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

12 December 2007
[8-07]

DRAFT ASSESSMENT REPORT

APPLICATION A595

FOOD DERIVED FROM INSECT-PROTECTED CORN LINE MON 89034

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 6 February 2008
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

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

Executive Summary

Food Standards Australia New Zealand (FSANZ) received a paid Application from Monsanto Australia Ltd (the Applicant) on 19 December 2006. The Applicant has requested an amendment to the *Australia New Zealand Food Standards Code* (the Code), specifically to Standard 1.5.2 – Food produced using Gene Technology, to permit the sale and use of food derived from a new genetically modified (GM) variety of corn, MON 89034. Standard 1.5.2 requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

MON 89034 corn has been genetically modified to be protected against feeding damage caused by the larvae of certain insect pest species. Protection is achieved through the expression in the plant of insecticidal proteins derived from *Bacillus thuringiensis*, a common soil bacterium.

Corn line MON 89034 is intended to be grown in North America. However, once commercialised, corn products imported into Australia and New Zealand could contain ingredients derived from MON 89034 corn. Approval is therefore necessary before these products may enter the Australian and New Zealand markets.

Safety Assessment

FSANZ has completed a comprehensive safety assessment of food derived from insect-protected corn line MON 89034, as required under Standard 1.5.2. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel proteins; and (iii) the composition of MON 89034 corn compared with that of conventional corn varieties.

No public health and safety concerns were identified as a result of the safety assessment. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from insect-protected corn line MON 89034 is considered as safe and wholesome as food derived from other commercial corn varieties.

Labelling

If approved, food derived from insect-protected corn line MON 89034 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies conducted by the Applicant show that the novel proteins are present at low levels in the grain.

Labelling addresses the requirement of section 18(1)(b) of the Act; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval, or (2) approval of food derived from insect-protected corn line MON 89034 based on the conclusions of the safety assessment.

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

Preferred Approach

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from insect-protected corn line MON 89034 in the Table to clause 2.

Reasons for Preferred Approach

An amendment to the Code approving food derived from insect-protected corn line MON 89034 in Australia and New Zealand is proposed 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-protected corn line MON 89034;
- food derived from insect-protected corn line MON 89034 is equivalent to food from the conventional counterpart and other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food commodities derived from insect-protected corn line MON 89034 will be required if novel DNA and/or protein is present in the final food; and
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is option 2, an amendment to the Code.

Consultation

The Initial Assessment was advertised for public comment between 21 March and 2 May 2007. A total of fourteen submissions were received during this period and a summary of these is attached to this report (Attachment 3).

FSANZ has taken the submitters' comments into account in preparing the Draft Assessment of this application. Specific issues relating to insect-protected corn line MON 89034 have been addressed in the report.

Public submissions will be invited on this Draft Assessment Report.

CONTENTS

INVITATION FOR PUBLIC SUBMISSIONS

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 of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 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 confidential commercial information. Section 114 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) 6 February 2008.

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 standards.management@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.

INTRODUCTION

An Application was received from Monsanto Australia Limited on 19 December 2006 seeking an amendment to Standard 1.5.2 – Food produced using Gene Technology, in the *Australia New Zealand Food Standards Code* (the Code), to approve food derived from insect-protected corn line MON 89034.

The genetic modification involved the transfer of two *cry* genes into corn. These genes are from a common soil bacterium called *Bacillus thuringiensis* and encode insecticidal proteins (Cry proteins) which protect the plant against feeding damage caused by certain insect pest larvae.

This Draft Assessment includes a full scientific evaluation of food derived from MON 89034 corn to assess its safety for human consumption. Public comment is now sought on the safety assessment and proposed recommendations prior to a Final Assessment and completion of the Application.

1. Background

1.1 Current Standard

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

1.2 Description and Purpose of the Genetic Modification

The genetic modification in insect-protected corn line MON 89034 involves the introduction of the *cry1A.105* and *cry2Ab2* genes derived from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. These genes encode the Cry1A.105 and Cry2Ab2 insecticidal proteins which are selectively toxic to a range of lepidopteran insect larvae including European corn borer, Asian corn borer, Southwestern corn borer, sugarcane borer, fall armyworm and corn earworm.

Cry proteins exert their effect on the target insects by causing lysis of midgut epithelial cells, which leads to gut paralysis, cessation of feeding and eventual death of the insect. The lysis of the midgut epithelial cells is mediated by the binding of the activated Cry protein to specialised receptors on these cells.

The purpose of expressing two different Cry proteins in the corn is to provide extended control over a range of insect pest larvae and also to improve the management of insect resistance to the Cry proteins. Cry1A.105 and Cry2Ab2 have different modes of action, particularly in the way in which they bind to the insect midgut. Because of the dual effective dose and the distinct modes of action, the Applicant claims the likelihood of resistance being developed by target insects is low.

Hybrid corn lines containing the MON 89034 transformation event are intended for cultivation in North America and are not intended to be grown in either Australia or New Zealand. Food from MON 89034 corn will therefore be entering the Australian and New Zealand food supply as imported, largely processed food products.

1.3 Overseas Approvals

The Applicant made submissions for food and feed use to the United States Food and Drug Administration in late 2006 and also requested a Determination of Nonregulated Status for MON 89034 from the U.S. Department of Agriculture, as well as a tolerance exemption from the Environmental Protection Agency.

Regulatory submissions for import approvals have been or will be made to countries that import significant corn or corn products, including China, Japan, Canada, Korea, the Philippines and Taiwan. Feed approval was granted in Japan on 2 October 2007.

2. The Issue / Problem

The Applicant has developed corn line MON 89034 that is protected from feeding damage caused by lepidopteran insect pest larvae. Before food derived from insect-protected corn line MON 89034 can enter the Australian and New Zealand food supply, it must first be assessed for safety and an amendment to the Code must be approved by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted, once the Ministerial Council process has been finalised.

Monsanto Australia Limited has therefore applied to have Standard 1.5.2 amended to include food derived from corn line MON 89034.

3. Objectives

The objective of this assessment is to determine whether it would be appropriate to amend the Code to approve the sale and use of food derived from corn line MON 89034 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 18 of the FSANZ Act. These are:

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

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

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and

- any written policy guidelines formulated by the Ministerial Council.

4. Key Assessment Questions

Based on information provided by the Applicant on the nature of the genetic modification, the molecular characterisation, the characterisation of the novel proteins, the compositional analysis and any nutritional issues, is food derived from corn line MON 89034 comparable to food derived from conventional varieties of corn in terms of its safety for human consumption?

Is other information available, including from the scientific literature, general technical information, independent scientists, other regulatory agencies and international bodies, and the general community, that needs to be considered?

Are there any other considerations that would influence the outcome of this assessment?

RISK ASSESSMENT

Food from insect-protected corn line MON 89034 has been evaluated according to the FSANZ Guidance Document on the Safety Assessment of Genetically Modified Foods[1]. The summary and conclusions from the full safety assessment report (at **Attachment 2**) are presented below. In addition to information supplied by the Applicant, other available resource material including published scientific literature and general technical information was used for the assessment.

5. Risk Assessment Summary

5.1 Safety Assessment Process

The safety assessment applied to food from corn line MON 89034 addresses only food safety and nutritional issues. It therefore does not address: environmental risks related to the environmental release of genetically modified (GM) plants used in food production; the safety of animal feed or animals fed with feed derived from GM plants; or the safety of food derived from the non-GM (conventional) plant.

In conducting a safety assessment of food derived from insect-protected MON 89034 corn, a number of criteria have been addressed including: a characterisation of the transferred genes, their origin, function and stability in the corn genome; the changes at the level of DNA, protein and in the whole food; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic in humans.

5.2 Outcomes of the Safety Assessment

Detailed molecular analyses indicate that one copy of each of the *cry1A.105* and *cry2Ab2* genes have been inserted at a single site in the plant genome and are stably inherited from one generation to the next. No antibiotic resistance marker genes are present in MON 89034 corn.

[1] http://www.foodstandards.gov.au/_srcfiles/GM%20FINAL%20Sept%2007L%20_2_.pdf

MON 89034 corn expresses two novel proteins, Cry1A.105 and Cry2Ab2. Both proteins are expressed at relatively low levels in the grain, with the mean concentration for Cry1A.105 and Cry2Ab2 being 5.1 µg/g fresh weight and 1.1 µg/g fresh weight, respectively. Both proteins as expressed in the plant conform in size and amino acid sequence to that expected, do not exhibit any post-translational modification including glycosylation, and also demonstrate the expected insecticidal activity.

In relation to potential toxicity and allergenicity, *B. thuringiensis* has been extensively studied and has a long history of safe use as the active ingredient in a number of insecticide products for use in agriculture as well as home gardens. It is well established that the Cry proteins from *B. thuringiensis* are inherently non-toxic to mammals and have exhibited little potential to be allergenic to humans over their long history of use.

In addition, bioinformatic studies with the Cry1A.105 and Cry2Ab2 proteins have confirmed the absence of any significant amino acid sequence similarity to known protein toxins or allergens and digestibility studies have demonstrated that both proteins would be rapidly degraded in the stomach following ingestion, similar to other dietary proteins. Acute oral toxicity studies in mice with both proteins have also confirmed the absence of toxicity. Taken together, the evidence indicates that both proteins are highly unlikely to be toxic or allergenic in humans.

Compositional analyses were done to establish the nutritional adequacy of MON 89034 corn, and to compare it to conventional corn lines under typical cultivation conditions. No differences of biological significance were observed between MON 89034 corn and its conventional counterpart. Food from insect-protected MON 89034 corn is therefore considered to be compositionally equivalent to food from conventional corn varieties and its introduction into the food supply would therefore be expected to have little nutritional impact.

5.3 Conclusions

No potential public health and safety concerns have been identified in the assessment of insect-protected MON 89034 corn. On the basis of the data provided in the present application, and other available information, food derived from insect-protected MON 89034 corn is considered as safe and wholesome as food derived from other corn varieties.

RISK MANAGEMENT

6. Options

There are no non-regulatory options for this Application. The two regulatory options available for this Application are:

6.1 Option 1 – Prohibit food from corn line MON 89034

Maintain the *status quo* by not amending Standard 1.5.2 to approve food derived from insect-protected corn line MON 89034.

6.2 Option 2 – Approve food from corn line MON 89034

Amend Standard 1.5.2 to permit the sale and use of food derived from food derived from insect-protected corn line MON 89034, with or without specified conditions in the Table to clause 2 of the Standard.

7. Impact Analysis

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community in Australia and New Zealand.

7.1 Affected Parties

The affected parties may include the following:

- 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.

The cultivation of corn line MON 89034 in Australia or New Zealand could have an impact on the environment, which would need to be assessed by the Office of the Gene Technology Regulator (OGTR) in Australia, and by various New Zealand Government agencies including the Environmental Risk Management Authority (ERMA) and the Ministry of Agriculture and Fisheries (MAF) before growing in either country could be permitted. MON 89034 corn has been developed primarily for agricultural production overseas and, at this stage, the Applicant has no plans for cultivation in either Australia or New Zealand.

7.2 Benefit Cost Analysis

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

7.2.1 Option 1 – prohibit food from corn line MON 89034

Consumers: Possible restriction in the availability of corn products if MON 89034 corn is present in imported foods.
No impact on consumers wishing to avoid GM foods, as food from MON 89034 corn is not currently permitted in the food supply.

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

Industry: Possible restriction on corn imports once MON 89034 corn is commercialised overseas.

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

7.2.2 Option 2 – approve food from corn line MON 89034

Consumers: No restriction on imported corn products if derived from MON 89034 corn. Increased choice in the marketplace as a result of mandatory labelling of certain products derived from MON 89034.

Potential impact on consumers wishing to avoid GM corn by a possible restriction of choice of products, or increased prices for non-GM corn.

Government: Benefit that if MON 89034 corn was detected in corn imports, approval would ensure compliance of those products with the Code. This would ensure no potential for trade disruption on regulatory grounds.

Approval of MON 89034 corn would ensure no conflict with WTO responsibilities.

This option could impact on monitoring resources, as certain foods derived from MON 89034 corn will be required to be labelled as genetically modified.

Industry: Broader market access and increased choice in raw materials for food manufacturing.

Benefit to importers of processed foods containing corn as an ingredient as foods derived from MON 89034 corn would be compliant with the Code.

Possible cost to food industry as some food ingredients derived from MON 89034 corn would be required to be labelled as genetically modified.

7.3 Comparison of Options

As food from insect-protected corn line MON 89034 has been found to be as safe as food from conventional varieties of corn, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations. Option 1 would also offer little benefit to consumers wishing to avoid GM foods, as approval of MON 89034 corn by other countries could limit supplementation of the Australian and New Zealand market with imported corn products. Under Option 2, primary producers would benefit from an increased choice of crop lines with potentially lower production costs and higher yields, which could flow on to other sectors including consumers in Australia and New Zealand as lower food prices.

As MON 89034 corn has been found to be safe for human consumption and the potential benefits outweigh the potential costs, Option 2, an amendment to Standard 1.5.2 giving approval to insect-protected corn line MON 89034, is therefore the preferred option.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

FSANZ has applied a communication strategy to this Application that involves advertising the availability of assessment reports for public comment in the national press and placing the reports on the FSANZ website. In addition, FSANZ will issue a media release drawing journalists' attention to the matter.

