Background

Food derived from genetically modified (GM) corn line MIR604 has been assessed for its safety for human consumption. This corn line has been genetically modified to be resistant to insect attack and has been developed for cultivation in North America. Therefore, if approved, food derived from corn line MIR604 may enter the Australian and New Zealand food supply as imported 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 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. Products derived from MIR604 corn may include flour, breakfast cereals, high fructose corn syrup and other starch products.

Description of the Genetic Modification

Corn line MIR604 contains two novel genes. The first, *mcry3A,* derived from *Bacillus thuringiensis*, encodes the insecticidal protein Cry3A. The second, *pmi*, is present as a selectable marker and encodes the enzyme phosphomannose isomerase derived from *E. 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 MIR604 expresses two novel proteins – mcry3A and phosphomannose isomerase. mcry3A levels in corn grain ranged from 0.34 – 2.15 µg/g fresh weight (0.43 – 3.13 µg/g dry weight) and phosphomannose isomerase from <0.06 – 0.41 µg/g fresh weight (<0.07 – 0.60) µg/g dry weight). No mcry3A was detected in corn oil or corn chips produced from MIR604 grain.

Acute oral toxicity studies were conducted on both novel proteins – there was no evidence of toxicity in either case. Potential allergenicity was assessed by sequence comparison to known allergens, simulated digestion studies and by determining thermolability – these data did not indicate potential for allergenicity.

Comparative Analyses

Compositional analyses were done to establish the nutritional adequacy of grain from corn line MIR604, and to compare it to a non-transgenic control line and commercial varieties of corn. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites and anti-nutrients.

No differences of biological significance were observed between the transgenic corn grain and its non-GM counterpart. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very small differences and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it was concluded that food from corn line MIR604 is equivalent in composition to that from other commercial corn varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that food derived from corn line MIR604 is equivalent in composition to food from non-GM corn varieties. This was supported by the results of a 49-day feeding study in broiler chickens. The introduction of food produced from corn line MIR604 into the food supply is therefore expected to have minimal nutritional impact.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food produced from corn line MIR604. On the basis of the data provided in the present application, and other available information, food produced from this GM corn can be considered as safe and as wholesome as food produced from other corn varieties.

1. INTRODUCTION

Syngenta Seeds Pty Ltd has submitted an application to Food Standards Australia New Zealand (FSANZ) to vary Standard 1.5.2 – Food Produced Using Gene Technology – in the *Australia New Zealand Food Standards Code*, to include food from a new genetically modified (GM) corn variety. The GM corn variety is known as corn line MIR604.

Corn line MIR604 has been genetically modified to be resistant to Western corn rootworm (*Diabrotica vigifera vigifera*), Northern corn rootworm (*Diabrotica berberi*), and Mexican corn rootworm (*Diabrotica vigifera zeae*). These species are serious insect pests of dent corn in the major corn-producing states of the north-central United States and Canada. Protection is conferred by the expression in the plant of the bacterially derived protein toxin Cry3A, encoded by the *mcry3A* gene in the corn plants. A selectable marker gene, *pmi*, encodes phosphomannose isomerase and allows transformed cells to utilise carbon from phosphomannose media.

Commercial corn lines containing the *cry* genes from *Bacillus thuringiensis* (*Bt*) can provide growers with effective methods for controlling corn rootworm. *Bt* formulations are widely used as biopesticides on a variety of cereal and vegetable crops grown organically or under conventional agricultural conditions.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAO, 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used in industrial products, such as ethyl alcohol 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. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other corn products such as cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

Applications to permit the use of corn line MIR604 for food and feed use in the United States, Canada, Japan, the European Union and South Africa have been made. No approvals have been granted to date. Corn line MIR604 is not being developed for cultivation in Australia or New Zealand. Therefore, if approved, food from this line may enter the Australian and New Zealand food supply as imported food products.

2. HISTORY OF USE

2.1 Donor Organisms

Bacillus thuringiensis

The source of the *cry3A* gene used in this GM corn is the ubiquitous soil and plant bacterium *Bacillus thuringiensis* (*Bt*) subspecies *tenebrionis*. This gene is a synthetic version of the native bacterial gene.

The WHO International Program on Chemical Safety (IPCS) report on environmental health criteria for *Bt* concludes that '*Bt* has not been documented to cause any adverse effects on human health when present in drinking water or food' (IPCS, 1999).

Bt proteins are used widely as an insecticide in both conventional and organic agriculture. In Australia, various *Bt* insecticidal products are registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use on cotton, vegetables, fruits, vines, oilseeds, cereal grains, herbs, tobacco, ornamentals, forestry and turf. The very wide use of formulations containing the *Bt* insecticidal proteins indicates that people eating and handling fresh foods are commonly in contact with this protein.

Insecticidal products using *Bt* were first commercialised in France in the late 1930s (Nester et al 2002) and were first registered for use in the United States by the Environment Protection Agency (EPA) in 1961 (EPA, 1998).

The EPA thus has a vast historical toxicological database for *B. thuringiensis*, which indicates that no adverse health effects have been demonstrated in mammals in any infectivity/ pathogenicity/ toxicity study (McClintock *et al*., 1995; EPA, 1998; Betz *et al*., 2000). This confirms the long history of safe use of *Bt* formulations in general, and the safety of *B. thuringiensis* as a donor organism.

Escherichia coli

The bacterium *Escherichia coli* is the source of the *pmi* gene in MIR604. *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 in travellers 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 (Bogosian and Kane, 1991).

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 genome into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing root deformation mainly with 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.

2.2 Host Organism

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 (OECD, 2002b). Worldwide production of maize is 500 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 ethyl alcohol (by fermentation), and highly refined starch (by wet-milling) to produce starch and sweetener products.

In addition to milling, the maize germ can be processed to obtain corn oil and numerous other products (White and Pollak, 1995).

Corn plants usually reproduce sexually by wind-pollination. This provides for natural out-crossing between plants, but it also presents an opportunity for plant breeders to produce hybrid seed by controlling the pollination process. Open pollination of hybrids in the field leads to the production of grain with properties derived from different lines and, if planted, would produce lower yields (CFIA, 1994). Instead, by controlling the cross-pollination of inbred lines from chosen genetic pools (using conventional techniques), the combining of desired genetic traits into a controlled hybrid line results in improved agronomic performance and increased yields. This inbred-hybrid concept and resulting yield response is the basis of the modern seed industry in several food commodities including corn.

The commercial production of corn has seen many improvements, particularly since the 1920's when corn varieties were developed by conventional breeding between progeny of two inbred lines to give hybrid varieties that were known to be superior to open-pollinated varieties in terms of their agronomic characteristics. In present agricultural systems, hybrid corn varieties are used in most developed countries for consistency of performance and production.

The corn recipient line was a proprietary line derived from the publicly available line A188 from the University of Minnesota.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the genetic modification

Corn line MIR604 was produced by *Agrobacterium*-mediated transformation of a proprietary line of *Zea mays* A188, using the transformation vector pZM26. Between the right and left borders, this plasmid contains the *mcry3A* 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 *mcry3A* and *pmi* genes and the absence of the *spec* gene (an antibiotic resistant marker in the plasmid backbone). Plants meeting these criteria were transferred to the greenhouse for propagation.

Table 1: Genetic elements in the plasmid pZM26

Genetic element	Size (bp)	Function
Right border	25	T-DNA right border region
MTL promoter	2556	Promoter derived from the metallothionein-like gene from Zea mays. Provides preferential expression in roots of *Zea mays* (de Framond, 1991).
mcry3A	1797	Modified version of the native *cry3A* gene (maize optimised).
NOS	253	Terminator sequence from nopaline synthase gene from *A. tumefaciens* (Depicker *et al*., 1982).
ZmUbiInt	1993	Promoter region and intron from the *Zea mays* polyubiquitin gene. Provides constitutive expression (Christensen *et al*., 1992).
pmi	1176	Phosphomannose isomerase gene from *E. coli*. Selectable marker gene (Negrotto *et al*., 2000).
NOS	253	Terminator sequence from nopaline synthase gene from *A. tumefaciens* (Depicker *et al*., 1982).
Left Border	25	T-DNA left border region

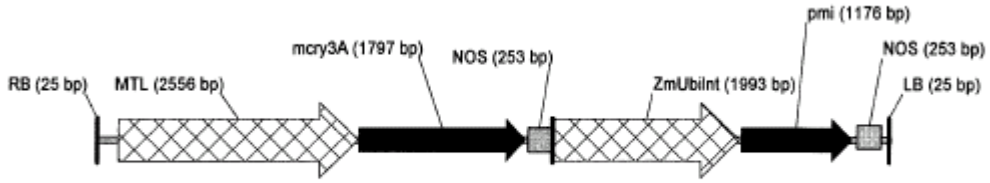

Figure 1: Genes and regulatory elements inserted in MIR604

3.2 Function and regulation of novel genes

mcry3A

The maize optimised modified *mcry3A* gene encodes a protein of 598 amino acids. The native *cry3A* gene was modified to incorporate a cathepsin-G serine protease recognition site within the expressed protein. The original N-terminal region of this protein has been removed and the mcry3A protein commences at a methionine residue in position 48 of the native protein. The entire coding region of the *mcry3A* gene was synthesised using codons that are preferred in maize. The amino acid sequence of the synthetic version of Cry3A is the same as the native protein, except for the modified serine-protease recognition site.

The regulatory elements are described in Table 1. The *mcry3A* gene is regulated by the promoter from the metallothionein-like gene from *Zea mays*, which is preferentially expressed in the root tissue and the nopaline synthase (NOS) terminator from *A. tumefaciens*.

The *mcry3A* gene confers protection against corn rootworm.