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

The Applicant and individuals and organisations that make submissions on this Application will be notified at each stage of the Application. After the FSANZ Board has considered the Final Assessment Report, if the draft variation to the Code is approved, we will notify that decision to the Ministerial Council. If the approval of food derived from insect-protected corn line MON 89034 is not subject to review, the Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website. In addition, FSANZ provides an advisory service to the jurisdictions on changes to the Code.

9. Consultation

9.1 Public Consultation

The Initial Assessment was advertised for public comment between 21 March and 2 May 2007. Fourteen 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. Responses to general issues regarding GM food are available from the FSANZ website[2]. Specific issues relating to food derived from corn line MON 89034 have been addressed in the report. The major issues raised are discussed here.

9.1.1 Animal feeding studies

Two submitters have suggested that the expression of two insecticidal proteins in MON 89034 corn should be enough to make the corn sufficiently different from an earlier GM corn variety (MON 810 corn, approved by FSANZ in November 2000) to warrant the undertaking of animal feeding studies.

9.1.1.1 Response

The similarity or otherwise of corn line MON 89034 to a previously approved corn line is not relevant to FSANZ's decision regarding the need for animal feeding studies.

[2] http://www.foodstandards.gov.au/newsroom/factsheets/factsheets2002/faqsongmfoods6august1632.cfm

FSANZ convened an expert group in June 2007 to consider the need for animal feeding studies in the safety assessment of GM foods. The report from that meeting is available on the FSANZ website[3].

FSANZ considers that a scientifically-informed comparative assessment of GM foods with their conventional counterparts can generally identify any potential adverse health effects or differences requiring further evaluation. In the majority of circumstances, animal toxicity studies with whole foods are not likely to contribute any further useful information to the safety assessment and are therefore not warranted. As a consequence, FSANZ does not require that animal toxicity studies with GM foods be undertaken on a routine basis.

FSANZ acknowledges there may be future GM applications, particularly for foods with intentional modifications to composition, where the results of animal toxicity studies may be informative, if considered appropriate by FSANZ. FSANZ will therefore continue to assess the need for whole food studies on a case-by-case basis, taking into account the nature of the genetic modification and the results of the comparative assessment.

For this Application, the comparative assessment indicates that food derived from corn line MON 89034 is equivalent to food from conventional corn in terms of its composition and nutritional adequacy. The corn grain contains low levels of two insecticidal proteins; however the evidence indicates these proteins are non-toxic to mammals, including humans and has limited potential to be a food allergen. FSANZ has therefore not identified any differences that raise potential safety or nutritional concerns. For this reason, FSANZ does not believe there would be any justification for undertaking additional testing in laboratory animals.

9.1.2 Monitoring and enforcement costs

Queensland Health and the NSW Food Authority have raised the issue of rising monitoring and enforcement costs for governments in terms of available resources, labour and reagent costs. Both jurisdictions consider that a national enforcement strategy for GM food approvals could be needed to address these issues.

9.1.2.1 Response

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

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

[3] http://www.foodstandards.gov.au/_srcfiles/Workshop%20Report%20FINAL.pdf

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

9.1.3 Claims about adverse effects in pigs fed GM corn

One submitter claims that pig farmers who have been feeding their pigs GM corn have noticed their pigs have become 'sterile, contracted cancer or died' and has asked why these findings have not been studied vigorously by the Australian and New Zealand Governments.

9.1.3.1 Response

FSANZ is aware that in 2002 some farmers in the United States claimed that the feeding of GM corn to their pigs caused infertility in their sows; effects such as cancer and death were not reported. FSANZ has not however been able to identify any credible scientific evidence which establishes GM corn as the cause of the infertility.

9.2 World Trade Organization (WTO)

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

The draft variation to the Code would have a trade enabling effect as it would permit food derived from MON 89034 corn to be imported into Australia and New Zealand and sold, where currently it is prohibited. For this reasons it was determined there is no need to notify this Application as a Sanitary and Phytosanitary (SPS) measure in accordance with the WTO Agreement on the Application of SPS Measures

CONCLUSION

10. Conclusion and Preferred Approach

Preferred Approach

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from insect-protected corn line MON 89034 in the Table to clause 2.

10.1 Reasons for Preferred Approach

An amendment to the Code to give approval to the sale and use of food derived from corn line MON 89034 in Australia and New Zealand is proposed 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-protected corn line MON 89034;

- food derived from insect-protected corn line MON 89034 is equivalent to food from the conventional counterpart and other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food commodities derived from insect-protected corn line MON 89034 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is option 2, an amendment to the Code.

11. Implementation and Review

Following the consultation period for this document, a Final Assessment of the Application will be completed and the draft variation considered for approval by the FSANZ Board. The FSANZ Board's decision will then be notified to the Ministerial Council.

Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for a review of FSANZ's decision.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Draft safety assessment report
3. Summary of submissions

Attachment 1

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

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

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

Food derived from insect-protected corn line MON 89034	

Attachment 2

Draft Safety Assessment

Food Derived From Insect-Protected Mon 89034 Corn

SUMMARY AND CONCLUSIONS

Background

Insect-protected MON 89034 corn has been genetically modified (GM) for protection against feeding damage caused by larvae of a number of insect species. Protection is conferred by expression in the plant of two *cry* genes, encoding insecticidal Cry proteins, derived from *Bacillus thuringiensis*, a common soil bacterium. The Cry proteins exert their effect on the insect by causing lysis of midgut epithelial cells, which leads to gut paralysis, cessation of feeding and eventual death of the insect.

Insect-protected MON 89034 corn is claimed to provide extended control over a range of insect pest larvae, compared to crops expressing a single cry gene. MON 89034 corn has been developed for commercial cultivation in North America and may therefore enter the Australian and New Zealand food supply as imported, largely processed food products.

In conducting a safety assessment of food derived from Insect-protected MON 89034 corn, a number of criteria have been addressed including: a characterisation of the transferred genes, their origin, function and stability in the corn genome; the changes at the level of DNA, protein and in the whole food; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic in humans.

This safety assessment report addresses only food safety and nutritional issues. It therefore does not address: environmental risks related to the environmental release of GM plants used in food production; the safety of animal feed or animals fed with feed derived from GM plants; or the safety of food derived from the non-GM (conventional) plant.

History of Use

Corn is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries. 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 MON 89034 corn may include flour, breakfast cereals, high fructose corn syrup and other starch products.

B. thuringiensis, the microorganism from which the *cry* genes were derived, has been extensively studied and commercially exploited for over 40 years as the active ingredient in a number of insecticide products used in agriculture as well as home gardens. *B. thuringiensis* therefore has had a long history of safe use and the Cry proteins it produces are not known to be toxic to any vertebrates, including humans and other mammals.

Molecular Characterisation

MON 89034 corn was generated through the transfer of the *cry1A.105* and *cry2Ab2* genes to the conventional inbred corn line LH172. The *cry1A.105* gene encodes the 133 kDa Cry1A.105 insecticidal protein, which is a chimeric protein consisting of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The *cry2Ab2* gene encodes the 61 kDa Cry2Ab2 insecticidal protein, which is a variant of the wild-type Cry2Ab2 protein from *B. thuringiensis* subspecies *kurstaki*.

Detailed molecular analyses indicate that one copy of each of the *cry1A.105* and *cry2Ab2* genes has been inserted at a single site in the plant genome and the genes are stably inherited from one generation to the next. No antibiotic resistance marker genes are present in MON 89034 corn.

Characterisation of Novel Protein

MON 89034 corn expresses two novel proteins, Cry1A.105 and Cry2Ab2. Both proteins are expressed at relatively low levels in the grain, with the mean concentration for Cry1A.105 and Cry2Ab2 being 5.1 µg/g fresh weight and 1.1 µg/g fresh weight respectively.

A large number of studies have been done to confirm the identity and physicochemical and functional properties of the expressed Cry1A.105 and Cry2Ab2 proteins, as well as to determine their potential toxicity and allergenicity. Both proteins conform in size and amino acid sequence to that expected, do not exhibit any post-translational modification including glycosylation, and also demonstrate the predicted insecticidal activity.

In relation to their potential toxicity and allergenicity, it is worth noting that Cry proteins from *B. thuringiensis* are inherently non-toxic to mammals and have exhibited little potential to be allergenic to humans over their long history of use. In addition, bioinformatic studies with the Cry1A.105 and Cry2Ab2 proteins have confirmed the absence of any significant amino acid sequence similarity to known protein toxins or allergens and digestibility studies have demonstrated that both proteins would be rapidly degraded in the stomach following ingestion, similar to other dietary proteins. Acute oral toxicity studies in mice with both proteins have also confirmed the absence of toxicity. Taken together, the evidence indicates that both proteins are unlikely to be toxic or allergenic in humans.

Compositional Analyses

Compositional analyses were done to establish the nutritional adequacy of MON 89034 corn, and to compare it to conventional corn lines under typical cultivation conditions. The components analysed were protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals, and the anti-nutrient phytic acid.

No differences of biological significance were observed between MON 89034 corn and its conventional counterpart. Some minor differences in key nutrients were noted, however the levels observed were within the range of values measured for commercial corn hybrids and other conventional corn varieties, and therefore they most likely reflect normal biological variability. Food from insect-protected MON 89034 corn is therefore considered to be compositionally equivalent to food from conventional corn varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from insect-protected MON 89034 corn. The introduction of MON 89034 corn into the food supply would therefore be expected to have little nutritional impact. The nutritional adequacy of food derived from MON 89034 corn was also confirmed using a feeding study in rapidly-growing broiler chicks, which demonstrated that MON 89034 corn is equivalent to its conventional counterpart and other commercial corn hybrids in its ability to support typical growth and well being.

Conclusion

No potential public health and safety concerns have been identified in the assessment of insect-protected MON 89034 corn. On the basis of the data provided in the present application, and other available information, food derived from insect-protected MON 89034 corn is considered as safe and wholesome as food derived from conventional corn varieties.

1. BACKGROUND

A safety assessment has been conducted on food derived from corn that has been genetically modified (GM) to be protected against feeding damage caused by the larvae of a number of insect species. The GM corn is referred to as MON 89034 corn.

MON 89034 corn is protected against a range of lepidopteran insect larvae including European corn borer, Asian corn borer, Southwestern corn borer, sugarcane borer, fall armyworm and corn earworm.

Protection is achieved through expression in the plant of two insecticidal Cry proteins, Cry1A.105 and Cry2Ab2, derived from *Bacillus thuringiensis*, a common soil bacterium. Cry1A.105, encoded by the *cry1A.105* gene, is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. The Cry2Ab2 protein is encoded by the *cry2Ab2* gene derived from *B. thuringiensis* subspecies *kurstaki*.

The Cry proteins exert their effect on the host insect by causing lysis of midgut epithelial cells, which leads to gut paralysis, cessation of feeding and eventual death of the insect. The lysis of the midgut epithelial cells is mediated by the binding of the activated Cry protein to specialised receptors on these cells.

Hybrid corn lines containing the MON 89034 transformation event are intended for cultivation in North America and are not intended to be grown in either Australia or New Zealand. Food from MON 89034 corn will therefore be entering the Australian and New Zealand food supply as imported, largely processed food products.

2. HISTORY OF USE

2.1 Host organism

The host organism is corn (*Zea mays L*), otherwise known as maize. Corn is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries (OECD 2002). In 2005, worldwide production of corn was over 700 million tonnes, with the United States and China being the major producers (FAOSTAT 2005).

Corn is naturally cross-pollinated and until about 1925, mainly open-pollinated varieties were grown, whereas today mainly hybrid varieties are grown. Hybrid varieties have more vigorous growth and higher yields.

Field corn has been grown in Mexico and Central America for some 8000 years and in Europe for 500 years and can thus be said to have a long history of safe use as food by humans. The majority of corn that is grown however is destined for use as animal feed.

Corn is processed using both wet and dry milling techniques. Dry milling is the oldest way of processing corn kernels for food and feed use. Three dry milling processes are used: (i) stone grinding, which is used widely in Latin America, Africa, Asia, and by small mills in North America; (ii) dry-grind, which is used for producing ethanol for commercial uses; and (iii) degermining, which is the process most widely used by the food industry.

The degermining process produces grits, meal, flour, oil and hominy feed[4]. Wet milling is used primarily to produce starch, which may be used directly in food products or converted to various sweeteners such as high fructose corn syrup. The starch also serves as a major source of sugar for the fermentation of beverage alcohol.

The corn line used as the recipient for transferred genes is the proprietary inbred line, LH172 (Eggerling 1994). This inbred line was used because it responds well to transformation with *Agrobacterium* and subsequent tissue culture steps. Inbred corn line LH172 is a yellow dent corn and is used as a parental line for producing first generation hybrid corn.

2.2 Donor Organism

The two Cry proteins expressed in MON 89034 corn are derived from *B. thuringiensis*. The effect of *B. thuringiensis* products on human health and the environment was the subject of a critical review by the WHO International Programme on Chemical Safety (WHO 1999). The review concluded that "*B. thuringiensis* products are unlikely to pose any hazard to humans or other vertebrates or the great majority of non-target invertebrates provided that they are free from non-*B. thuringiensis* microorganisms and biologically active products other than the insecticidal proteins".