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 (Negrotto *et al.,* 2000). 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*.

No other genes were transferred to corn line MIR604.

3.3 Characterisation of the genes in the plant

Insert and copy number

Southern blot analysis was used to determine the insert and copy number of the *mcry3A* and *pmi* genes and to confirm the absence of DNA sequence from outside the T-DNA borders of the transformation vector.

Southern blot analyses of leaf tissue from plants in MIR604 backcross generation six (BC6) and negative segregants from BC4 demonstrate that insertion event MIR604 occurred as an integration of a single intact T-DNA from plasmid pZM26. Plasmid backbone DNA is not present.

PCR and sequence analysis

To further characterise the integrity of the inserted T-DNA, the sequence of the insert was determined. The entire insert was sequenced and this sequence compared to the DNA sequence of the transforming plasmid (pZM26). In total, 8416 bp of T-DNA had become inserted into the corn genome. Forty-four and 43 bp were found to be missing from the Right and Left border regions, respectively. While T-DNA border sequences are known to play a critical role in T-DNA insertion into the genome, this result is not unexpected since insertions are often imperfect, particularly at the Left T-DNA border (Tinland B. and Hohn B., 1995). Three base pair changes were noted in the MIR604 insert. One occurred within the MTL promoter, a regulatory region that does not encode a protein. The other two sequence changes occurred within the *pmi* gene and resulted in two amino acid changes; valine at position 61 has been substituted by alanine (V61A) and glutamine at position 210 has been substituted by histidine (Q210H). The first of these changes is a conservative one (both aliphatic amino acids) and the second change results in the substitution of an acidic residue for a basic residue. The *pmi* gene is still functional and these changes are discussed in Section 4.1.

Conclusion

Molecular analyses have been performed on corn line MIR604 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 pZM26. Sequence analysis showed that three single nucleotide changes had occurred within the insert, one in a non-coding region of the insert and two within the *pmi* gene.

3.4 Stability of the genetic changes

Generational stability

To determine whether the insert in corn line MIR604 is stable over a number of generations, Southern blot analysis was conducted on samples from three generations – BC_4, BC_5 and BC_6. The expected band was identified in each of these generations when probed with DNA specific to the *mcry3A* gene, which indicates that the insert is stably integrated into the corn genome.

Segregation analysis

Segregation analysis was performed on MIR604 plants from generation T_5 (original transformant was out-crossed and progeny were selfed twice, out-crossed once and selfed again to produce the T_5 generation). Individual plants from this generation were assayed for the presence of the mcry3A protein by enzyme-linked immuno-sorbent assay (ELISA) and both the *mcry3A* and *pmi* genes by PCR analysis. The expected and observed ratios of positive and negative plants were analysed by Chi square analysis to determine if the trait is segregating in a Mendelian fashion. The expected ratio was 3:1 positive to negative for the introduced traits. The results are shown in Table 2.

Table 2: Observed vs. expected ratios for T_5 generation as determined by ELISA for mcry3A and PCR for *mcry3A* and *pmi*

		ELISA	PCR
	Expected	Observed	Observed
Trait positive	313.5	317	315
Trait negative	104.5	101	103
Total	418	418	418
X^2		0.1148 ^{ns}	0.0128 ^{ns}

ns = not significant (critical X^2 value > 3.84 (p = 0.05))

No significant difference was observed for either the ELISA assay or the PCR assay between observed and expected. This indicates that the insert in MIR604 is segregating according to Mendelian principles. It is noted that there was a difference in two plants between the two assays (ELISA and PCR). Although the same 418 plants were assayed, it is not unusual to have a small number of false calls from the fluorescence output generated by the TaqMan PCR probe.

Conclusion

The studies indicate that the T-DNA insert is stably integrated into the corn genome in corn line MIR604 and segregates as expected over the generations examined.

3.5 Antibiotic resistance genes

No antibiotic resistance marker genes are present in corn line MIR604.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Biochemical function and phenotypic effects

Corn line MIR604 contains two novel proteins: mcry3A and PMI.

mcry3A

mcry3A is an insecticidal δ-endotoxin based on the native Cry3A protein derived from *B. thuringiensis* subspecies *tenebrionis*. It is 598 amino acids in length (molecular weight of approximately 67,700 Daltons). During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 μm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxin-containing crystals.

The protoxin is then activated by trypsin-like gut proteases, which cleave off domains from the carboxy- and amino- termini, leaving a protease resistant core, which is the active toxin. The active toxin binds to a highly specific glycoprotein receptor on the surface of midgut epithelial cells in the insect. Aggregation of the core toxins results in the formation of a pore through the cell membrane. These cells eventually swell and burst causing loss of gut integrity and resulting in larval death within 1 to 2 days (Hofte and Whiteley, 1989; Schnepf *et al*., 1998).

mcry3A differs from the wild-type Cry3A protein in two ways. Firstly, the amino-terminus has been shortened and the mcry3A amino acid sequence begins only at the methionine residue in position 48 of the wild-type protein. Naturally, *B. thuringiensis* produces both the full-length Cry3A protein and the truncated one, and both are active. For MIR604, rather than include the extra residues at the N-terminus, it was decided only to transfer the DNA necessary for the shorter protein.

Secondly, the amino acid sequence has been altered to introduce a cathepsin-G serine protease recognition site within the expressed protein. Native Cry3A does not show activity to corn rootworm as it does not have the appropriate protease recognition site for cleavage and activation in the insect's gut. The cathepsin-G serine protease recognition site has been added for this purpose as serine protease is the dominant protease in the corn rootworm gut and the most widely recognised serine recognition site was that of cathepsin-G. This site has the sequence alanine-alanine-proline-phenylalanine and has replaced the amino acids valine-155, serine-156 and serine-157 in the wild-type protein.

Phosphomannose isomerase

Phosphomannose isomerase (PMI, 391 amino acids) catalyses the interconversion of mannose 6-phosphate and fructose 6-phosphate and is not present in many plants. Plants lacking this enzyme are unable to survive on culture media containing mannose.

Mannose is converted to mannose 6-phosphate by hexokinase in the plant. In the absence of PMI, this accumulates, inhibiting the enzyme phosphoglucose isomerase and blocking glycolysis. In addition, depletion of the pyrophosphate required for ATP production and transcriptional repression of genes involved in photosynthesis and glyoxylate cycle occurs (Freeze, 2002; Privalle, 2002a). It has also been reported that accumulation of mannose 6-phosphate may induce apoptosis (Privalle, 2002a).

Introduction of the PMI enzyme from *E. coli* into plant cells allows them to utilise mannose as a carbon source and is therefore useful as a selectable marker gene in transgenic plants.

The PMI enzyme in corn line MIR604 has two amino acid changes from the sequence coded by the transformation plasmid pZM26. One of these (V61A) is conservative, the second (Q210H) resulted in a substitution of an acidic residue for a basic one. These changes do not affect the function of the enzyme.

4.2 Protein expression analysis

The expression levels of the two novel proteins (mcry3A and PMI) in corn line MIR604 were determined by enzyme-linked immuno-sorbent assay (ELISA) (Joseph and Hill, 2003). Plants from one MIR604 inbred line (referred to as MIR604-A) and two MIR604 hybrid lines (referred to as MIR604-B and MIR604C) were analysed. The hybrid plants were hemizygous for event MIR604 and are representative of the maize varieties that would (upon regulatory approval) be grown commercially. The transgenic inbred line was homozygous for event MIR604.

Ten plants per genotype were harvested at each of four growth stages: whorl (6 weeks), anthesis (10-11 weeks), seed maturity (18-20 weeks) and senescence (23-24 weeks). Protein was extracted from samples of leaves, roots, kernels, silk, pollen, whole plant and silage and analysed quantitatively for mcry3A and PMI by ELISA. mcry3A extraction efficiency was also measured and determined to be approximately 76.6% across all tissues, indicating good extraction efficiency. ELISA values provided were not corrected for extraction efficiency.

mcry3A

mcry3A levels measured in kernels are shown in Table 3. Across all growth stages, mean mcry3A levels measured in leaves, roots and whole plants ranged from 3 – 23 µg/g fresh wt (4 – 94 µg/g dry wt), 2 – 14 µg/g fresh wt (7 – 62 µg/g dry wt) and 0.9 – 11 µg/g fresh wt (3 – 28 µg/g dry wt), respectively. No mcry3A protein was detected in pollen from either the inbred or hybrid corn lines.

Table 3: mcry3A levels in corn kernels (µg/g)

Genotype	Developmental stage			
	Seed maturity		Senescence	
	fresh weight	dry weight	fresh weight	dry weight
MIR604-A (inbred)	1.18 ± 0.47 (0.66 – 1.56)	1.73 ± 0.75 (0.89 – 2.35)	1.28 ± 0.53 (0.60 – 1.89)	1.50 ± 0.61 (0.71 – 2.20)
MIR604-B (hybrid)	0.78 ± 0.33 (0.52 – 1.34)	1.09 ± 0.45 (0.74 – 1.83)	0.63 ± 0.17 (0.49 – 0.89)	0.77 ± 0.20 (0.59 – 0.99)
MIR604-C (hybrid)	1.37 ± 0.53 (0.84 – 2.15)	1.95 ± 0.75 (1.26 – 3.13)	0.77 ± 0.38 (0.34 – 1.30)	0.94 ± 0.47 (0.43 – 1.59)

Data is expressed as mean ± standard deviation (range)

PMI

PMI was detected at low levels in most samples. PMI levels measured in kernels are shown in Table 4. Across all plant stages, mean PMI levels measured in leaves, roots and whole plants ranged from not detectable (ND) to 0.4 µg/g fresh wt (ND – 2.1 µg/g dry wt), below LOQ (<0.03 µg/g fresh wt) – 0.2 µg/g fresh wt (<0.1 – 1.0 µg/g dry wt), and below the LOQ (<0.02 µg/g fresh wt) – 0.3 µg /g fresh wt (<0.04 – 2 µg/g dry wt), respectively.

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

Genotype	Developmental stage			
	Seed maturity		Senescence	
	fresh weight	dry weight	fresh weight	dry weight
MIR604-A (inbred)	0.14 ± 0.01 (0.13 – 0.15)	0.20 ± 0.03 (0.18 – 0.23)	<0.06 DNQ[1]	< 0.07 DNQ
MIR604-B (hybrid)	0.28 ± 0.08 (0.20 – 0.41)	0.40 ± 0.12 (0.28 – 0.60)	<0.06 DNQ	<0.07 DNQ
MIR604-C (hybrid)	0.35 ± 0.01 (0.34 – 0.36)	0.50 ± 0.03 (0.47 – 0.54)	0.21 ± 0.02 (0.18 – 0.23)	0.26 ± 0.02 (0.23 – 0.28)

Data is expressed as mean ± standard deviation (range)
DNQ: Detectable but not quantifiable

The stability of expression of novel proteins was determined in leaves at anthesis stage over four backcross generations and is presented in Table 5.

Table 5: Novel protein expression in leaf tissue over 4 generations

Backcross generation	Mean mcry3A µg/g fresh wt	Mean mcry3A µg/g dry wt	Mean PMI µg/g fresh wt	Mean PMI µg/g dry wt
BC3	3.01	15.54	0.26	1.32
BC4	3.12	15.30	0.25	1.20
BC5	2.33	11.83	0.23	1.19
BC6	2.51	12.33	0.23	1.14

Conclusion

Both novel proteins are expressed in the grain of MIR604 corn at low levels. mcry3A levels in corn grain ranged from 0.34 – 2.15 µg/g fresh weight (0.43 – 3.13 µg/g dry weight) and PMI from <0.06 – 0.41 µg/g fresh weight (<0.07 – 0.60) µg/g dry weight).

Analysis for the presence of mcry3A in processed corn products

Wet and dry-milled fractions of MIR604 corn grain were quantitatively analysed for mcry3A protein. In addition, flaking grits and flour produced during dry milling were further processed to oil and corn chips, respectively. All samples were quantitatively analysed by ELISA. The lower limit of quantitation was calculated to be 0.06 µg mcry3A/g.

The starting material (MIR604 corn grain) contained approximately 1.1 µg mcry3A/g. Levels of mcry3A found in the wet-milled and dry-milled samples are shown in Table 6. The highest concentration was found in the flaking grits (2.12 µg/g). No mcry3A was detected in the corn chips or in the corn oil (Joseph and Kramer, 2003).

Table 6: mcry3A levels in processed fractions of MIR604 corn grain

Fractions	µg mcry3A/g fresh weight
Wet milled fractions	
Grain (starting material)	1.06
Starch	<0.06
Course fibre	<0.06
Medium fibre	0.46
Fine fibre	0.26
Germ	<0.06
Gluten meal	0.24
Steep water	nd[1]
Dry-milled fractions	
Fine grit	0.69
Course grit	0.92
Flaking grit	2.12
Corn cones	0.34
Corn flour	0.32
Corn hulls	1.42
Corn meal	0.40
Processed samples	
Wet-milled oil	nd
Corn chips	nd

[1] mcry3A was considered not detectable when the mean absorbance did not exceed that of the control samples

4.3 Potential toxicity of novel proteins

Proteins which cause toxicity act via acute mechanisms and generally at very low doses (Sjoblad *et al.*, 1992). Therefore, when a protein demonstrates no acute oral toxicity at a high dose level using a standard laboratory mammalian test species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

The Applicant submitted two acute oral toxicity studies in mice to support the safety of the novel proteins.

As it is very difficult to extract and purify sufficient quantities of the subject protein from transgenic corn plants for the acute oral toxicity studies, it has become standard practice to instead use equivalent proteins that have been produced using bacterial expression systems. Prior to use, the bacterially produced proteins are compared to the proteins produced *in planta* in order to establish their equivalence. mcry3A and PMI were produced in recombinant *E. coli* (Hill, 2004; Joseph and Graser, 2003a).

The molecular identity and biochemical characteristics of the proteins expressed *in planta* and in the bacterial-expression systems were examined by analysis of biochemical and functional parameters, including molecular weight determination, immunoreactivity, glycosylation analysis and biological activity. SDS-PAGE and Western blotting confirmed the expected molecular weight of mcry3A to be approximately 67,700 Daltons. In the plant derived protein sample, a second band was present and was thought to represent mcry3A that had been degraded in the plant cell. PMI was confirmed to be approximately 45,000 Daltons. No glycosylation was observed for either protein.

The insecticidal activity of plant-derived mcry3A was similar to the bacterially produced protein and the enzyme activity of plant-derived PMI was equivalent to that of the bacterially produced enzyme. The two amino acid changes in the plant expressed PMI do not affect the function of this enzyme.

These studies established that bacterially produced mcry3A and PMI are equivalent to those proteins produced in corn line MIR604, thus support the use of the bacterial proteins in the toxicity testing.

Potential toxicity of mcry3A

Acute Oral Toxicity Study of Modified Cry3A Protein (MCRY3A-0102) in the Mouse. Study Director Ian Johnson. Syngenta Seeds Inc, Study No. AM7301. November 11, 2003.

Test material	mcry3A protein preparation produced in *E. coli* (90.3% mcry3A protein w/w)
Vehicle	1% w/v aqueous methylcellulose
Test Species	AP_fCD-1 mice (five males and five females)
Dose	2632 mg/kg bw protein preparation, equivalent to 2377 mg/kg bw mcry3A by gavage
Control	vehicle only

The mice received a single dose of 2377 mg/kg bw mcry3A and were observed for two weeks. Parameters evaluated included body weights, food consumption and detailed clinical observations. At the end of the study all animals were killed and examined post mortem. Brain, liver, kidneys and spleen were weighed and selected tissues were taken for histopathological examination.

One female mouse was killed on day 2 of the study due to clinical signs consistent with a dosing injury and not related to the test substance. No test substance-related mortalities occurred. There were no test substance-related effects on body weight, food consumption, organ weights or macroscopic and microscopic pathology.

Therefore, under the conditions of this study, the acute oral LD_{50} of mcry3A in mice is greater than 2377 mg/kg bw, the highest dose tested

Potential toxicity of PMI

Phosphomannose isomerase: Acute Oral Toxicity Study in Mice Study Director Janice Kuhn. Stillmeadow Inc. Sugar Land TX. Study No. 4708-98. 11 August 1999

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

The mice received a single dose of 3080 mg/kg bw PMI and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. At the end of the study all animals were killed and examined post mortem. 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 is greater than 3080 mg /kg bw.

Similarities with known protein toxins

<u>mcry3A</u>

To determine whether mcry3A has any significant homology with known protein toxins, its amino acid sequence was systematically compared to the latest posting of the National Centre for Biotechnology Information (NCBI) Entrez Protein Database (NCBI, 2004) containing all the publicly available protein sequences. The appropriate cut-off expectation (E) value was determined to be 0.38 and amino acid sequences with E values lower than this were considered to be significant.

Two hundred and twenty three entries in GenBank returned E values below 0.38. Most of these (216) were identified as known or putative delta-endotoxins. None of other entries were identified as known or putative toxins other than delta-endotoxins (Hart and Rabe, 2004a).

PMI

To determine whether the PMI protein sequence has any significant homology with known protein toxins, it was systematically compared to the latest posting of the National Centre for Biotechnology Information (NCBI) Entrez Protein Database (NCBI, 2004) containing all the publicly available protein sequences. The sequence used in this study did not take into account the two amino acid changes present in PMI in MIR604. The appropriate cut-off expectation (E) value was determined to be 0.17 and amino acid sequences with E values lower than this were considered to be significant.

One hundred and thirty three protein entries in GenBank returned E values below 0.17. One hundred and fourteen of these were identified as known or putative PMI proteins. Sixteen entries were hypothetical proteins and 3 were unnamed proteins. No association with known or putative toxins was described for these entries (Hart and Rabe, 2003b)

Conclusion

The data from the acute oral toxicity studies and bioinformatics analyses of the novel proteins indicate that neither of the proteins are toxic at high levels in mice, nor do they show any similarity with known protein toxins.

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 (Jones and Maryanski, 1991; Lehrer and Reese, 1998).

Similarity to known allergens

A comparison on the amino acid sequence of the introduced proteins to known protein allergens is one of the steps in a multilevel decision tree to assess allergenic potential (Metcalfe *et al.*, 1996).

mcry3A

To determine whether mcry3A has any significant homology with allergenic proteins, the protein sequence was systematically compared to the Syngenta Biotechnology Inc (SBI) Allergen database.

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.

Overall similarity was examined by comparing sequential 80-amino acid sequences covering the entire mcry3A 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 (FAO/WHO, 2001).

The mcry3A 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 sequence homology was found between any of the sequential mcry3A 80-amino acid peptides and any entries in the database. No alignments of eight or more contiguous identical amino acids between mcry3A and any of the proteins in the database were identified (Hart and Rabe, 2004c).

PMI

To determine whether PMI as expressed in corn line MIR604 has any significant homology with allergenic proteins, the MIR604 protein sequence was systematically compared to the SBI Allergen database. The sequence used in this study is the amino acid sequence of PMI as expressed in corn line MIR604 (that is with the two changes to amino acid sequence).

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 (FAO/WHO, 2001).