B. thuringiensis is a facultative anaerobic, gram-positive spore-forming bacterium that produces characteristic insecticidal proteins, as parasporal crystals, during the sporulation phase. These crystals are predominantly comprised of one or more Crystal (Cry) and Cytolytic (Cyt) toxins, also called δ-endotoxins.

These toxins are highly specific to their target insect species, are innocuous to humans, vertebrates and plants, and are completely biodegradable (Bravo et al 2007).

Over 60 subspecies of *B. thuringiensis* have been described. *B. thuringiensis* subspecies can synthesise more than one type of Cry protein, which are specifically toxic to the insect orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera, and also to nematodes. The Cyt toxins are mostly found in *B. thuringiensis* strains that are active against Diptera.

A number of different commercial *B. thuringiensis* formulations have been registered worldwide for use as an insecticide to be applied to foliage, soil, and water or food storage facilities. While the *B. thuringiensis* spores or vegetative cells may persist in the environment for weeks, months or years, the Cry proteins become inactive within hours or days.

Studies on mammals, particularly laboratory animals, demonstrate that *B. thuringiensis* is mostly non-pathogenic and non-toxic. *B. thuringiensis* has been demonstrated to be highly specific in its insecticidal activity and has demonstrated little, if any, direct toxicity to non-target insects.

[4] A mixture of the bran, germ, and some of the starchy portion of the corn kernel, used as a feed for livestock

The use of *B. thuringiensis* products in the field can result in considerable aerosol and dermal exposure in humans. With the exception of case reports on ocular and dermal irritation, no adverse health effects have been documented after occupational exposure to *B. thuringiensis* products (McClintock et al 1995).

Studies with human volunteers who ingested and inhaled large quantities of a Btk formulation (*B. thuringiensis* subspecies *kurstaki*) did not reveal any adverse effects (Fisher & Rosner 1959). Similarly, *B. thuringiensis* present in drinking water or food has not been reported to cause adverse effects on human health (WHO 1999).

3. MOLECULAR CHARACTERISATION

Molecular characterisation is necessary to provide an understanding of the genetic material introduced into the host genome and helps to frame the subsequent parts of the safety assessment. The molecular characterisation addresses three main aspects: the transformation method together with a detailed description of the DNA sequences introduced to the host genome; a characterisation of the inserted DNA including any rearrangements that may have occurred as a consequence of the transformation; and the genetic stability of the inserted DNA and any accompanying expressed traits.

3.1 Transformation method

MON 89034 was developed through *Agrobacterium*-mediated transformation of the proprietary inbred corn line, LH172 (Eggerling 1994), using the transformation vector, PV-ZMIR245. PV-ZMIR245 contains two separate T-DNAs. The first T-DNA, designated T-DNA I, contains the *cry1A.105* and the *cry2Ab2* gene expression cassettes. T-DNA II contains the *nptII* gene expression cassette that encodes the neomycin phosphotransferase II (NPTII) enzyme which confers tolerance to antibiotics such as neomycin and paromomycin.

The use of two separate T-DNAs enables the generation of marker free plants by allowing insertion of the T-DNA with the traits of interest (T-DNA I) and the T-DNA with the selectable marker (T-DNA II) into two independent loci within the corn genome. Following selection of the transformants, the inserted T-DNA encoding the selectable marker can be segregated away through subsequent breeding and genetic selection, while the inserted T-DNA containing the trait(s) of interest is maintained.

Freshly isolated immature corn embryos were used in the transformation. Following inoculation with *Agrobacterium* containing plasmid PV-ZMIR245, the immature embryos were transferred to a co-culture medium for 1-3 days to ensure transformation of individual cells. The immature embryos were then transferred to selection medium containing carbenicillin to eliminate *Agrobacterium*, and paromomycin to eliminate cells that were not transformed so that only cells containing T-DNA II, with or without T-DNA I, survived. The resulting transformed cells were then subcultured several times on selection medium and regenerated into R_0 plants.

During subsequent breeding at the F_1 generation, the unlinked insertions of T-DNA I and T-DNA II were segregated. Plants containing only the *cry1A.105* and *cry2Ab2* gene cassettes were selected using molecular analysis, while plants containing the *nptII* gene cassette were discarded.

The absence of the *nptII* gene and the NPTII protein was further confirmed by both Southern blot and ELISA. The steps used in the generation of MON 89034 corn are depicted in Figure 1.

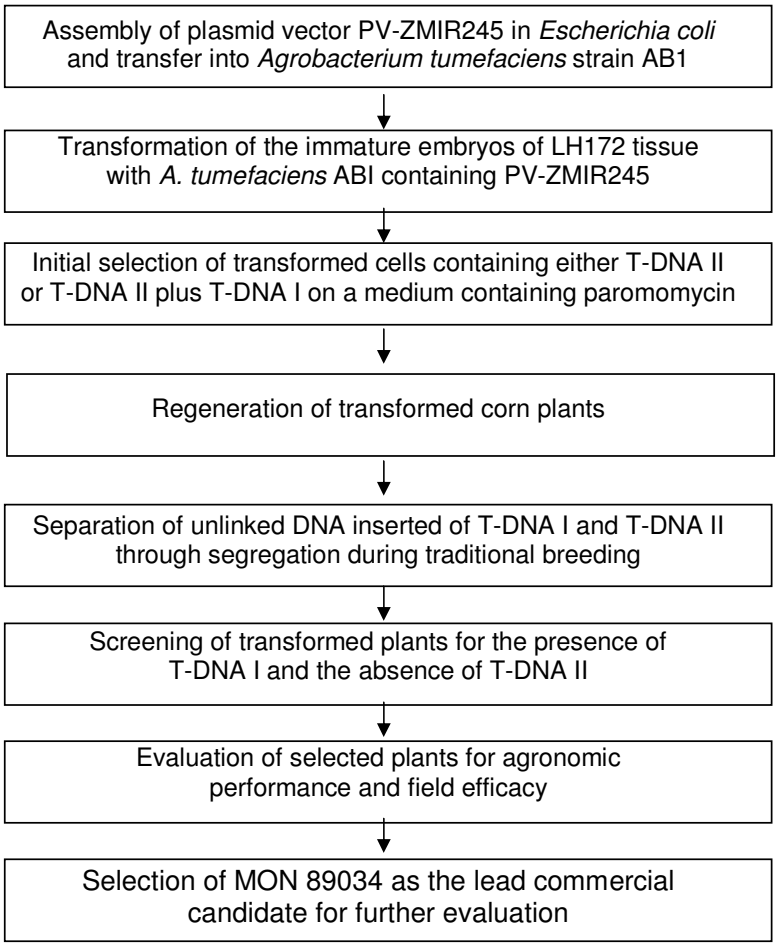

Figure 1: Steps used in the generation of MON 89034 corn

3.2 Description of the breeding process

The MON 89034 breeding process is briefly described below and depicted in Figure 2.

The primary transformant (R_0) was cross-pollinated with the recurrent parent LH172 to produce the LH172 $BC0F_1$ generation. During this breeding step, the T-DNA I and T-DNA II insertions were segregated. Only plants positive for the *cry1A.105* gene and negative for the *nptII* gene were selected for subsequent breeding steps.

The selected LH171 $BC0F_1$ plants were self-pollinated to generate LH172 $BC0F_2$. The segregation in the LH172 $BC0F_2$ generation was examined using ELISA for Cry2Ab2 on leaf tissue. The selected LH172 $BC0F_2$ plants were self-pollinated to generate LH172 $BC0F_3$. The segregation in LH172 $BC0F_3$ generation was determined using ELISA for Cry1A.105 and immunoassay for CryAb2 in leaf tissue.

The F_3 generation was fixed for the DNA insert and not expected to segregate further. The stability of the F_4 and F_5 generations was confirmed using an immunoassay for Cry2Ab in leaf tissue.

The LH172 $BC0F_4$ generation was crossed to 12 different inbred corn lines for subsequent commercial development. Generations LH172 $BC0F_6$ and LH172 $BC0F_7$ were crossed to LH198 (hybrid seed) and were used in all other analyses (composition and protein expression analyses) and additional field trials.

3.3 Description of the gene construct

The transformation vector PV-ZMIR245 contains three gene expression cassettes (Figure 3). The *cry1A.105* and *cry2Ab2* gene expression cassettes are contained in T-DNA I and the *nptII* gene expression cassette is contained in T-DNA II.

T-DNA I in PV-ZMIR245 contains the *cry1A.105* and *cry2Ab2* genes. The *cry1A.105* gene encodes the 133 kDa Cry1A.105 insecticidal protein, which is a chimeric protein consisting of domains I and II from Cry1Ab or Cry1Ac[5], a substantial portion of domain III from Cry1F and the C-terminal domain from Cry1Ac. Cry 1Ac and Cry1Ab are derived from *B. thuringiensis* subsp. *kurstaki* and Cry1F is derived from *B. thuringiensis* subsp. *aizawai*. The *cry1A.105* coding sequence was optimised for expression in monocotyledonous plants. The *cry2Ab2* gene encodes the 61 kDa Cry2Ab2 insecticidal protein, which is a variant of the wild-type Cry2Ab2 protein from *B. thuringiensis* subsp. *kurstaki*. The *cry2Ab2* coding sequence has been modified to change codon usage for optimised expression in plants.

T-DNA II in PV-ZMIR245 contains the *nptII* gene from *E. coli* transposon Tn5 (Beck et al 1982). The *nptII* gene encodes the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the aminoglycoside antibiotics, including neomycin, paromomycin, kanamycin and geneticin (G418). The *nptII* gene in T-DNA II functions as a dominant selectable marker in the initial stages of plant cell selection following transformation but is segregated away in subsequent generations.

[5] Cry1Ab and Cry1Ac share 100% amino acid sequence identity in domains I and II.

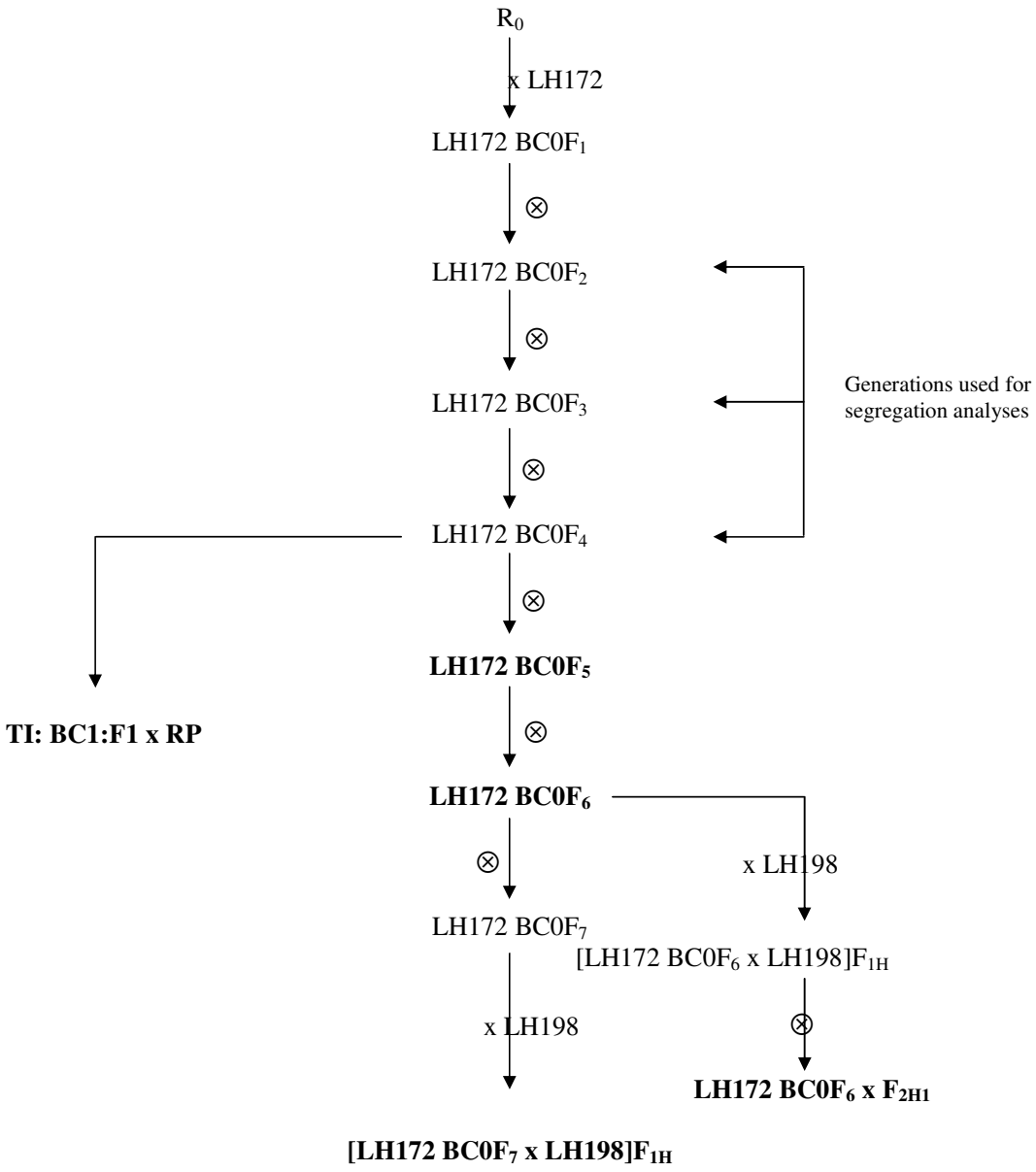

Figure 2: Breeding Tree for MON 89034 corn

The LH172 $BC0F_6$ x F_{2H1} generation was used for all the molecular analyses. Generations in bold were used for molecular stability analyses.
Key: R_0 = primary transformant; F(#) = filial generation; ⊗ = self pollination; BC(#) = backcross generation; RP = recurrent parent; H = hybrid; TI = trait integration.