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 PMI 80-amino acid peptides and any entries in the database. There was one region of eight identical amino acids between PMI and the known allergen α-parvalbumin from *Rana species* CH2001 (Rabe, 2004)(Hilger *et al*., 2002).

One case of severe food-induced anaphylaxis in a single individual who consumed Indonesian frogs legs has been shown to be due to the protein α-parvalbumin from *Rana species* (Hilger *et al.,* 2002). The same protein from *Rana esculenta* (the common edible frog) elicited no response in serum from the same individual. To determine if IgE antibodies present in this patient's serum recognised PMI, Syngenta sent a sample of PMI to Dr Hilger's laboratory for cross-reactivity analysis (Hilger, 2004).

The PMI sample was bacterially derived and did not contain the two amino acid changes present in MIR604 PMI, however, the region of identity between PMI and α-parvalbumin was not affected by this change and therefore the use of bacterially derived PMI would not affect the outcome of this test.

No cross reactivity between the human serum IgE and PMI occurred. This indicates that the allergic patient's serum IgE does not recognise any portion of the PMI protein as an allergenic epitope. Therefore, the similarity between PMI and *Rana species* α-parvalbumin is not biologically relevant.

In vitro digestibility

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Astwood and Fuchs, 1996; Metcalfe *et al.,* 1996; Kimber *et al*., 1999). The mcry3A and phosphomannose isomerase proteins were therefore investigated for their digestibility in simulated digestion models.

mcry3A

mcry3A from two sources (MIR604 corn-derived and bacterially-derived) was digested in simulated gastric fluid, at pH 1.2 and 10 units pepsin / µg protein. Samples were examined by SDS-PAGE and Western blot analysis. No intact mcry3A (approximately 67,600 Daltons) or immunoreactive fragments were detected following digestion for two minutes. This indicates that mcry3A is not stable in the peptic and acidic conditions of the digestive system and therefore unlikely to be a food allergen (Joseph and Graser, 2003b).

PMI

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 0.0001X of 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. In the unlikely event that PMI survived digestion by pepsin, it would be digested in the mammalian intestinal environment by pancreatin. This indicates that PMI is not stable to digestion and is unlikely to be a food allergen (Privalle, 1999).

Glycosylation

mcry3A

mcry3A was assessed for glycosylation by DIG Glycan analysis. The limit of detection was 2.5 ng. mcry3A samples of 1000 ng were tested and neither the *E. coli*-expressed mcry3A nor the corn-expressed mcry3A were found to be glycosylated.

PMI

The PMI amino acid sequence contains no consensus sequences for N-glycosylation, although O-glycosylation could theoretically occur (Privalle, 2002b). Mass spectrometric analysis of human PMI indicates that this protein is not post-translationally modified (Freeze, 2002).

Thermolability

mcry3A

The effect of temperature on mcry3A was determined by incubation for 30 minutes at a range of temperatures (4ºC, 25ºC, 37ºC, 65ºC, and 95ºC) followed by a bioassay against Western corn rootworm larvae. At 95ºC mcry3A was completely inactivated. Some reduction in activity was observed after incubation at 65ºC and temperatures of 4ºC, 25ºC and 37ºC had no effect on mcry3A bioactivity (Joseph, 2003).

PMI

The stability of PMI 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 protein (Hill, 2003).

Conclusion

No significant homology was found between the novel proteins and known protein allergens. *In vitro* digestibility studies indicate that the novel proteins are quickly digested. Glycosylation and thermolability studies did not indicate a cause for concern. It is unlikely that either mcry3A or PMI have any allergenic potential.

4.5 Conclusion regarding characterisation of the novel proteins

Corn line MIR604 expresses two novel proteins – mcry3A and PMI, expressed at low levels in the corn grain.

A number of studies have been done on these proteins to determine their potential toxicity and allergenicity. These studies demonstrate that the proteins are non-toxic to mammals, and have limited potential to be allergenic.

5. COMPARATIVE ANALYSES

Most crops, including oilseed crops, exhibit considerable variability in their nutrient composition. Environmental factors and the genotype of the plant have an enormous impact on composition. Thus, variation in these nutrient parameters is a natural phenomenon and is considered to be normal.

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 (WHO, 2000). The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO, 1996).

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 comparison include protein, fat, carbohydrates, amino acids, fatty acids, vitamins, minerals, and phytic acid (OECD, 2002b).

5.1 Nutrient analysis

To determine whether unexpected changes had occurred in the nutrient composition of corn line MIR604 as a result of the genetic modification, and to assess the nutritional adequacy of 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 2002: C = control grain and D = MIR604 grain
E = control grain and F = MIR604 grain

In 2003 E1 = control grain and E3 = MIR604 grain
E2 = control grain and E4 = MIR604 grain

The transgenic corn and control lines were grown at three replicate plots at four locations in 2002 and nine locations in 2003. Grain samples were from pooled ears harvested from 10-15 plants from each genotype from each replicate plot at each location.

Analyses performed include:

Analyte	**2002**	**2003**
Proximates	✓	✓
ash	✓	✓
fat	✓	✓
moisture	✓	✓
protein	✓	✓
carbohydrate		✓
crude fibre	✓	
Acid detergent fibre		✓
Neutral detergent fibre		✓
Total dietary fibre		✓

Minerals; Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn, Cr, Se.	✓	✓
Beta-carotene	✓	✓
Cryptoxanthin		✓
Folic acid	✓	✓
Vitamin B1	✓	
Vitamin B2	✓	✓
Vitamin B3	✓	✓
Analyte	**2002**	**2003**
Vitamin B5		✓
Vitamin B6	✓	✓

Vitamin C		✓
Vitamin E	✓	✓
Amino acid composition	✓	✓
Fatty acid profile	✓	✓
Ferulic and p-Coumaric acids		✓
Furfural		✓
Inositol		✓
Phytic acid		✓

Raffinose		✓
Trypsin inhibitor		✓
Phytosterols		✓
cholesterol		✓
campestrol		✓
stigmasterol		✓
beta-sitosterol		✓

Proximates

Results of the proximate analysis are shown in Table 7 and literature ranges in Table 8. Where statistically significant differences were observed, these were small (e.g. carbohydrates analysed in 2003 differed by only 1 – 1.5% between control and transgenic grain), were not consistently observed between pairs and over the different growing seasons, or were within the available literature ranges and not considered to be biologically significant.

Minerals

Results of the mineral analysis are shown in Table 9 and literature ranges in Table 10. Where statistically significant differences were observed, these were small, were not consistently observed between pairs and over the different growing seasons, or were within the available literature ranges and not considered to be biologically significant.

Vitamins

Vitamin analyses are shown in Table 11 and literature ranges in Table 12. Scattered significant differences were observed. Most are not consistent across growing season or the direction of the difference is inconsistent. A significant difference was noted for gamma-tocopherol for both hybrid pairs in 2003, however values for both transgenic and control samples are within the range reported in the literature.

Amino acids

Eighteen amino acids were analysed in 2002 and 2003. The results are shown in Table 13 and the literature range in Table 14. Several statistically significant differences were noted in 2003, however these were not observed in 2002. The degree of difference between average values for the transgenic and control samples ranged from 1 – 10%. All values of amino acids were within the ranges reported in the literature.

Fatty Acids

The five most abundant fatty acids in corn were analysed in the grain. The results are presented in Table 15 and literature ranges in Table 16.

No difference was observed between the transgenic and control grain for palmitic acid. Some significant differences were observed for stearic, linoleic and linolenic acids (in one or two of the four hybrid pairs), however these were within the range of literature values and not considered to be relevant. A significant difference was observed for oleic acid for three of the four hybrid pairs and some of the values were outside of the literature range for oleic acid. In all cases, the levels of oleic acid in MIR604 grain were closer to the literature range than those of the control and therefore this result is considered to be normal variability rather than the genetic modification.

Secondary metabolites and anti-nutrients

Secondary metabolites are defined as those natural products which do not function directly in the primary biochemical activities which support the growth, development and reproduction of the organism in which they occur. One class of secondary metabolites, anti-nutrients, is responsible for deleterious effects related to the absorption of nutrients and micronutrients from foods. There are no generally recognised anti-nutrients in maize at levels considered to be harmful, but for the purposes of assessment of substantial equivalence, the OECD has asked for analytical data for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor.

These selected secondary metabolites and anti-nutrients were included in the compositional analysis of the 2003 grain samples and are shown in Table 17. Statistically significant differences are noted for both ferulic acid and p-coumaric acid, with lower levels of both in the transgenic samples as compared to the control samples, but all values are within ranges reported in the literature (Table 18).

Phytosterols

Grain from the 2003-growing season was analysed for the phytosterols campesterol, β-sitosterol and stigmasterol (Table 19). Small but statistically significant differences were observed for campesterol and stigmasterol for both hybrid pairs, with the transgenic samples having higher amounts of both these phytosterols, compared to the control samples. Levels of phytosterols in both the transgenic and control grain are below the levels published in the literature, but there is very limited historical data available, and it is insufficient to determine if these levels are within conventional ranges (Table 20).

5.2 Conclusion

The comparative analyses do not indicate that there are any compositional differences of biological significance in corn grain from transgenic corn line MIR604, compared to the non-GM control. 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. On the whole, it can be concluded that MIR604 corn grain is equivalent in composition to non-GM corn grain.

Table 7: Proximate Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons

2003	Moisture %fw	Protein %dw	Total Fat % dw	Ash %dw	Carbohydrates %dw	Starch %dw	NDF [a] %dw	ADF [b] %dw	Total Dietary Fibre %dw
E3 (MIR604)	9.84	10.88	3.53	1.55	84.0	55.2	13.4	5.5	13.4
E1 (Control)	9.84	10.42	3.38	1.51	84.7	56.7	12.9	4.9	14.1
Standard Deviation	0.40	0.61	0.37	0.15	0.7	2.5	1.2	0.8	0.9
Coefficient of Variation	4.1%	5.8%	10.8%	9.6%	0.8%	4.5%	9.4%	15.4%	6.8%
F-Test Probability for Genotype	97.4%	1.6%	15.5%	40.7%	0.3%	6.3%	12.7%	1.5%	1.2%
F-Test Probability for Location x Genotype Interaction	0.2%	81.7%	76.3%	49.2%	91.8%	35.6%	11.5%	2.3%	18.6%

2003	Moisture %fw	Protein %dw	Total Fat % dw	Ash %dw	Carbohydrates %dw	Starch %dw	NDF [a] %dw	ADF [b] %dw	Total Dietary Fibre %dw
E4 (MIR604)	9.90	11.80	3.88	1.50	82.8	54.5	12.9	5.2	13.1
E2 (Control)	10.32	11.00	3.46	1.48	84.0	55.7	13.2	4.9	13.5
Standard Deviation	0.35	0.70	0.32	0.11	0.6	2.0	2.0	0.9	0.8
Coefficient of Variation	3.5%	6.1%	8.6%	7.1%	0.8%	3.7%	15.7%	17.5%	5.7%
F-Test Probability for Genotype	0.2%	0.3%	0.1%	60.0%	<0.1%	9.9%	65.6%	26.7%	11.0%
F-Test Probability for Location x Genotype Interaction	2.6%	10.5%	4.9%	22.6%	0.6%	4.9%	31.7%	11.9%	14.2%

a. Fibre by neutral detergent fiber method
b. Fibre by acid detergent fiber method

Table 7: Proximate Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Moisture %fw	Protein %dw	Total Fat % dw	Ash %dw	Starch %dw	Crude Fibre %dw
D (MIR604)	9.18	10.44	2.65	1.56	68.70	3.49
C (Control)	8.90	10.02	2.63	1.55	68.90	3.39
Standard Deviation	0.33	0.49	0.08	0.10	1.40	0.21
Coefficient of Variation	3.7%	4.8%	2.9%	6.1%	2.0%	6.0%
F-Test Probability for Genotype	12.4%	12.2%	60.5%	85.0%	77.2%	34.1%
F-Test Probability for Location x Genotype Interaction	11.7%	11.3%	18.5%	41.4%	15.7%	35.9%

2002	Moisture %fw	Protein %dw	Total Fat % dw	Ash %dw	Starch %dw	Crude Fibre %dw
F (MIR604)	8.93	11.21	3.09	1.59	67.99	3.49
E (Control)	9.37	11.41	2.89	1.62	69.09	3.46
Standard Deviation	0.43	0.39	0.24	0.07	1.44	0.25
Coefficient of Variation	4.7%	3.5%	8.0%	4.2%	2.1%	7.1%
F-Test Probability for Genotype	7.1%	32.1%	12.2%	38.4%	15.6%	78.3%
F-Test Probability for Location x Genotype Interaction	17.4%	5.5%	8.3%	2.9%	87.1%	49.4%

Table 8: Proximate Composition of Maize Grain Reported in the Literature

Source		Moisture % fw	Protein % dw	Total Fat % dw	Ash % dw	Carbohydrate % dw	Starch % dw	NDF [a] % dw	ADF [b] % dw	Total Dietary Fibre % dw
(OECD, 2002a)	**Range**	7.0 - 23	6 - 12.7	3.1 - 5.8	1.1 - 3.9	82.2 - 82.9		8.3 - 11.9	3.0 - 4.3	11.1
(ILSI 2003)	**Range**	6.1 - 26.2	6.15 - 15.01	1.742 - 5.564	0.616 - 6.282	77.4 - 89.5	67.8 - 73.8	5.59 - 22.64	1.82 - 11.34	11.8 - 25.63
	Average	11.2	10.25	3.554	1.44	84.7	71.8	11.01	3.79	16.22
	N	773	773	719	749	749	24	725	725	80
(USDA 2004)[a]	**Average**	10.37	9.42	4.74	1.2	74.26				
	N	10	7	5	4					
(Watson, 1987)	**Range**	7 - 23	6 - 12	3.1 - 5.7	1.1 - 3.9		61 - 78	8.3 - 11.9	3.3 - 4.3	
	Average	16	9.5	4.3	1.42		71.7	9.5	3.3	
(Souci *et al*., 1994))	**Range**	12 - 13.2	7.61 - 9.84	3.20 - 4.30			60.98 - 63.80			
	Average	12.5	8.54	3.8			61.45			

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

Table 9: Mineral Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons

2003	Calcium mg/100g	Copper mg/100g	Iron mg/100g	Magnesium mg/100g	Manganese mg/100g	Phosphorus mg/100g	Potassium mg/100g	Sodium mg/100g	Zinc mg/100g	Selenium [a] mg/100g
E3 (MIR604)	4.76	0.174	2.92	123	0.692	331	405	<LOQ[c] -12.8	2.52	<LOQ[c] - 0.0281
E1 (Control)	4.40	0.195	2.76	111	0.680	303	390	<LOQ[c] -12.8	2.49	<LOQ[c] - 0.0372
Standard Deviation	0.40	0.022	0.27	10	0.106	26	26		0.23	
Coefficient of Variation	8.7%	12.0%	9.7%	8.7%	15.5%	8.1%	6.6%		9.3%	
F-Test Probability for Genotype	0.5%	0.3%	5.9%	<0.1%	68.2%	0.1%	5.0%		75.5%	
F-test Probability for Location x Genotype Interaction	<0.1%	23.5%	31.0%	75.6%	74.0%	68.8%	13.0%		40.7%	

2003	Calcium mg/100g	Copper mg/100g	Iron mg/100g	Magnesium mg/100g	Manganese mg/100g	Phosphorus mg/100g	Potassium mg/100g	Sodium mg/100g	Zinc mg/100g	Selenium[a] mg/100 g
E4 (MIR604)	4.96	0.236	2.88	127	0.690	336	385	<LOQ[c] - 17.2	2.48	<LOQ[c] - 0.0312
E2 (Control)	4.68	0.237	2.61	121	0.753	331	388	<LOQ[c] - 12.7	2.41	<LOQ[c] - 0.0285
Standard Deviation	0.32	0.055	0.39	13	0.068	31	28		0.28	
Coefficient of Variation	6.7%	23.1%	14.1%	10.3%	9.3%	9.2%	7.4%		11.3%	
F-Test Probability for Genotype	1.7%	95.8%	4.3%	23.0%	1.2%	61.7%	74.3%		42.5%	
F-test Probability for Location x Genotype Interaction	6.5%	61.3%	32.2%	51.5%	10.9%	52.1%	51.2%		33.1%	

Table 9: Mineral Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Calcium mg/100g	Copper[b] mg/100 g	Iron mg/100g	Magnesium[b] mg/100 g	Manganese[b] mg/100 g	Phosphorus mg/100g	Potassium mg/100g	Sodium mg/100g	Zinc mg/100g	Chromium[b] mg/100 g
D (MIR604)	3.09	0.3	2.82	91.1	0.64	305	347	4.38	1.64	0.1
C (Control)	2.96	0.3	2.79	92.2	0.67	303	340	4.90	1.90	0.1
Standard Deviation	0.28		0.51			21	21	0.71	0.08	
Coefficient of Variation	9.4%		18.1%			7.1%	6.0%	15.2%	4.8%	
F-Test Probability for Genotype	35.9%		89.4%			83.4%	52.0%	16.8%	<0.1%	
F-test Probability for Location x Genotype Interaction	77.4%		84.4%			22.1%	43.0%	53.7%	60.9%	

2002	Calcium mg/100g	Copper[b] mg/100g	Iron mg/100g	Magnesium[b] mg/100 g	Manganese[b] mg/100 g	Phosphorus mg/100g	Potassium mg/100g	Sodium mg/100g	Zinc mg/100g	Chromium[b] mg/100 g
F (MIR604)	2.99	0.3	3.08	92.2	0.67	307	311	3.88	1.98	0.1
E (Control)	2.71	0.3	2.82	93.3	0.68	307	327	4.11	2.25	0.1
Standard Deviation	0.29		0.45			8	20	0.86	0.17	
Coefficient of Variation	10.0%		15.1%			2.5%	6.3%	21.6%	8.2%	
F-Test Probability for Genotype	8.5%		27.0%			100.0%	15.0%	58.7%	1.7%	
F-test Probability for Location x Genotype Interaction	13.8%		53.8%			77.0%	77.7%	99.1%	19.6%	

a. Selenium analysis only for five locations, due to insufficient sample size.

b. Due to the limits of quantitation of the analytical methods for these analytes, these data were not suited to statistical analysis.

c. Levels were below the limit of quantitation (<LOQ) for the analytical methods used

Table 10: Mineral Composition of Maize Grain Reported in the Literature (dry weight)