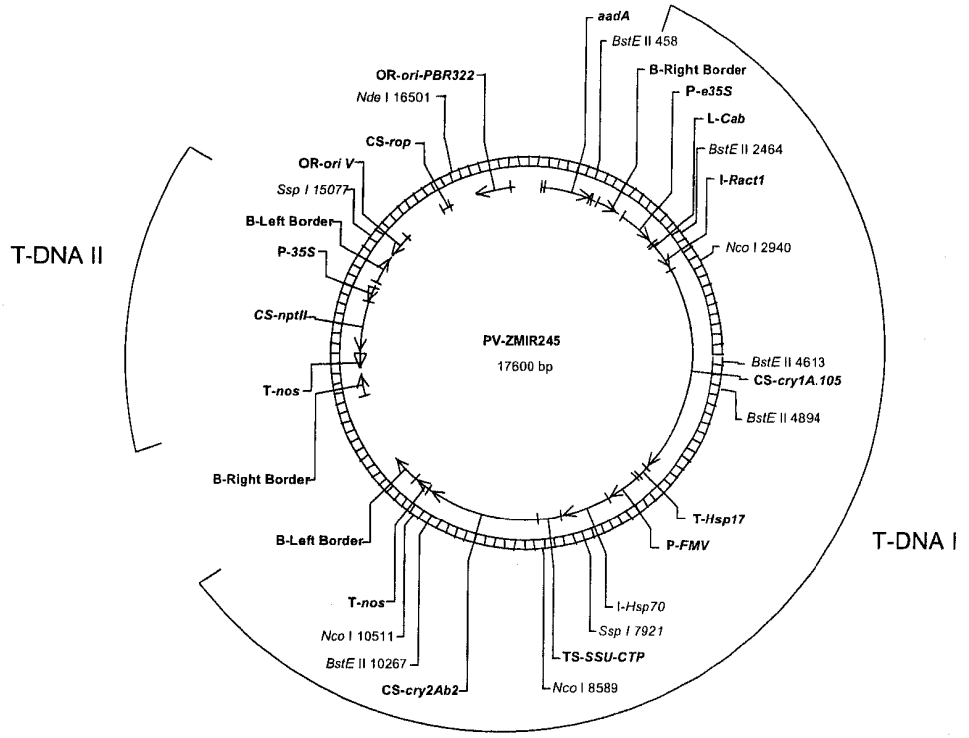

Figure 3: Map of transformation vector PV-ZMIR245

A full description of all the genetic elements in the transformation vector is provided in Table 1.

Table 1: Description of the genetic elements in PV-ZMIR245

Genetic Element	Location in PV-ZMIR245	Function
Vector Backbone		
Intervening sequence	1-257	Sequence used in DNA cloning.
aadA	258-1146	Bacterial gene encoding aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from transposon Tn7 (Fling et al 1985). Confers resistance to the antibiotics streptomycin and spectinomycin.
Intervening sequence	1147-1261	Sequence used in DNA cloning.
T-DNA I		
Right border	1262-1618	DNA region from Agrobacterium tumefaciens containing the 24 bp right border sequence used for the transfer of the T-DNA (Depicker et al 1982).
Intervening sequence	1619-1728	Sequence used in DNA cloning.
e35S	1729-2349	Promoter and 9 bp leader for the cauliflower mosaic virus (CaMV) 35S RNA (Odell et al 1985) containing the duplicated enhancer region (Kay et al 1987).
Intervening sequence	2350-2375	Sequence used in DNA cloning

Genetic Element	Location in PV-ZMIR245	Function
Cab	2376-2436	5' untranslated leader of the wheat chlorophyll a/b-binding protein (Lamppa et al 1985).
Intervening sequence	2437-2452	Sequence used in DNA cloning.
Ract1	2453-2932	Intron from the rice actin gene (McElroy et al 1991).
Intervening sequence	2933-2941	Sequence used in DNA cloning.
cry1A.105	2942-6475	Coding sequence for the *B. thuringiensis* Cry1A.105 protein (Monsanto unpublished information). The coding sequence has been modified for optimised codon usage in monocotyledonous plants.
Intervening sequence	6476-6506	Sequence used in DNA cloning.
Hsp17	6507-6716	3' nontranslated region of the coding sequence for wheat heat shock protein 17.3, which ends transcription and directs polyadenylation (McElwain & Spiker 1989).
Intervening sequence	6717-6783	Sequence used in DNA cloning.
FMV	6784-7347	Figwort mosaic virus 35S promoter (Rogers 2000).
Intervening sequence	7348-7369	Sequence used in DNA cloning.
Hsp70	7370-8173	First intron from the maize heat shock protein 70 gene (Brown & Santino 1995).
Intervening sequence	8174-8189	Sequence used in DNA cloning.
SSU-CTP	8190-8590	DNA region containing the targeting sequence for the transit peptide region of maize ribulose 1,5-bisphosphate carboxylase small subunit and the first intron (Matsuoka et al 1987).
cry2Ab2	8591-10498	Coding sequence for the Cry2Ab2 protein from B. thuringiensis (Widner & Whitely 1989, Donovan 1991). The coding sequence has been modified for optimised codon usage.
Intervening sequence	10499-10524	Sequence used in DNA cloning.
nos terminator	10525-10777	3' transcript termination sequence of the nopaline synthase (*nos*) gene from *A. tumefaciens* which terminates transcription and directs polyadenylation (Bevan et al 1983).
Intervening sequence	10778-10844	Sequence used in DNA cloning.
Left border	10845-11286	DNA region from *A. tumefaciens* containing the 25 bp left border sequence used for the transfer of T-DNA (Barker et al 1983).
Vector Backbone		
Intervening sequence	11287-12489	Sequence used in DNA cloning.
T-DNA II		
Right border	12490-12846	DNA region from *A. tumefaciens* containing the 24 bp right border sequence used for transfer of the T-DNA (Depicker et al 1982).
Intervening sequence	12847-12971	Sequence used in DNA cloning.
nos terminator	12972-13224	3' termination sequence of the nopaline synthase (nos) gene from *A. tumefaciens* which terminates transcription and directs polyadenylation (Bevan et al 1983).
Intervening sequence	13225-13255	Sequence used in DNA cloning.
nptII	13256-14050	Coding sequence for neomycin phosphotransferase II protein that confers resistance to aminoglycoside antibiotics (Beck et al 1982).
35S	14084-14407	Promoter and 31 bp leader for the CaMV 35S RNA (Odell et al 1985).
Intervening sequence	14408-14457	Sequence used in DNA cloning.
Left border	14458-14899	DNA region from A. tumefaciens containing the left border sequence used for transfer of the T-DNA (Barker et al 1983).

Genetic Element	Location in PV-ZMIR245	Function
Vector Backbone		
Intervening sequence	14900-14985	Sequence used in DNA cloning.
ori V	14986-15382	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2 (Stalker et al 1981).
Intervening sequence	15383-16119	Sequence used in DNA cloning.
rop	16120-16311	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza & Huang 1989).
Intervening sequence	16312-16738	Sequence used in DNA cloning.
ori-PBR322	16739-17327	Origin of replication from pBR322 allowing maintenance of the plasmid in *E. coli* (Sutcliffe 1978).
Intervening sequence	17328-17600	Sequence used in DNA cloning.

3.4 Characterisation of the genes in the plant

A number of molecular analyses were done to characterise the inserted DNA in MON 89034 corn (LH172 BC0 F_6 x F_{2H1} in Figure 2). A summary of each of the molecular analyses and the findings are given below.

3.4.1 Southern blot analyses

Genomic DNA from MON 89034 corn was analysed using Southern blot analysis to determine the insert number, the copy number, the integrity of the inserted *cry1Ab.105* and *cry2Ab2* gene cassettes (T-DNA I), and evaluate the presence or absence of plasmid backbone and selectable marker sequences (T-DNA II). Conventional corn with the same genetic background as the MON 89034 line was used as the control for these analyses, with the transformation vector, PV-ZMIR245, used as a positive hybridisation control.

Isolated genomic DNA from MON 89034 corn and conventional corn was digested with various restriction enzymes, separated on agarose gels and then subjected to Southern blot analysis. These analyses demonstrated the presence of a single copy of T-DNA I and confirmed a single site of insertion. .

Further Southern blot analyses were done to separately confirm the presence of the *cry1A.105* and *cry2Ab2* genes as well as each of the associated regulatory elements. Genomic DNA was digested with selected restriction enzymes and probed with probes specific to each of the different genetic elements within T-DNA I. Hybridising bands of the expected size were observed for each of the probes used, confirming the presence of intact *cry1A.105* and *cry2Ab2* genes as well as all associated regulatory elements. No unexpected bands were detected.

Southern blot analysis was also used to determine whether any vector backbone sequence is present in the inserted DNA, and also to demonstrate the absence of the *nptII* gene. Genomic DNA was probed with overlapping probes spanning the vector backbone of PV-ZMIR245 as well as the *nptII* coding region. No detectable hybridisation bands were detected with any of the probes used, indicating the absence of any vector backbone sequence as well as the *nptII* gene. The absence of other T-DNA II sequences was also confirmed by probing genomic DNA with overlapping probes spanning T-DNA II. Hybridising bands consistent with that expected for the genetic elements common to both T-DNA I and T-DNA II were detected (e.g. the 35S promoter, *nos* terminator, border sequences). No other hybridising bands were detected.

3.4.2 Polymerase chain reaction and DNA sequence analyses

The organisation of the genetic elements within the insert in MON 89034 corn was confirmed using PCR analysis by amplifying seven overlapping regions of DNA spanning the entire length of the insert (Figure 4). The PCR products generated, following PCR of genomic DNA from MON 89034 corn, were all of the expected size, indicating that the arrangement of the genetic elements within the insert is the same as in the transformation vector, PV-ZMIR245.

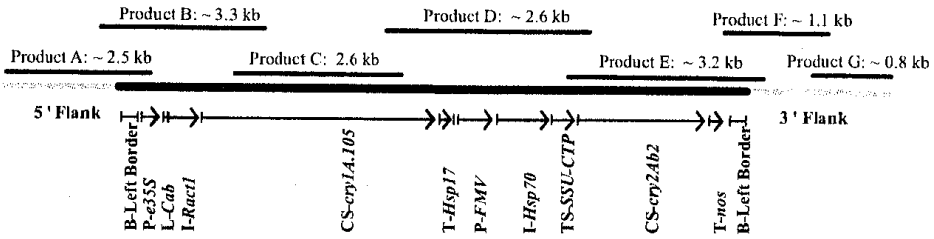

Figure 4: Overlapping PCR products generated across the insert in MON 89034 corn

The PCR products generated from MON 89034 corn genomic DNA were sequenced to further confirm the organisation of genetic elements within the insert, and determine the 5' and 3' insert-to-genomic DNA junctions, and the complete DNA sequence of the inserted DNA and adjacent genomic DNA regions. A consensus sequence of the inserted DNA was generated by compiling the results of numerous sequencing reactions performed on each of the overlapping PCR products. This consensus sequence was then aligned to the DNA sequence of T-DNA I in the transformation vector, PV-ZMIR245, to determine, what, if any, changes had occurred during the transformation process.

The sequence analysis indicated that the insert DNA in MON 89034 corn is 9317 nucleotides in length. The sequence of the inserted T-DNA I was almost identical to that in the transformation vector with one exception. The *e35S* promoter that regulates the expression of the *cry1A.105* gene has been modified and the right border sequence of the T-DNA has been replaced by a left border sequence. The modified *e35S* promoter in MON 89034 corn no longer has the duplicated enhancer elements which are present in the original *e35S* promoter in PV-ZMIR245. The Applicant considers this molecular rearrangement has most likely occurred through a recombination event between the two *35S* promoters in T-DNA I and T-DNA II, either prior to or during the process of T-DNA transfer to the plant cell.

In addition to the entire insert sequence, the sequence of 2060 base pairs of genomic DNA flanking the 5' end of the insert and 905 base pairs of genomic DNA flanking the 3' end of the insert were also determined.

3.4.3 Bioinformatic analysis of the 5' and 3' junction regions

Bioinformatic analyses were performed to assess the potential for allergenicity, toxicity and bioactivity of putative polypeptides encoded by the 5' and 3' insert-genomic DNA junction regions of MON 89034 corn. These analyses are entirely theoretical, that is, no empirical evidence exists to suggest that the 5' and 3' junction regions in MON 89034 corn are transcribed.