		Calcium	Copper	Iron	Magnesium	Manganese	Phosphorus
(OECD, 2002a)	**Range**	3 - 100 mg/100g	0.09 - 1.0 mg/100g	0.1 - 10 mg/100g	82 - 1000 mg/100g		234 - 750 mg/100g
(ILSI 2003)	**Range**	21.6 - 208.4 ppm	0.73 - 5.01 ppm	10.42 - 49.07 ppm	788.3 - 1605.5 ppm	2.61 - 11.25 ppm	2080.5 - 4341.8 ppm
	Average	47.3	1.72	21.78	1199.4	6.25	3341.8
	N	720	625	632	633	632	725
(USDA 2004)	**Average**	7 mg/100g	0.314 mg/100g	2.71 mg/100g	127 mg/100g	0.485 mg/100g	210 mg/100g
	N	4	6	6	1	3	5
(Watson, 1987)	**Range**	0.01 - 0.1 %dw	0.9 - 1.0 mg/kg	1 - 100 mg/kg	0.09 - 1.0 %dw	0.7 - 54 mg/kg	0.26 - 0.75 %dw
	Average	0.03 %dw	4.0 mg/kg	30.0 mg/kg	0.14 %dw	5.0 mg/kg	0.29 %dw
(Souci *et al*., 1994)	**Range**	10.0 - 19.0 mg/g	70.0 - 250.0 ug/g	0.5 - 2.40 mg/g		150 - 800 ug/100g	
	Average	15.0 mg/g			120.0 mg/g	480 ug/100g	256 mg/100g

		Potassium	Sodium	Zinc	Selenium	Chromium
(OECD, 2002a)	**Range**	320 - 720 mg/100g	0 - 150 mg/100 g	1.2 - 3.0 mg/100g	0.001 - 0.1 mg/100g	
(ILSI 2003)	**Range**	2710.0 - 5275.6 ppm	5.08 - 440.18 ppm	6.5 - 37.2 ppm	0.07 - 0.36 ppm	
	Average	3808.4	111.86	21.4	0.18	
	N	633	43	633	7	
(USDA 2004)	**Average**	287 mg/100 g	35 mg/100g	2.21 mg/100g	15.5 ug/100g	
	N	1	1	5	5	
(Watson, 1987)	**Range**	0.32 - 0.72 % dw	0.0 - 0.15 %dw	12 - 30 mg/kg	0.01 - 1.00 mg/kg	0.06 - 0.16 mg/kg
	Average	0.37 %dw	0.03 %dw	14.0 mg/kg	0.08 mg/kg	0.07 mg/kg
(Souci *et al*., 1994)	**Range**	310.0 - 350.0 mg/100g	1.0 - 10.0 mg/100g			27.0 - 37.0 ug/100g
	Average	330.0 mg/100g	6.0 mg/100g	2.5 mg/100g	16 ug/100g	32.0 ug/100g

Table 11: Vitamin Analysis of MIR604 Maize Grain from 2002 and 2003 Growing Seasons

2003	Beta Carotene RE/g[a]	Cryptoxanthin RE/g[a]	Folic Acid mg/kg	Vitamin B1 Thiamine mg/kg	Vitamin B2 Riboflavin mg/kg	Vitamin B3 Niacin mg/kg	Vitamin B5 Pantothenic Acid µg/g	Vitamin B6 mg/kg	Vitamin C mg/kg	Alpha Tocopherol mg/100g	Beta Tocopherol mg/100g	Gamma Tocopherol mg/100g	Delta Tocopherol mg/100g
E3 (MIR604)	0.157	0.0624	0.588	4.86	1.44	27.74	8.05	6.85	<LOQ	<LOQ - 0.990	<LOQ	3.12	<LOQ
E1 (Control)	0.130	0.0546	0.584	4.59	1.43	25.57	8.50	6.68	<LOQ	<LOQ - 1.270	<LOQ	3.80	<LOQ
Standard Deviation	0.023	0.0103	0.090	0.37	0.17	1.99	1.17	0.52				0.43	
Coefficient of Variation	16.4%	17.6%	15.5%	7.8%	11.9%	7.5%	14.1%	7.7%				12.3%	
F-Test Probability for Genotype	<0.1%	1.4%	87.5%	1.6%	92.4%	0.1%	20.0%	26.5%				<0.1%	
F-Test Probability Location x Genotype Interaction	34.4%	51.1%	20.2%	52.0%	60.1%	38.8%	74.4%	4.4%				54.5%	

Table 11: Vitamin Analysis of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2003 [b]	Beta Carotene RE/g [a]	Cryptoxanthin RE/g [a]	Folic Acid mg/kg	Vitamin B1 Thiamine mg/kg	Vitamin B2 Riboflavin mg/kg	Vitamin B3 Niacin mg/kg	Vitamin B5 Pantothenic Acid µg/g	Vitamin B6 mg/kg	Vitamin C mg/kg	Alpha Tocopherol mg/100g	Beta Tocopherol mg/100g	Gamma Tocopherol mg/100g	Delta Tocopherol mg/100g
E4 (MIR604)	0.125	0.0442	0.715	4.71	1.66	24.31	8.83	6.43	<LOQ	0.989	<LOQ	2.80	<LOQ
E2 (Control)	0.200	0.0715	0.679	4.26	1.35	23.75	8.28	6.02	<LOQ	1.098	<LOQ	3.09	<LOQ
Standard Deviation	0.031	0.0136	0.059	0.25	0.35	1.33	0.99	0.33		0.098		0.41	
Coefficient of Variation	18.8%	23.2%	8.4%	5.6%	23.4%	5.5%	11.6%	5.3%		9.5%		13.8%	
F-Test Probability for Genotype	<0.1%	<0.1%	8.9%	<0.1%	1.6%	21.0%	13.7%	0.2%		0.4%		4.6%	
F-Test Probability Location x Genotype Interaction	11.7%	45.8%	2.2%	31.3%	21.4%	0.4%	38.6%	0.4%		88.4%		57.7%	

Table 11: Vitamin Analysis of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Beta Carotene IU/kg [c]		Folic acid mg/kg	Vitamin B1 Thiamine mg/kg	Vitamin B2 Riboflavin mg/kg	Vitamin B3 Niacin mg/kg		Vitamin B6 mg/kg		Vitamin E IU/kg [d]
D (MIR604)	922		<LOQ - 0.163	4.30	1.89	24.40		4.19		30.6
C (Control)	1052		<LOQ - 0.148	3.90	1.57	25.75		3.56		27.0
Standard Deviation	306			1.09	0.04	2.52		0.84		4.3
Coefficient of Variation	30.9%			26.7%	20.8%	10.1%		21.7%		14.9%
F-Test Probability for Genotype	39.5%			47.7%	10.6%	29.1%		16.4%		12.5%
F-Test Probability Location x Genotype Interaction	88.2%			46.3%	70.8%	51.3%		61.1%		12.8%

Table 11: Vitamin Analysis of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Beta Carotene IU/kg [c]		Folic acid mg/kg	Vitamin B1 Thiamine mg/kg	Vitamin B2 Riboflavin mg/kg	Vitamin B3 Niacin mg/kg		Vitamin B6 mg/kg		Vitamin E IU/kg [d]
F (MIR604)	1086		0.169	4.49	1.85	22.21		4.83		26.7
E (Control)	1048		<LOQ - 0.153	4.77	1.80	21.19		4.69		26.1
Standard Deviation	262			0.572	0.19	2.38		0.81		6.8
Coefficient of Variation	24.6%			12.3%	10.5%	11.0%		17.1%		25.8%
F-Test Probability for Genotype	76.8%			33.1%	52.7%	38.7%		72.9%		86.3%
F-Test Probability Location x Genotype Interaction	52.2%			47.9%	20.0%	82.8%		47.0%		83.0%

a. 6 ug of beta-carotene = 1 RE (example calculation: 0.157 RE x 6 ug/g = 0.942 ug/g = 0.942 mg/100g)

b. No data from Hawaii field due to insufficient sample for analysis

c. 3.333 IU (International Unit) = 1 RE (example calculation: 922 IU/3.33 = 277 RE/kg x 6 ug/kg = 1662 ug/kg = 1.662 mg/kg)

d. 0.67 mg alpha-tocopherol = 1 IU Vitamin E (example calculation 30.6 IU/kg x 0.67 mg = 20.5 mg/kg = 2.05 mg/100g)

Table 12: Vitamin Analysis of Maize Grain Reported in the Literature (dry weight)

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

Table 12: Vitamin Analysis of Maize Grain Reported in the Literature (dry weight) (continued)

		Vitamin B5 Pantothenic acid	Vitamin B6 Pyridoxine	Vitamin C	Vitamin E	Beta Tocopherol	Gamma Tocopherol	Delta Tocopherol
OECD (2002)	**Range**		4.6 - 9.6 mg/kg	0 mg/100g				
ILSI (2004)	**Range** **Average** **N**		0.457 - 0.732 mg/100g 0.625 80	0 mg/100g		0.081 - 2.280 mg/100g 0.849 30	1.920 - 6.100 mg/100g 3.565 30	0.196 - 1.610 mg/100g 0.679 28
USDA (2004)	**Average** **N**	0.424 mg/100g 1	0.622 mg/100g 2					
Watson (1987)	**Range** **Average**	3.5 - 14 mg/kg 6.6 mg/kg	5.3 mg/kg		17 - 47 IU/kg[a] 30 IU/kg			
Souci (1994)	**Range** **Average**	600 - 700 ug/100g 650 ug/100g	400 ug/100g					

a. Vitamin E IU/kg calculated by multiplying 1.49 x (mg of alpha-tocopherol/ug + 0.1 gamma-tocopherol/kg). One IU = 1 mg of standard DL-alpha-tocopherol

Table 13: Amino Acid Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons

2003	Asp %dw	Thr %dw	Ser %dw	Glu %dw	Pro %dw	Gly %dw	Ala %dw	Cys %dw	Val %dw
E3 (MIR604)	0.776	0.368	0.574	2.10	0.894	0.408	0.829	0.188	0.514
E1 (Control)	0.717	0.350	0.534	1.96	0.879	0.398	0.774	0.202	0.481
Standard Deviation	0.052	0.020	0.036	0.15	0.058	0.026	0.055	0.009	0.033
Coefficient of Variation	7.0%	5.7%	6.5%	7.2%	6.6%	6.3%	6.9%	4.6%	6.7%
F-Test Probability for Genotype	<0.1%	0.6%	0.1%	0.3%	36.8%	16.3%	0.3%	<0.1%	0.3%
F-test Probability for Location x Genotype Interaction	96.9%	81.4%	89.1%	94.2%	88.4%	85.8%	91.9%	12.3%	89.2%