The DNA sequence spanning the 5' and 3' junction regions of the MON 89034 corn insertion site were analysed for the presence of translational stop codons (TGA, TAG, TAA) and all six reading frames originating or terminating within the MON 89034 corn insertion were translated from stop codon to stop codon. Following translation, putative polypeptide sequences greater than or equal to eight amino acids in length were selected for further analysis. At the 5' junction region, five deduced putative polypeptides were identified, with another five being identified at the 3' junction region.

Putative polypeptides from each reading frame were compared to three different databases:

(i) AD6 database – contains allergen, gliadin and glutenin sequences and was assembled from sequences found on the FARRP allergen database[6]. Gene identification numbers for the 1537 sequences found on the FARRP database were used to assemble a list which was used to batch query the NCBI protein sequence database[7];

(ii) TOXIN5 database – this database was assembled from public sequence databases, including GenBank and EMBL release 124 and SwissProt release 1. Protein sequences were retrieved using the STRINGSEARCH function (keyword = toxin) of the Wisconsin package (version 10). This search was used to identify and retrieve 12,771 separate entries which were then used to compile the TOXIN5 database. The actual number of unique toxin sequences will be less than 12,771 because of the redundancy associated with the public database and because some entries may contain the word 'toxin' but may not be relevant protein toxins; and

(iii) ALLPEPTIDES – this database was used to represent all currently known publicly available protein sequences and consisted of SwissProt release 40.0 (May 4, 2005) and NRAA release 65.0 (October 24, 2005).

The overall structural similarity of the putative polypeptides to sequences in each database was assessed using the FASTA algorithm. The extent of structural relatedness was evaluated using visual inspection of the aligned sequences, the calculated percent identity, and *E* (expectation) score. Related protein sequences are considered to be potentially cross-reactive if linear identity is 35% or greater in a segment of 80 or more amino acids (FAO/WHO 2001). The *E* score is a statistical measure of the likelihood that the observed similarity could have occurred by chance. A larger *E* score indicates a lower degree of similarity. Typically, alignments between two sequences will need to have an *E* score of less than 1×10^{-5} to be considered to have significant homology.

In addition to the FASTA comparisons, searches were also done to identify short amino acid sequences that may represent linear IgE binding epitopes. An algorithm (ALLERGENSEARCH) was developed to identify matches of eight contiguous amino acids or more. Segments of eight amino acids were chosen by the Applicant because they consider this to be the smallest number of amino acids that will identify immunologically relevant matches. The Applicant states that searches using small segments (e.g. 6 or 7 amino acids) lead to high rates of false positive matches and therefore have little predictive value. The ALLERGENSEARCH algorithm compares the query sequence to each sequence in the allergen database using a sliding window of eight amino acids.

[6] www.allergenonline.com, accessed in January 2006.
[7] www.ncbi.nlm.nih.gov/entrez/batchentrez.cgi

No biologically relevant sequence similarity to know allergens, toxins or other proteins was identified for any of the putative polypeptides. No short peptide matches, representing putative IgE binding epitopes, were shared between any of the putative polypeptides and proteins in the allergen database.

3.4.4 Conclusion

Detailed molecular analyses indicate that one copy of T-DNA I, containing the *cry1A.105* and *cry2Ab2* gene expression cassettes, has been inserted at a single genomic locus in MON 89034 corn. Both coding regions are intact, although during the transformation process, a small rearrangement occurred to the *e35S* promoter which directs expression of the *cry1A.105* gene. This minor modification to the *e35S* promoter has not affected the expression of the gene or the trait (see Section 4). The molecular analyses also confirmed the absence of the *nptII* selectable marker in MON 89034 corn, which was introduced in the original transformation on a second T-DNA and subsequently selectively segregated away.

3.5 Stability of the genetic changes

A number of analyses were done to demonstrate the stability of the genetic changes in MON 89034 corn. Segregation analysis over multiple generations was done to determine the heritability and stability of the new trait (the *cry1A.105* and *cry2Ab2* genes, and Cry1A.105 and Cry2Ab2 proteins). Southern blot analysis over multiple generations was done to determine the stability of the inserted DNA.

3.5.1 Segregation analyses

For the segregation analysis, Chi-square test of inheritance data over four generations of MON 89034 corn was done to determine the heritability and stability of the new traits. The confirmation of the presence of the gene and stability of the trait was based on: (i) ELISA to detect the Cry2Ab2 and Cry1A.105 proteins; and (ii) PCR assay to detect the presence of the *cry1A.105* and *cry2Ab2* genes. The Chi-square test is based on testing the observed segregation ratio of the Cry proteins to the ratio that is expected according to Mendelian principles as shown in Table 2.

The results of the Chi-square test[8] are summarised in Table 3. All Chi-square values indicate no significant differences between observed and expected genetic ratios across all tested generations of MON 89034 corn. These results are consistent with the molecular characterisation data indicating a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes.

[8] Computed as $\chi^2 = \Sigma [(| o - e | - 0.5)^2/e]$ where o = observed frequency of the genotype, e = expected frequency of the genotype, and 0.5 = Yates correction factor for analysis with one degree of freedom (Little & Hills, 1978).

Table 2: Expected segregation ratios for MON 89034 corn generations

Generation[a]	Expected ratio[b]	Comment
LH172BC0F_1	n.a	Screened for copy number and absence of *nptII* (segregation data not shown)
LH172BC0F_2	3:1	Positive:negative (product of self pollination)
LH172BC0F_3	1:0	Positive:negative (homozygous plant selection)
LH172BC0F_4	1:0	Positive:negative (homozygous plant selection)
LH172BC1F_1[c]	1:1	Positive:negative (product of backcrossing)
LH172BC1F_2[d]	3:1	Positive:negative (product of self pollination)
LH172BC1F_2[d]	3:1	Positive:negative (product of self pollination)

[a]See breeding tree in Figure 2.
[b]n.a. = not applicable.
[c]To confirm segregation, LH172BC0F1 plants were backcrossed to the recurrent parent (LH172) to produce this generation (not shown in Figure 2).
[d]To confirm segregation, the LH172BC1F1 plants were self pollinated to produce two different plant populations of this generation (not shown in Figure 2).

Table 3: Segregation analyses of MON 89034 corn

Generation	No. of Plants	Observed positives	Expected positives	Observed negatives	Expected negatives	Chi-square	Probability
LH172BC0F_2	11	7	8.25	4	2.75	0.2727	>0.05
LH172BC0F_3	24	24	24	0	0	Fixed +	n.a
LH172BC0F_4	30	30	30	0	0	Fixed +	n.a
LH172BC1F_1	28	13	14	15	14	0.0357	>0.05
LH172BC1F_2	24	20	18	4	6	0.5	>0.05
LH172BC1F_2	24	17	18	7	6	0.0556	>0.05

3.5.2 Stability of the inserted DNA

To determine the stability of the inserted DNA, Southern blot analyses were done using genomic DNA isolated from multiple generations of MON 89034 corn (see Figure 2; the generations used are in bold). For these analyses, DNA samples were digested with *Ssp* I which cleaves once within the inserted DNA and in both the 5' and 3' genomic flanking regions. This produces two DNA fragments of ~8.2 and >4.3 kb. The stability of the inserted DNA was confirmed using overlapping T-DNA I probes spanning the entire inserted DNA sequence. Genomic DNA isolated from corn with the same genetic background as MON 89034 corn was used as a negative control, and was also spiked with DNA from PV-ZMIR245 to serve as a positive hybridisation control.

Genomic DNA extracted from the different MON 89034 generations produced two bands of ~8.2 kb and ~7.4 kb. The ~8.2 kb band is the expected size for the 5' border fragment and the ~7.4 kb band is consistent with the expected band size of >4.3 kb for the 3' border fragment. These bands were consistent with bands detected in other Southern analyses and are also identical across multiple generations.

3.5.3 Conclusion

The results of the segregation analysis are consistent with a single site of insertion for the *cry1A.105* and *cry2Ab2* gene expression cassettes and confirm the results of the molecular characterisation. Molecular analysis of both self pollinated and cross-fertilised lines, representing a total of seven different generations, indicates that the inserted DNA is stably transformed and inherited from one generation to the next.

3.6 Presence of antibiotic resistance genes

No genes that encode resistance to antibiotics are present in the genome of MON 89034 corn. The molecular characterisation confirmed the absence of both the *aad* and *nptII* genes, which were used in the cloning and transformation process.

4. CHARACTERISATION OF THE NOVEL PROTEIN

In considering the safety of novel proteins it is important to consider that a large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects, although a small number have the potential to impair health, e.g. because they are allergens or anti-nutrients.

As proteins perform a wide variety of functions, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutritional and allergenic effects. To effectively identify any potential hazards requires knowledge of the characteristics, concentration and localisation of all novel proteins expressed in the organism as well as a detailed understanding of their biochemical function and phenotypic effects. It is also important to determine if the novel protein is expressed as expected, including whether any post-translational modifications have occurred.

4.1 Description and function of novel protein

MON 89034 corn expresses two different Cry proteins, Cry1A.105 and Cry2Ab2. A number of different analyses were done to determine the identity, physiochemical properties, *in planta* expression, bioactivity and potential toxicity and allergenicity of the two proteins. Because the expression of proteins *in planta* is usually too low to allow purification of sufficient quantities for safety assessment studies, a bacterial expression system was used to generate large quantities of the Cry1A.105 and Cry2Ab2 proteins. The Cry1A.105 and Cry2Ab2 proteins produced in *E. coli* were engineered so their amino acid sequence matched that of the plant-produced Cry1A.105 and Cry2Ab2 proteins. The equivalence of the bacterial-produced proteins to the plant-produced proteins was determined as part of the protein characterisation.

4.1.1 Mode of action of Cry proteins

The general mechanism of insecticidal activity of Cry proteins is well understood (Gill et al 1992, Schnepf et al 1998, Zhuang & Gill 2003, Bravo et al 2007), with the mode of action being characterised principally in lepidopteran insects. The Cry proteins belong to a class of bacterial toxins known as pore-forming toxins (PFT) that are secreted as water-soluble proteins, which after undergoing conformational change, are able to insert into, or translocate across, the cell membranes of their host. There are two main groups of PFT: (i) the α-helical toxins in which the α-helix regions form the trans-membrane pore; and (ii) the β-barrel toxins, that insert into the membrane by forming a β-barrel composed of β-sheet hairpins from each monomer (Parker & Feil 2005). The Cry proteins belong to the α-helical group of PFT, along with other toxins such as exotoxin A (from *Pseudomonas aeruginosa*) and diphtheria toxin.

The primary action of Cry toxins is to lyse midgut epithelial cells in the target insect by forming pores in the apical microvilli membrane of the cells, which subsequently leads to ion leakage and cell lysis. The crystal inclusions ingested by susceptible larvae dissolve in the alkaline environment of the gut, and the solubilised inactive protoxins are cleaved by midgut proteases yielding 60-70 kDa protease resistant proteins (Bravo et al 2005). Toxin activation involves the proteolytic removal of an N-terminal peptide (25 – 30 amino acids for Cry1 toxins, 58 residues for the Cry3A and 49 for Cry2Aa) and approximately half of the remaining protein from the C-terminus in the case of the long Cry protoxins. The activated toxin then binds to specific receptors on the brush border membrane of the midgut epithelium columnar cells (de Maagd et al 2001, Bravo et al 2005) before inserting into the membrane. Toxin insertion leads to formation of lytic pores in microvilli apical membranes (Aronson & Shai 2001, Bravo et al 2005). Subsequently cell lysis and disruption of the midgut epithelium releases the cell contents providing spores a germinating medium leading to a severe septicaemia and insect death.

4.1.2 Cry1A.105

Cry1A.105 is a full-length Cry protein consisting of 1177 amino acids with a molecular weight of 133 kDa. It is a chimeric protein consisting of domains I and II from Cry1Ab or Cry1Ac[9], substantially domain III from Cry1F, and the C-terminal domain from Cry1Ac (Figure 5).

Cry1A.105 was designed using a domain exchange strategy to achieve high levels of activity against the target insect pests. Domain exchange is a naturally occurring mechanism that increases protein diversity in *B. thuringiensis* (De Maagd et al 2001, Masson 2002, De Maagd et al 2003). A domain exchange strategy has been used to switch the functional domains of Cry1 proteins to develop a commercial biopesticide with improved specificity to lepidopteran insect pests.

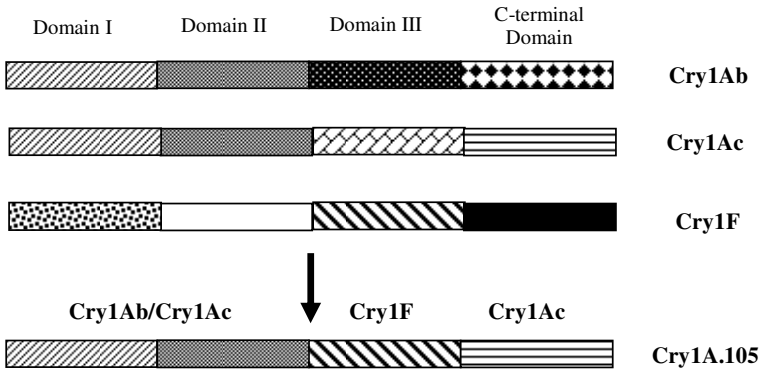

Figure 5: Schematic representation of the origin of Cry1A.105 protein domains.

The overall amino acid sequence identity between Cry1A.105 and Cry1Ac, Cry1Ab, and Cry1F is 93.6%, 90.0% and 76.7%, respectively (see Table 4). According to this, Cry1A.105 is most closely related to Cry1Ac, but is also has a high degree of amino acid identity with Cry1Ab.

[9] Cry1Ab and Cry1Ac share 100% amino acid sequence identity in domains I and II.

Table 4: Amino acid sequence identity between Cry1A.105 and Cry1Ac, Cry1Ab and Cry1F proteins.

	Amino acid identity to Cry1A.105 (%)		
Domain	**Cry1Ac**	**Cry1Ab**	**Cry1F**
I	100	100	57
II	100	100	37
III	57	46	99
C-terminal	100	92	93
Overall	**93.6**	**90**	**76.7**

4.1.3 Cry2Ab2

Cry2Ab2 is an insecticidal protein consisting of 637 amino acids with a predicted molecular weight of 61 kDa and is a variant of the wild-type Cry2Ab2 protein from *B. thuringiensis* subsp. *kurstaki*. The *cry2Ab2* gene expressed in MON 89034 has been modified to change the codon usage for optimised expression in the plant. The Cry2Ab2 variant protein expressed in MON 89034 has 88% amino acid sequence identity to Cry2Aa, which is present in a number of registered pest control products in Australia and New Zealand.

Cry2Ab2 is targeted to the chloroplast using a chloroplast transit peptide (CTP) from the maize ribulose 1,5-bisphosphate carboxylase small subunit which is fused to the N-terminus of Cry2Ab2. CTPs are typically cleaved from the mature protein upon translocation into the chloroplast, and then rapidly degraded (Bruce 2000). The CTP used in MON 89034 has a potential cleavage site located three amino acids upstream from the start of the Cry2Ab2 protein sequence (Figure 6); therefore the Cry2Ab2 protein expressed in MON 89034 has an additional three amino acids (MQA) at the N-terminus, compared to the wild-type protein. An additional amino acid (aspartic acid) was also inserted in the N-terminus of the plant expressed Cry2Ab2 in order to facilitate the cloning of the *cry2Ab2* gene. These additional amino acids were also included in the N-terminus of the *E. coli*-produced Cry2Ab2 protein.

MON 89034 Cry2Ab2	**M**-**Q**-**A**[1]-M-**D**[2]-N-S-V-L-N
E. coli-produced Cry2Ab2	**M**-**Q**-**A**[1]-M-**D**[2]-N-S-V-L-N
Wild-type Cry2Ab2	-M- -N-S-V-L-N

[1] **M**-**Q**-**A** - predicted amino acids from chloroplast transit peptide
[2] **D** - an additional amino acid included to facilitate cloning

Figure 6: Comparison of the N-terminus of the MON 89034, E. coli and wild-type Cry2Ab2 proteins.

4.2 Protein characterisation

A range of analytical techniques was used to determine the identity as well as the physicochemical and functional properties of the plant-produced Cry1A.105 and Cry2Ab2 proteins isolated from MON 89034 and to compare them to *E. coli*-produced proteins. These techniques included sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry, Western blot analysis, matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, N-terminal sequencing (Cry2Ab2 only), glycosylation analysis, and insect bioactivity assays.

The *E. coli*-produced proteins were used as reference standards for determination of protein concentration and immunoblot analysis using N-terminal peptide antibody. These proteins were also used as reference standards to evaluate equivalence between plant- and *E. coli*-produced proteins for molecular weight and functional activity assays, as a reference and negative control in glycosylation analyses, and as a reference and a positive control in immunoblot analyses using anti-Cry1A.105 and anti-Cry2Ab2 antibodies.

4.2.1 Cry1A.105

The Cry1A.105 protein was purified at 4ºC from an extract of ground grain from MON 89034 corn using a combination of ammonium sulphate fractionation, anion exchange chromatography, and immunoaffinity chromatography.

Protein identity

The identity of the plant-produced Cry1A.105 was confirmed by Western blot analysis using anti-Cry1A.105 antibody and the N-terminal peptide antibody, and MALDI-TOF MS tryptic mass map analysis:

(i) Western blot using anti-Cry1A.105 antibody – the plant-produced Cry1A.105 protein and the *E. coli*-produced reference standard were loaded onto the same gel. Immunoreactive bands migrating between approximately 85 and 130 kDa were observed in both samples. The major protein band, migrating with an apparent molecular weight of ~130 kDa was present in both the plant-produced and *E. coli*-produced samples and is consistent with the molecular weight of 133 kDa expected for the full length protein. The bands of lower molecular weight are partial Cry1A.105 fragments, which the Applicant believes represent proteolytic degradation products;

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(ii) Western blot using N-terminal peptide antibody – the antibodies were raised against a synthetic peptide consisting of the first 14 amino acids of the Cry1A.105 N-terminus (MDNNPNINECIPYN). The *E. coli*-produced Cry1A.105 protein containing the same N-terminal sequence, a Cry1A.105 trypsin-resistant core protein lacking the N-terminus, and the plant-produced Cry1A.105 protein were all loaded onto the same gel. The *E. coli*-produced protein served as the positive control and the trypsin-resistant core of Cry1A.105 served as the negative control. A ~130 kDa immunoreactive band was observed in both the plant- and *E. coli*-produced Cry1A.105 samples. An immunoreactive band of ~85 kDa was also observed in both samples, which suggests it represents a C-terminal degradation of the Cry1A.105 protein. No immunoreactive bands were observed for the trypsin-resistant core Cry1A.105 protein. These results indicate that the plant-produced Cry1A.105 protein contains an intact N-terminus and are consistent with published literature which shows that proteolytic fragments of Cry1 proteins have intact N-termini (Gao et al 2006);

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(iii) Tryptic peptide mapping by MALDI-TOF – the plant-produced protein was heat denatured, chemically reduced, alkylated and digested with trypsin, and the masses of the tryptic fragments were measured. The ability to identify a protein using this method is dependent on matching a sufficient number of observed tryptic mass fragments to expected (theoretical) mass fragments.

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In general, a protein identification made by peptide mapping is considered to be reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al 1997). There were 52 tryptic mass fragments identified that matched the expected tryptic peptides generated *in silico* based on the predicted trypsin cleavage sites in the amino acid sequence. Overall, the confirmed sequence accounts for 43.8 % (516 of 1177 amino acids) of the full length Cry1A.105 protein.

Glycosylation analysis

To assess whether post-translational glycosylation of the plant-produced Cry1A.105 protein occurred, the purified protein sample was subjected to glycosylation analysis. As prokaryotic organisms lack the capacity for protein glycosylation, the *E. coli*-produced Cry1A.105 protein was used as the negative control. Transferrin and horse radish peroxidase (HRP) proteins, which are known to have multiple covalently-linked carbohydrate modifications, were used as positive controls. The test and control protein samples were separated by SDS-PAGE and protein-bound carbohydrate moieties were detected using a fluorescent stain. The transferrin and HRP proteins were detected at the expected molecular weights in a concentration dependent manner, with no signal being detected for either the plant-produced or *E. coli*-produced Cry1A.105 proteins.

These results indicate that the plant-produced Cry1A.105 protein is not glycosylated and is equivalent to the *E. coli*-produced Cry1A.105 protein in terms of its lack of glycosylation.

Insect bioassays

The insecticidal activity of the plant- and *E. coli*-produced Cry1A.105 protein was determined using an insect bioassay with corn earworm. The bioassay was repeated three times, in replicate, on separate days with separate batches of larvae. Each replicate consisted of a series of five protein levels yielding a dose series ranging from 0.00048 – 0.039 µg Cry1A.105 protein/ml of diet with a 3 fold separation factor between dose levels. Insect larvae (16 larvae per treatment) were allowed to feed on the diet for 6-7 days. The combined weight of the surviving insects at each dose level for each source of protein was recorded at the end of the 6-7 day incubation period. The mean EC_{50} value[10] for the plant-produced Cry1A.105 protein was 0.0074 ± 0.0017 µg protein/ml diet, and for the *E. coli*-produced Cry1A.105 protein was 0.012 ± 0.0062 µg protein/ml diet. Both protein sources also produced similar dose response curves. These results indicate that the plant- and *E. coli*-produced Cry1A.105 proteins can be regarded as equivalent in terms of their insecticidal activity.

4.2.2 Cry2Ab2

The Cry2Ab2 protein was purified from an extract of ground grain from MON 89034 corn using a combination of ammonium sulphate fractionation, anion exchange chromatography, and immunoaffinity chromatography.

[10] The Effective Concentration necessary to reduce the growth of the target insect by 50 %.

Protein identity

The identity of the plant-produced Cry2Ab2 protein was confirmed by Western blot analysis using anti-Cry2Ab2 antibody, MALDI-TOF mass spectrometry, and N-terminal sequence analysis:

(i) Western blot analysis using anti-Cry2Ab2 antibodies – samples of the plant-produced Cry2Ab2 protein and *E. coli*-produced Cry2Ab2 reference protein were loaded onto the same gel. Two immunoreactive bands of approximately 61 kDa and 50 kDa were observed in plant-produced Cry2Ab2 sample, with a single band of 61 kDa being observed in the *E. coli*-produced sample. The predicted molecular weight of the full length Cry2Ab2 is 61 kDa. N-terminal sequence analysis of the ~ 50 kDa band demonstrated that its N-terminal sequence begins at amino acid position 145, indicating it is the product of a N-terminal truncation;

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(ii) Tryptic peptide mapping by MALDI-TOF – the two major immunoreactive bands cross-reacting with anti-Cry2Ab2 antibodies in the Western blot analysis were further assessed using MALDI-TOF mass spectrometry. For the ~61 kDa band, a total of 32 observed tryptic peptide masses matched the predicted (theoretical) tryptic peptide masses of the Cry2Ab2 protein. The overall peptide sequence coverage was 44.4 % out of the 637 amino acid residues of the full length Cry2Ab2 protein. For the lower molecular weight band migrating at ~50 kDa, a total of 24 observed peptide masses matched the expected tryptic peptide masses of the Cry2Ab2 protein, which represents 47.7 % out of the 493 amino acid residues of this truncated protein;

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(iv) N-terminal sequence analysis – the N-terminus of the ~61 kDa immunoreactive band from MON 89034 corn was blocked therefore it was not possible to obtain the N-terminal sequence of the protein. N-terminal sequence of this immunoreactive band did however result in N-terminal sequence starting from amino acid position 24, indicating that a minor portion of the protein co-migrating with the full length protein was proteolytically degraded. As discussed above, N-terminal sequence analysis of the lower immunoreactive band migrating at ~50 kDa revealed this fragment starts from amino acid residue no.143. N-terminal sequence analysis of the *E. coli*-produced Cry2Ab2 protein confirmed the N-terminus as MQAMDN, as expected (see Figure 6). The results of the N-terminal sequence analysis are summarised in Table 5 below.

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Table 5: Summary of N-terminal sequence analysis of Cry2Ab2 protein from MON 89034 corn[1,2]

Amino acid residue #	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**145**	**146**	**147**	**148**	**149**	**150**
Predicted sequence[3]	A	H	D	P	F	S	F	Q	A	V	P	L	S	I
61 kDa band sequence	A	H	D	P	F	S	F	Q						
50 kDa band sequence									X	V	P	L	(S)	I

[1] Undesignated amino acid assignments are shown as "X", tentative assignments are shown in parentheses

[2] The single letter amino acid code is A, alanine; D, aspartic acid; F, phenylalanine; H, histidine; I, isoleucine; L, leucine; Q, glutamine; P, proline; S, serine; T, threonine; and V, valine

[3] The amino acid sequence deduced from the coding region of the full length *cry2Ab2* gene present in MON 89034 corn

Glycosylation analysis

To assess whether post-translational glycosylation of the plant-produced Cry2Ab2 protein occurred, the purified protein sample was subjected to glycosylation analysis. The *E. coli*-produced Cry2Ab2 protein was used as the negative control.

Transferrin, which is known to have multiple covalently-linked carbohydrate modifications, was used as the positive control. The transferrin protein was detected at the expected molecular weight in a concentration dependent manner, with no signal being detected for the plant-produced Cry2Ab2 protein. These results confirm that the plant-produced Cry2Ab2 protein is not glycosylated and also that it is equivalent to the *E. coli*-produced Cry2Ab2 in terms of its lack of glycosylation.