2003	Asp %dw	Thr %dw	Ser %dw	Glu %dw	Pro %dw	Gly %dw	Ala %dw	Cys %dw	Val %dw
E4 (MIR604)	0.780	0.376	0.605	2.27	0.972	0.416	0.898	0.216	0.534
E2 (Control)	0.717	0.352	0.561	2.12	0.939	0.401	0.838	0.223	0.500
Standard Deviation	0.039	0.020	0.035	0.15	0.056	0.025	0.057	0.019	0.029
Coefficient of Variation	5.2%	5.6%	6.0%	6.9%	5.9%	6.2%	6.6%	8.6%	5.5%
F-Test Probability for Genotype	<0.1%	0.3%	0.2%	0.8%	9.3%	8.6%	0.7%	27.1%	0.2%
F-test Probability for									

Location x Genotype Interaction	23.4%	33.4%	4.6%	25.3%	34.9%	70.0%	26.1%	90.0%	48.8%

2002	**Asp %dw**	**Thr %dw**	**Ser %dw**	**Glu %dw**	**Pro %dw**	**Gly %dw**	**Ala %dw**	**Cys %dw**	**Val %dw**
D (MIR604)	0.735	0.368	0.499	1.857	0.860	0.395	0.758	0.232	0.464
C (Control)	0.706	0.361	0.485	1.792	0.842	0.385	0.716	0.229	0.447
Standard Deviation	0.063	0.026	0.033	0.106	0.054	0.022	0.040	0.014	0.027
Coefficient of Variation	8.8%	7.2%	6.8%	5.8%	6.4%	5.7%	5.4%	5.9%	5.9%
F-Test Probability for Genotype	36.8%	60.8%	39.3%	24.5%	51.2%	37.7%	6.5%	62.0%	21.2%
F-test Probability for Location x Genotype Interaction	64.1%	55.1%	19.4%	19.0%	12.1%	66.4%	17.2%	48.5%	35.3%

Table 13: Amino Acid Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Asp %dw	Thr %dw	Ser %dw	Glu %dw	Pro %dw	Gly %dw	Ala %dw	Cys %dw	Val %dw
F (MIR604)	0.785	0.388	0.540	2.037	0.930	0.401	0.828	0.237	0.497
E (Control)	0.809	0.401	0.558	2.083	0.964	0.411	0.836	0.246	0.512
Standard Deviation	0.057	0.028	0.038	0.143	0.048	0.028	0.060	0.014	0.034
Coefficient of Variation	7.1%	7.1%	7.0%	6.9%	5.1%	6.9%	7.2%	5.8%	6.7%
F-Test Probability for Genotype	41.7%	35.0%	36.5%	51.4%	18.3%	47.6%	76.2%	22.5%	36.4%
F-test Probability for Location x Genotype Interaction	70.1%	49.5%	90.2%	91.6%	93.7%	76.6%	92.7%	52.7%	98.1%

2003	Met %dw	Ile %dw	Leu %dw	Tyr %dw	Phe %dw	His %dw	Lys %dw	Arg %dw	Trp %dw
E3 (MIR604)	0.201	0.384	1.407	0.392	0.573	0.303	0.347	0.498	0.0630
E1 (Control)	0.196	0.358	1.289	0.352	0.525	0.297	0.343	0.481	0.0633
Standard Deviation	0.012	0.027	0.107	0.059	0.040	0.018	0.029	0.046	0.0045
Coefficient of Variation	5.8%	7.2%	7.9%	15.9%	7.2%	5.9%	8.4%	9.3%	7.2%
F-Test Probability for Genotype	15.3%	0.3%	0.1%	2.5%	<0.1%	27.2%	64.7%	18.3%	84.4%
F-test Probability for Location x Genotype Interaction	18.8%	82.9%	90.7%	78.7%	91.2%	95.7%	84.0%	63.7%	93.3%

2003	Met %dw	Ile %dw	Leu %dw	Tyr %dw	Phe %dw	His %dw	Lys %dw	Arg %dw	Trp %dw
E4 (MIR604)	0.243	0.404	1.529	0.412	0.601	0.319	0.340	0.506	0.0685
E2 (MIR604)	0.239	0.376	1.408	0.385	0.547	0.313	0.328	0.492	0.0695
Standard Deviation	0.024	0.023	0.113	0.047	0.037	0.019	0.025	0.037	0.0034
Coefficient of Variation	9.9%	5.9%	7.7%	11.9%	6.5%	6.0%	7.4%	7.5%	4.9%
F-Test Probability for Genotype	58.2%	0.2%	0.5%	9.6%	<0.1%	33.1%	14.4%	25.5%	37.9%
F-test Probability for Location x Genotype Interaction	58.8%	39.8%	22.8%	28.3%	16.6%	58.9%	65.3%	30.8%	4.7%

Table 13: Amino Acid Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons (continued)

2002	Met %dw	Ile %dw	Leu %dw	Tyr %dw	Phe %dw	His %dw	Lys %dw	Arg %dw	Trp %dw
D (MIR604)	0.247	0.326	1.188	0.244	0.472	0.293	0.313	0.452	0.089
C (Control)	0.224a	0.318	1.139	0.235	0.448	0.294	0.310	0.438	0.092
Standard Deviation	0.013	0.031	0.066	0.019	0.030	0.023	0.028	0.038	0.010
Coefficient of Variation	5.3%	9.6%	5.7%	7.9%	6.6%	7.8%	8.9%	8.6%	11.0%
F-Test Probability for Genotype	1.3%	61.4%	16.7%	35.9%	13.7%	92.1%	80.8%	45.3%	50.6%
F-test Probability for Location x Genotype Interaction	9.9%	61.6%	7.0%	22.8%	14.9%	69.1%	86.4%	83.5%	29.6%

2002	Met %dw	Ile %dw	Leu %dw	Tyr %dw	Phe %dw	His %dw	Lys %dw	Arg %dw	Trp %dw
F (MIR604)	0.251	0.359	1.327	0.241	0.521	0.319	0.308	0.461	0.093
E (Control)	0.239	0.366	1.349	0.273	0.529	0.333	0.332	0.495	0.099
Standard Deviation	0.015	0.023	0.092	0.025	0.036	0.023	0.024	0.042	0.012
Coefficient of Variation	6.2%	6.4%	6.9%	9.6%	6.9%	7.0%	7.5%	8.8%	12.3%
F-Test Probability for Genotype	13.7%	56.7%	62.8%	3.3%	66.3%	22.9%	7.4%	14.5%	35.6%
F-test Probability for Location x Genotype Interaction	45.4%	97.0%	95.8%	22.0%	97.1%	47.7%	64.6%	63.7%	11.6%

a. One data point (outlier) excluded

Table 14: 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 % dw	0.27 - 0.58 % dw	0.35 - 0.91 %dw	1.25 - 2.58 % dw	0.63 - 1.36 % dw	0.26 - 0.49 % dw	0.56 - 1.04 % dw	0.08 - 0.32 % dw	0.21 - 0.85 % dw
ILSI (2004)	**Range**	4.17 - 9.5 mg/g	2.24 - 6.5 mg/g	2.35 - 7.66 mg/g	10.41 - 30.35 mg/g	5.76 - 14.57 mg/g	2.8 - 4.98 mg/g	4.39 - 12.03 mg/g	1.48 - 3.16 mg/g	3.16 - 7.23 mg/g
	Av	6.82	3.53	5	19.8	9.44	3.810	7.9	2.17	4.98
	N	725	725	725	725	725	725.000	725	725	725
USDA (2004)	**Av**	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 mg/100g	320 - 510 mg/100g	500 - 530 g/100g	1.74 - 1.88 g/100g	0.93 - 1.19 g/100g	430 - 440 g/100g	770 - 830 mg/100g	70 - 280 mg/100g	430 - 740 mg/100g
	Av	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 % dw	0.22 - 0.71 % dw	0.79 - 2.41 % dw	0.12 - 0.79 % dw	0.29 - 0.64 % dw	0.15 - 0.38 % dw	0.05 - 0.55 % dw	0.22 - 0.64 % dw	0.04 - 0.13 %dw
ILSI (2004)	**Range**	1.30 - 3.44 mg/g	2.04 - 5.96 mg/g	6.42 - 21.74 mg/g	1.10 - 5.95 mg/g	2.63 - 8.30 mg/g	1.97 - 4.18 mg/g	2.36 - 5.57 mg/g	2.58 - 6.23 mg/g	0.355 - 0.900 mg/g
	Av	2.03	3.74	13	3.5	5.26	2.970	3.1	4.45	0.60
	N	725	725	725	725	725	725.000	725	725	725
USDA (2004)	**Av**	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 mg/100g	350 - 620 mg/100g	0.91 - 2.11 g/100g	190 - 690 mg/100g	320 - 510 mg/100g	130.0 - 330.0 mg/100g	40 - 480 mg/100g	190 - 560 mg/100g	40 - 100 mg/100g
	Av	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 15: Fatty Acid Composition of MIR604 Maize Grain from 2002 and 2003 Growing Seasons