Insect bioassays

The insecticidal activity of the plant- and *E. coli*-produced Cry2Ab2 proteins was determined using an insect bioassay with corn earworm. The bioassay was repeated three times, in replicate, on separate days with separate batches of larvae. Each replicate consisted of a series of seven dilutions yielding a dose series ranging from 0.016 – 1.0 µg Cry2Ab2 protein/ml of diet with a 2-fold separation factor between dose levels. Insect larvae (16 larvae per treatment) were allowed to feed on the diet for 7 days. The combined weight of the surviving insects at each does level for each source of protein was recorded at the end of the 7 day incubation period. The mean EC_{50} values for the plant-produced Cry2Ab2 protein was 0.16 ± 0.01 µg protein/ml diet and for the *E. coli*-produced Cry2Ab2 protein was 0.16 ± 0.04 µg protein/ml diet. Both protein sources also produced similar dose response curves. These results indicate that the plant- and *E. coli*-produced Cry2Ab2 proteins can be regarded as equivalent in terms of their insecticidal activity.

4.2.3 Conclusion

A large number of studies have been done on the Cry1A.105 and Cry2Ab2 proteins to confirm their identity and physicochemical and functional properties as well as to determine their equivalence to *E. coli*-produced Cry1A.105 and Cry2Ab2 proteins. These studies have demonstrated that the two proteins expressed in MON 89034 corn both conform in size and amino acid sequence to that expected and also both exhibit the expected insecticidal activity. The *E. coli*-produced proteins were also shown to be equivalent to the plant produced proteins in terms of their size, amino acid sequence, physicochemical properties as well as their insecticidal activity. The *E. coli*-produced proteins are therefore suitable to act as substitutes for the plant-produced protein for safety assessment purposes.

4.3 Protein expression levels

Validated ELISA methods were used to quantify the levels of the Cry1A.105 and Cry2Ab2 proteins in tissues from MON 89034 corn grown in field trials in the United States in 2005. Field sites were selected which represent the major corn growing region of the U.S. and which provide a range of environmental conditions that would be encountered during commercial production. At each site, three replicated plots of MON 89034 corn (the [LH172 $BC0F_7$ x LH198]F_{1H} and LH172 $BC0F_6$ x F_{2H1}generations, see Figure 2) were grown along with a conventional hybrid corn variety with a similar genetic background to the test plants.

Over season leaf (OSL 1-4), over season root (OSR 1-4), over season whole plant (OSWP 1-4), forage, stover[11], forage-root, senescent root, pollen, silk and grain were collected from each replicated plot at all field sites.

The over season leaf and whole plant samples were collected four times at different growth stages: (1) V2 – V4 stage (2-4 leaf stage); (2) V6 – V8 stage (6-8 leaf); (3) V10 – V12 (10-12 leaf) stage; and (4) pre-VT stage (pre-tasseling). The over season root samples were collected at: (1) V2 – V4 stage; (2) V6 – V8 stage; (3) V10 – V12 stage; (4) pre-VT stage; (5) early dent stage; and (6) after harvest. Pollen and silks were collected at approximately 100 – 120 days after planting and grain was harvested at maturity. Stover was collected following harvest at approximately 130 – 160 days after planting.

Goat polyclonal antibodies were used for the Cry1A.105 ELISA and a mouse monoclonal antibody was used for the Cry2Ab2 ELISA. The limits of detection (LOD) and limits of quantitation (LOQ) for each of the tissues are provided in Table 6 below.

Table 6: LOD and LOQ for the Cry1A.105 and Cry2Ab2 proteins in the tissues analysed

	Cry1A.105 (µg/g fwt[1])		Cry2Ab2 (µg/g fwt)	
Tissue	**LOD**	**LOQ**	**LOD**	**LOQ**
Forage	0.372	0.44	0.191	0.44
Leaf	0.568	0.66	0.081	0.44
Pollen	0.412	1.1	0.055	0.11
Root	0.254	0.33	0.056	0.22
Silk	0.275	0.44	0.40	0.22
Grain	0.262	1.1	0.123	0.22

[1] fresh weight

The Cry1A.105 and Cry2Ab2 protein levels in the various tissues are summarised in Table 7. The mean concentration of Cry1A.105 in MON 89034 corn was highest in young leaf (V2 – V4 stage; 85 µg/g fwt) with the mean level in grain being 5.1 µg/g fwt. For Cry2Ab2, the highest levels were also in young leaf (29 µg/g fwt), with the mean level in grain being 1.1 µg/g fwt. Data on over season protein levels is not presented in this report but in general shows that the levels of both proteins declined over the growing season.

Table 7: Summary of Cry1A.105 and Cry2Ab2 protein levels in tissues from MON 89034 corn

Tissue Type	**Growth Stage**	**Cry1A.105 Mean (SD) [Range], n=15**		**Cry2Ab2 Mean (SD) [Range], n=15**	
		µg/g fwt	**µg/g dwt**	**µg/g fwt**	**µg/g dwt**
Young leaf	V2 – V4	85 (21)	520 (130)	29 (6.8)	180 (59)
		[56 – 130]	[380 – 850]	[19 – 43]	[94 – 270]
Pollen	R1	6.4 (1.5)	12 (1.7)	0.34 (0.084)	0.64 (0.091)
	(silking)	[3.8 – 8.8]	[8.5 – 16]	[0.21 – 0.47]	[0.49 – 0.79]
Silk	R1	3.0 (0.57)	26 (3.9)	8.2 (3.6)	71 (35)
	(silking)	[2.0 – 3.8]	[20 – 31]	[3.3 – 16]	[33 – 160]

[11] stalk and leaf material remaining after harvest

Tissue Type	Growth Stage	Cry1A.105 Mean (SD) [Range], n=15		Cry2Ab2 Mean (SD) [Range], n=15	
		µg/g fwt	µg/g dwt	µg/g fwt	µg/g dwt
Forage	R4 – R6	14 (3.6)	42 (9.4)	12 (4.0)	38 (14)
	(early dent)	[8.3 – 24]	[20 – 56]	[6.5 – 18]	[15 – 55]
Forage root	R4 – R6	2.2 (0.35)	12 (3.1)	4.1 (1.4)	21 (5.9)
	(early dent)	[1.3 – 2.7]	[6.2 – 16]	[2.2 – 6.5]	[14 – 33]
Grain	R6	5.1 (0.67)	5.9 (0.77)	1.1 (0.31)	1.3 (0.36)
	(maturity)	[4.1 – 6.0]	[4.7 – 7.0]	[0.67 – 1.8]	[0.77 – 2.1]
Stover	R6	17 (4.4)	50 (17)	22 (3.6)	62 (15)
	(after harvest)	[9.5 – 26]	[26 – 85]	[17 – 29]	[46 – 97]
Senescent root	R6	2.2 (0.36)	11 (1.4)	5.3 (2.0)	26 (8.8)
	(after harvest)	[1.7 – 3.1]	[9.4 – 15]	[2.4 – 9.1]	[13 – 43]

4.4 Potential toxicity of novel proteins

While the vast majority of proteins ingested as part of the diet are not typically associated with toxic effects, a small number may be harmful to health. Therefore, if a GM food differs from its conventional counterpart by the presence of one or more novel proteins, these proteins should be assessed for their potential toxicity. The main purpose of an assessment of potential toxicity is to establish, using a weight of evidence approach, that the novel protein will behave like any other dietary protein. The assessment focuses on: whether the novel protein has a prior history of safe human consumption, or is sufficiently similar to proteins that have been safely consumed in food; amino acid sequence similarity with known protein toxins and anti-nutrients; structural properties of the novel protein including whether it is resistant to heat or processing and/or digestion. Appropriate oral toxicity studies in animals may also be considered, particularly where results from the biochemical, bioinformatic, digestibility or stability studies indicate a concern.

4.4.1 History of use

Cry1A.105

Cry1A.105 is a chimeric protein made up of different functional domains derived from three wild-type Cry proteins from *B. thuringiensis* subspecies *kurstaki* and *aizawai*. Cry1A.105 consists of domains I and II from Cry1Ab or Cry1Ac[12], substantially domain III from Cry1F, and the C-terminal domain from Cry1Ac. So while Cry1A.105 does not itself have a history of use, it shares 93.6% amino acid sequence similarity to Cry1Ac, which does have an extensive history of prior human exposure, including consumption in food. Microbial pesticide products based on *B. thuringiensis* producing Cry1Ac (e.g. DIPEL®) have been used in both Australia and New Zealand since 1989 and a microbial pesticide that contains a Cry1Ac/Cry1F chimeric protein (Lepinox™) has been approved and used for control of lepidopteran pests in the United States since 1997. The potential toxicity of both Cry1Ac and Cry1F has been previously assessed by FSANZ and no safety concerns were identified.

[12] Cry1Ab and Cry1Ac chare 100% amino acid sequence identity in domains I and II.

Cry2Ab2

The amino acid sequence of the Cry2Ab2 protein expressed in MON 89034 corn is 88 % identical to the wild type Cry2Ab2 protein produced by *B. thuringiensis* subsp. *kurstaki*.

This protein is present in various microbial pesticide products which have been used for a number of years (e.g. Dipel, Cutlass, Crymax). In addition, food from insect-protected cotton expressing an identical Cry2Ab2 to that in MON 89034 was approved in Australia and New Zealand in 2002 (Application A436). In that assessment it was concluded that Cry2Ab2 is unlikely to be toxic to humans.

4.4.2 Specificity

The Cry proteins are a diverse group of proteins which have a defined spectrum of insecticidal activity within particular insect orders (Lepidoptera, Diptera, Coleoptera, and Hymenoptera). The Cry proteins are not known to be toxic to any vertebrates, including humans and other mammals. This high degree of specificity is determined by a number of different factors, including (i) activation of the protein by specific proteolytic enzymes in the insect midgut; (ii) binding of the activated protein to specific midgut receptors; and (iii) changes in protein configuration, which enable the protein to insert into and form pores in the insect midgut membrane. As a consequence, only insects with specific receptors are affected, and no toxicity is observed in species that lack these receptors, including other insects. For example, the Cry1Ab, Cry1Ac and Cry1F proteins are active against lepidopteran but not coleopteran insects.

As the Cry1A.105 protein is a chimeric protein, insect bioassays were done to confirm that it retained its specificity to lepidopteran insects. Insects used in the bioassay were representative of the Lepidoptera (European corn borer, black cutworm, corn earworm, fall armyworm), Coleoptera (boll weevil, southern corn rootworm) and Hemiptera (western tarnished plant bug, green peach aphid). These bioassays confirmed that the Cry1A.105 protein is toxic only to lepidopteran insects.

4.4.3 Similarities with known protein toxins

Bioinformatic analyses were done to assess the Cry1A.105 and Cry2Ab2 proteins for any amino acid sequence similarity with known protein toxins. The TOXIN5 data base, as described in Section 3.4.3 of this report, was assembled for this purpose and the FASTA algorithm was used to assess structural similarity. Although the FASTA program directly compares amino acid sequences and thus is mainly being used to determine similarity in primary protein structure, the alignment data may also be used to infer both secondary and tertiary structure of proteins. In addition to the TOXIN5 database, comparisons were also made to the ALLPEPTIDES database (described in Section 3.4.3) which represents all currently known publicly available protein sequences. Although it may be redundant to search both the TOXIN5 and the ALLPEPTIDES databases for potential similarity to protein toxins, the ALLPEPTIDES database search was used to assess for similarity to other biologically active proteins (e.g. prions), which may not have been annotated with the keyword “toxin”.

The extent of similarity was evaluated using visual inspection of the aligned sequences, the calculated percent identity, and *E* score. The *E* score reflects the degree of amino acid similarity between a pair of sequences and can be used to evaluate the significance of the alignment. A larger *E* score indicates a lower degree of similarity. Typically, alignments between two sequences will need to have an *E* score of less than 1×10^{-5} for them to be considered to have significant homology.

For Cry1A.105, the most significant similarity observed was to Cry1Ac from *B. thuringiensis*, with 92 % identity over 1182 amino acids and with an *E* score of zero. This degree of similarity is expected because the Cry1A.105 protein contains domains I and II as well as the C-terminal domain from Cry1Ac. For Cry2Ab2, the most significant similarity observed was to Cry2Ab2 from *B. thuringiensis* (subsp. *kurstaki*), with 100% identity over 632 amino acids with an *E* score of zero. All of the remaining alignments with significant *E* scores were to other Cry proteins. The analyses did not demonstrate any significant similarity between the Cry1A.105 and Cry2Ab2 proteins and other proteins that may potentially be toxic to humans or other animals.

4.4.4 Digestibility

See Section 4.5.

4.4.5 Acute oral toxicity study

Acute oral toxicity studies using CD-1 mice were conducted to examine the potential toxicity of the Cry1A.105 and Cry2Ab2 proteins. For these studies, *E. coli*-produced Cry1A.105 and Cry2Ab2 were used as the test substances because it was not possible to purify sufficient quantities of the Cry1A.105 and Cry2Ab2 proteins from plant material. The equivalence of the *E. coli*- and MON 89034 corn-produced proteins was established using a range of methods including Western blot analysis, MALDI-TOF mass spectrometry, glycosylation analysis and insect bioassay (see Section 4.2).

<u>Cry1A.105</u>

E. coli-produced Cry1A.105 protein was administered by gavage to 10 male and 10 female young adult CD-1 mice as two separate doses administered approximately 4 hours apart on day 0. The total dose administered was 2072 mg/kg bodyweight. Separate groups of 10 male and 10 female CD-1 mice were administered vehicle only (carbonate-bicarbonate buffer with reduced glutathione, pH 10 – 11) or bovine serum albumin (1998 mg/kg body weight) as controls.