2003	16:0 Palmitic % dw	18:0 Stearic % dw	18:1 Oleic % dw	18:2 Linoleic % dw	18:3 Linolenic % dw
E3 (MIR604)	0.427	0.061	0.801	1.827	0.052
E1 (Control)	0.419	0.064	0.754	1.738	0.054
Standard Deviation	0.043	0.007	0.094	0.201	0.004
Coefficient of Variation	10.2%	10.8%	12.1%	11.3%	8.1%
F-Test Probability for Genotype	52.0%	14.9%	9.6%	13.1%	13.9%
F-test Probability for Location x Genotype Interaction	76.4%	69.4%	39.4%	61.4%	48.0%

2003	16:0 Palmitic % dw	18:0 Stearic % dw	18:1 Oleic % dw	18:2 Linoleic % dw	18:3 Linolenic % dw
E4 (MIR604)	0.492	0.082	0.783	2.051	0.051
E2 (Control)	0.474	0.076	0.633	1.855	0.055
Standard Deviation	0.043	0.007	0.064	0.172	0.005
Coefficient of Variation	8.9%	8.4%	9.1%	8.8%	9.1%
F-Test Probability for Genotype	23.2%	2.6%	<0.1%	0.5%	1.2%
F-test Probability for Location x Genotype Interaction	15.6%	3.1%	2.2%	2.2%	6.9%

2002	16:0 Palmitic % dw	18:0 Stearic % dw	18:1 Oleic % dw	18:2 Linoleic % dw	18:3 Linolenic % dw
D (MIR604)	0.320	0.061	0.658	1.806	0.035
C (Control)	0.311	0.064	0.594	1.844	0.034
Standard Deviation	0.008	0.002	0.012	0.056	0.001
Coefficient of Variation	2.5%	3.9%	1.9%	3.1%	3.6%
F-Test Probability for Genotype	5.2%	3.8%	<0.1%	19.7%	10.8%
F-test Probability for Location x Genotype Interaction	8.2%	76.6%	1.8%	29.3%	11.1%

2002	16:0 Palmitic % dw	18:0 Stearic % dw	18:1 Oleic % dw	18:2 Linoleic % dw	18:3 Linolenic % dw
F (MIR604)	0.397	0.077	0.823	2.007	0.035
E (Control)	0.365	0.073	0.656	2.010	0.033
Standard Deviation	0.036	0.006	0.065	0.162	0.003
Coefficient of Variation	9.5%	8.3%	8.8%	8.1%	9.2%
F-Test Probability for Genotype	11.8%	21.4%	0.2%	96.9%	18.1%
F-test Probability for Location x Genotype Interaction	18.0%	6.1%	12.7%	9.8%	10.5%

Table 16: 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 %dw	0.04 - 0.17 %dw	0.70 - 1.39 %dw	0.67 - 2.81 %dw	0.03 - 0.10 % dw
ILSI (2004)	**Range**	8.51 - 17.46 % of total FA	1.02 - 2.76% of total FA	18.6 - 40.1 % of total FA	43.1 - 65.6 % of total FA	0.70 - 1.92 % of total FA
	Average	11.03	1.8	26	57.6	1.13
	N	719	719	719	719	719
USDA (2004)	**Average**	0.569 g/100g	0.075 g/100g	1.247 g/100g	2.097 g/100g	0.065 g/100g
	N	197	197	197	197	197
Souci (1994)	**Range**	250 - 690 mg/100g	36 - 145 mg/100g		0.59 - 2.46 g/100g	30 - 70 mg/100g
	Average	470 mg/100g	90 mg/100g	1.10 g/100g	1.63 g/100g	40.0 mg/100g

Table 17: Secondary Metabolites and Anti-Nutrients in MIR604 Maize Grain from 2003 Growing Season

2003	Ferulic Acid ppm	p-Coumaric ppm	Furfural ppm	Inositol µg/g	Phytic Acid[b] %dw	Raffinose[b] %dw	Trypsin Inhibitor TIU/mg
E3 (MIR604)	1815	131	<LOQ[a]	2783	<LOQ[a] - 0.816	<LOQ[a] - 0.197	2.64
E1 (Control)	2069	188	<LOQ[a]	2784	<LOQ[a] - 0.767	<LOQ[a] - 0.165	2.79
Standard Deviation	202	23		247			0.35
Coefficient of Variation	10.4%	14.0%		8.8%			12.9%
F-Test Probability for Genotype	<0.1%	<0.1%		99.6%			15.6%
F-test Probability for Location x Genotype Interaction	98.1%	44.0%		0.5%			58.1%

2003	Ferulic Acid ppm	p-Coumaric ppm	Furfural ppm	Inositol µg/g	Phytic Acid[b] %dw	Raffinose[b] %dw	Trypsin Inhibitor TIU/mg
E4 (MIR604)	1794	99	<LOQ[a]	2943	<LOQ[a] - 0.937	<LOQ[a] - 0.161	2.75
E2 (Control)	2278	151	<LOQ[a]	2906	<LOQ - 0.792	<LOQ[a] - 0.131	2.85
Standard Deviation	101	17		222			0.28
Coefficient of Variation	5.0%	13.7%		7.6%			10.1%
F-Test Probability for Genotype	<0.1%	<0.1%		61.1%			27.3%
F-test Probability for Location x Genotype Interaction	6.0%	41.0%		5.7%			19.9%

[a] Levels were below the limits of quantitation for the analytical method used (<LOQ)

[b] Some values were <LOQ, calculation of averages and statistical analysis not possible. Range of values is presented instead.

Table 18. Secondary Metabolites and Anti-Nutrients in Maize Grain Reported in the Literature (dry weight)[a]

Source		Ferulic Acid	p- Coumaric	Furfural	Inositol	Phytic Acid	Raffinose	Trypsin Inhibitor TIU/mg dw
(OECD, 2002a)	**Range**	0.02 - 0.3 %dw	0.003 - 0.03 %dw	<0.01 ppm		0.45 - 1.0 %dw	0.21 - 0.31 %dw	
(ILSI 2003)	**Range** **Average** **N**	1340 - 3725.5 mg/kg 2454.6 275	90.7 - 576.2 mg/kg 247.5 275	3.000 - 5.000 mg/kg 3.5 12	138 - 2570 ppm 1374 154	0.290 - 1.287 %dw 0.754 609	0.040 - 0.290 % dw 0.131 270	1.10 - 7.18 TIU/mg dw 2.72 311
(EuropaBio 2003)	**Average**					0.89 %dw		1.9 TIU/mg dw
(Naczk *et al*., 1997)	**Average**						0.21 - 0.31 g/100 gdw	
(Souci *et al*., 1994)	**Range** **Average**					890 - 990 mg/100g 940 mg/100g	190 - 270 mg/100g 230 mg/100g	

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

Table 19: Phytosterol Composition of MIR604 Grain from 2003 Growing Season

2003	Cholesterol mg/100g	Campesterol mg/100g	Stigmasterol mg/100g	Beta-Sitosterol mg/100g
E3 (Mir604)	<LOQ[a]	14.0	6.33	45.5
E1 (Control)	<LOQ[a]	11.8	5.98	45.2
Standard Deviation		2.0	0.53	4.0
Coefficient of Variation		15.6%	8.7%	8.8%
F-Test Probability for Genotype		0.1%	3.5%	78.2%
F-test Probability for Location x Genotype Interaction		16.1%	44.7%	90.0%

2003[b]	Cholesterol mg/100g	Campesterol mg/100g	Stigmasterol mg/100g	Beta-Sitosterol mg/100g
E4 (MIR604)	<LOQ[a]	12.4	5.28	42.6
E2 (Control)	<LOQ[a]	10.4	4.96	43.4
Standard Deviation		1.3	0.29	2.3
Coefficient of Variation		11.6%	5.6%	5.4%
F-Test Probability for Genotype		0.1%	0.7%	37.5%
F-test Probability for Location x Genotype Interaction		92.9%	2.3%	35.6%

[a] Levels were below the limit of quantitation for the analytical methods used

[b] Hawaii data excluded from analysis due to insufficient sample

Table 20: Phytosterol Analysis of Maize Grain Reported in the Literature (dry weight)

Source		Campesterol	Stigmasterol	Beta-Sitosterol
Souci (1994)	**Average**	32 mg/100g	21 mg/100g	120 mg/100g

6. NUTRITIONAL IMPACT

In assessing the safety and suitability 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.

In the case of corn line MIR604, the extent of the compositional and other available data is considered to be adequate to establish the nutritional adequacy of the food. However, the Applicant supplied the results of a 49-day feeding study with MIR604 corn in commercial broiler chickens. Broiler chickens are highly sensitive to small nutrient changes within their diets because of their extremely rapid growth. The MIR604 grain supported growth at low mortality rates and good feed conversion ratios without significant impact on overall carcass yield or quality. This confirms the nutritional quality of MIR604 corn.

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Attachment 3

Summary of first round of public consultation

Submitter	Option	Comments
Food Technology Association of Victoria Inc	2	-
Australian Food & Grocery Council	2	Supports option 2 contingent on the food being shown to be safe at draft assessment
New Zealand Food Safety Authority	-	Reserves comment for after the draft assessment
Department of Health, SA	-	Supports this Application being progressed to draft assessment
National Council of Women of New Zealand	-	Believes this issue to be important to the health and economy of New Zealand Will consider this application further at draft assessment
Private (Mr Paul Elwell-Sutton)	1	Is concerned about the effects of Bt toxins on human health, in particular that Bt toxins may not be easily digested Concerned that the use Bt toxins with the novel antibiotic zwittermicin A may increase toxicity Believes that the current GM labelling requirements are not sufficient Does not agree with the impact analysis that prohibiting GM foods in New Zealand may lead to increased food prices Requests that this application be considered without regard to the WTO and its directives.
Private (Mr Ivan Jeray)	1	Does not support GM food Does not believe that there is evidence for the safety of GM foods