Following dosing, all mice were observed daily, with body weights and food consumption measured weekly (days 0, 7 and 14). A gross necropsy examination was performed on all animals at the time of death or at the termination of the study (day 14).

One male animal in the Cry1A.105 treated group was euthanased on day 1 with a suspected gavage injury, which was confirmed on necropsy. No test article related mortality or clinical signs were observed. There were no significant differences in body weight, cumulative body weight, or food consumption between the control groups and the Cry1A.105 treated group. No treatment-related gross pathological findings were observed at necropsy on day 14.

Cry2Ab2

E. coli-produced Cry2Ab2 protein was administered by gavage to 10 male and 10 female young adult CD-1 mice as two separate doses administered approximately 4 hours apart on day 0. The total dose administered was 2198 mg/kg bodyweight. Separate groups of 10 male and 10 female CD-1 mice were administered vehicle only (2 mM carbonate-bicarbonate buffer containing 2 mM reduced glutathione, pH 10.5) or bovine serum albumin (2442 mg/kg body weight) as controls.

Following dosing, all mice were observed daily, with body weights and food consumption measured weekly (days 0, 7 and 14). A gross necropsy examination was performed on all animals at the time of death or at the termination of the study (day 14).

There were no unscheduled deaths. No treatment- related mortality or clinical signs were observed during the study. There were no significant differences in body weight, cumulative body weight, or food consumption between the control groups and the Cry2Ab2-treated group. No treatment-related gross pathological findings were observed at necropsy on day 14.

4.5 Potential allergenicity of novel proteins

The potential allergenicity of novel proteins 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 sequence similarity between the novel protein and known allergens; the structural properties of the novel protein, including susceptibility to digestion, heat stability and/or enzymatic treatment; and specific serum screening if the novel protein is derived from a source known to be allergenic or has amino acid sequence similarity with a known allergen. Applying this approach systematically provides reasonable evidence about the potential of the novel protein to act as an allergen.

4.5.1 Source of novel proteins

The Cry1A.105 and Cry2Ab2 proteins are both derived from *B. thuringiensis* subsp. *kurstaki* and *aizawai*. *B. thuringiensis* has been used as the active ingredient in insecticidal sprays for the last 40 years and during that period has not been associated with any reported allergic reactions associated with its use. Humans using the insecticidal sprays have been shown to develop antibodies to the expressed Cry proteins, but in no case has the presence of these antibodies been linked with any clinical, including allergic, symptoms (Nester et al 2002).

4.5.2 Similarity to known allergens

Bioinformatic analyses were done to assess the Cry1A.105 and Cry2Ab2 proteins for any amino acid sequence similarity with known allergens, gliadins or glutenins. The AD6 data base, as described in Section 3.4.3 of this report, was assembled for this purpose and the FASTA algorithm was used to assess structural similarity. In addition to the FASTA comparisons, searches were also done to identify short amino acid sequences that may represent linear IgE binding epitopes. An algorithm (ALLERGENSEARCH) was developed to identify matches of eight contiguous amino acids or more (see Section 3.4.3).

As with the assessment of similarity to known protein toxins (Section 4.4.3), the extent of structural relatedness was evaluated using visual inspection of the aligned sequences, the calculated percent identity, and *E* score. *E* scores of ~1 or greater are expected to occur for alignments between random, non-homologous sequences (Pearson 2000).

In terms of the calculated % identity, related protein sequences are considered to be potentially cross-reactive if linear identity is 35% or greater in a segment of 80 or more amino acids (FAO/WHO 2001).

For Cry1A.105, no significant amino acid sequence similarity was observed, with none of the alignments generating an *E* score of less than 1×10^{-5}. The most significant alignment was to a Kiwi fruit allergen, actinidin. This alignment demonstrated 24.2 % identity over a 318 amino acid window, with an *E* score of 2.3. This alignment does not meet the criteria established by the FAO/WHO (2001) for potential cross-reactivity, nor is the *E* score reflective of significant homology. Visual inspection also indicates the alignment is of low significance, with the insertion of many gaps being necessary to optimise the alignment. When searched using the ALLERGENSEARCH algorithm, no matches, representing putative IgE binding epitopes, were shared between the Cry1A.105 protein and proteins in the AD6 database.

For Cry2Ab2, no significant amino acid sequence similarity was observed, with none of the alignments generating an *E* score of less than 1×10^{-5}. The most significant alignment was to the *Coprinus comatus*[13] protein Cop c1, where there was 32.7 % identity over a 52 amino acid window, with an *E* score of 0.89. As the E score is close to 1, this alignment is not reflective of significant homology between Cry2Ab2 and the Cop c1 allergen. In addition, the length of the overlap between the two proteins is relatively short (52 amino acids) when compared to the full length of 637 amino acids of the Cry2Ab2 protein and does not meet the criteria of 35 % identity over 80 amino acids established by the FAO/WHO (2001) for potential cross reactivity. When searched using the ALLERGENSEARCH algorithm, no matches, representing putative IgE binding epitopes, were shared between the Cry2Ab2 protein and proteins in the AD6 database.

4.5.3 Digestibility

Resistance to hydrolysis by digestive proteases has been observed in several food allergens (Astwood et al 1996), therefore a correlation exists between resistance to digestion by pepsin and allergenic potential. As a consequence, one of the criteria for assessing potential allergenicity is to determine the stability of novel proteins in conditions mimicking human digestion. Proteins that are rapidly degraded in such conditions are considered less likely to be involved in eliciting an allergic response. Recently, a pepsin digestibility assay protocol was standardised in a multi-laboratory evaluation (Thomas et al 2004). This protocol was followed to determine the digestive stability of the Cry1A.105 and Cry2Ab2 proteins.

[13] The edible mushroom, Shaggy mane; known to be a source of aeroallergens associated with allergy and asthma. Cop c1 was the first putative allergen cloned from the genus *Coprinus* and has since been shown to be a clinically relevant allergen.

In addition to the pepsin protocol using simulated gastric fluid (SGF), a second digestibility study was done using simulated intestinal fluid (SIF) containing pancreatin, which is a mixture of enzymes including amylase, trypsin, lipase, ribonuclease and protease. The relevance of the SIF study however is limited because ordinarily an ingested protein would first be exposed to pepsin-mediated hydrolysis in the acidic environment of the stomach before being subject to further digestion in the small intestine.

Because it was not possible to purify sufficient quantities of the Cry1A.105 and Cry2Ab2 proteins from plant material for use in these studies, *E. coli*-produced Cry1A.105 and Cry2Ab2 were used as the test substances in both the SGF and SIF studies.

Simulated gastric fluid study

Digestibility of the Cry1A.105 and Cry2Ab proteins in SGF was assessed separately using SDS-PAGE and Western blot analysis. The limit of detection (LOD) of the full length Cry1A.105 and Cry2Ab2 proteins on SDS-PAGE was 0.005 µg, and for Western blot was 1.0 ng for Cry1A.105 and 0.2 ng for Cry2Ab2.

Digestibility of the two proteins in SGF was measured by taking samples at selected time points (0, 0.5, 2, 5, 10, 20, 30 and 60 minutes) and subjecting these to SDS-PAGE. Proteins were visualized by staining the gel or by transferring the protein to a nitrocellulose membrane for Western blot analysis. The full length Cry1A.105 and Cry2Ab2 proteins were digested below the LOD within 30 seconds, which equates to > 95% of the full length proteins being digested. Very faint low molecular weight bands (~4.5 kDa for Cry1A.105 and ~5 kDa for Cry2Ab2) were observed on the stained gel after the 30 second time point, but these disappeared fully by the 20 minute time point for Cry1A.105 and 2 minute time point for Cry2Ab2. The lower molecular weight bands were not visible on Western blot.

Simulated intestinal fluid study

Digestibility of the Cry1A.105 and Cry2Ab2 proteins in SIF was assessed separately using Western blot analysis. The LOD for the Cry1A.105 protein was 0.1 ng and for Cry2Ab2 was 0.5 ng.

Digestibility of the two proteins in SIF was measured by taking samples at selected time points (0, 5, 15, 30 minutes, 1, 2, 4, 8, 12, and 24 hours) and then subjecting these to SDS-PAGE then transferring to polyvinylidene difluoride membrane for Western blot analysis. The full length Cry1A.105 was digested below the LOD within 5 minutes of incubation in SIF. Proteolytic fragments of approximately 60, 32 and 30 kDa were observed at the 5 minute time point. The 60 kDa band, migrating as a doublet, represents the trypsin-resistant core of the Cry1A.105 protein and was observed for up to 24 hours of digestion. The 32 kDa fragment was digested below LOD by the 2 hour time point, whereas the 30 kDa band, migrating as a doublet, was observed for up to 24 hours of digestion, however the intensity of the signal decreased over time. In the case of Cry2Ab2, the full length protein was digested below the LOD within 15 minutes of incubation in SIF. Several proteolytic fragments, with approximate molecular weights of 60, 55, 50, 40, 12 and 10 kDa, were observed, and displayed varying degrees of stability over the time course although the majority, with the exception of the 50 kDa band, were not detectable by the 24 hour time point.

4.6 Conclusion

MON 89034 corn expresses two novel proteins, Cry1A.105 and Cry2Ab2. Both proteins are expressed at relatively low levels in the grain, with the mean concentration for Cry1A.105 being 5.1 μg/g fresh weight and for Cry2Ab2, the mean concentration was 1.1 μg/g fresh weight.

A large number of studies have been done with the Cry1A.105 and Cry2Ab2 proteins to confirm their identity and physicochemical and functional properties as well as to determine their potential toxicity and allergenicity. These studies have demonstrated that both proteins conform in size and amino acid sequence to that expected, do not exhibit any post-translational modification including glycosylation, and also demonstrate the expected insecticidal activity.

In relation to their potential toxicity and allergenicity, it is worth noting that Cry proteins from *B. thuringiensis* are inherently non-toxic to mammals and have exhibited little potential to be allergenic to humans over their long history of use. In addition, bioinformatic studies with the Cry1A.105 and Cry2Ab2 proteins have confirmed the absence of any significant amino acid sequence similarity to known protein toxins or allergens and digestibility studies have demonstrated that both proteins would be rapidly degraded in the stomach following ingestion, similar to other dietary proteins. Acute oral toxicity studies in mice with both proteins have also confirmed the absence of toxicity in animals. Taken together, the evidence indicates that both proteins are unlikely to be toxic or allergenic to humans.

5. COMPOSITIONAL ANALYSES

The main purpose of compositional analysis is to determine if any unexpected changes in composition have occurred to the food and to establish its nutritional adequacy. Compositional analysis can also be important for evaluating the intended effect where there has been a deliberate change to the composition of food.

The classic approach to the compositional analysis of GM food is a targeted one; rather than analysing every single constituent, which would be impractical, the aim is to analyse only those constituents most relevant to the safety of the food or that may have an impact on the whole diet. Important analytes therefore include the key nutrients, toxicants and anti-nutrients for the food in question. The key nutrients and anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates or enzyme inhibitors as anti-nutrients) or minor constituents (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in an organism, such as compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes).

In the case of corn, the key components that should be considered in the comparison include protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals, and the anti-nutrient phytic acid (OECD 2002).

5.1 Study design and conduct

To determine whether unexpected changes have occurred in the composition of MON 89034 corn as a result of the modification, and to assess its nutritional adequacy, compositional analyses were done on forage and grain samples collected from MON 89034, a conventional control line with the same genetic background as MON 89034, and 15 commercial corn hybrids grown under field conditions.

The MON 89034 corn lines used for this study are hybrids between the F_6 and F_7 generations and a conventional inbred corn line, LH198 (see Section 3.2). As a consequence, the line used as the comparator for this study is a conventional LH198 x LH172 hybrid, which has a genetic background representative of the MON 89034 hybrid lines, but without the transferred genes. Commercial corn hybrids were also included in the study as reference lines to provide data for the development of a 99 % tolerance interval for each component analysed. The commercial hybrids used were all conventional lines[14].

Field trials were conducted in the United States in 2004 at five replicated sites. The field sites were located in regions of the U.S. that are suitable for the growth of corn and which are representative of commercial corn production[15]. Seed was planted in a randomised complete block design with three replicates per block of each MON 89034, control and reference line. All the corn lines at each of the field sites were grown under normal agronomic field conditions for their respective geographic regions.

Following compositional analysis, the results were statistically analysed using a mixed model analysis of variance. Data from the five replicated sites were analysed separately and as a combined data set. For each component analysed for MON 89034 corn, the result was compared with that from the conventional counterpart, in this case the LH198 x LH172 hybrid line, for the combination of all five sites (i.e., the combined-site) and for each individual site. A range of measured values from the reference lines was also determined for each component and used as a basis for comparison. Results were also compared to ranges for individual components in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI-CCD 2006), as well as to published literature ranges.

A total of 90 ground forage and grain samples were analysed for 77 different components (9 in forage, and 68 in grain). Compositional analysis of the forage samples included proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium and phosphorus) and carbohydrates by calculation. For the grain samples, compositional analysis included proximates, ADF, NDF, total dietary fibre, amino acids, fatty acids, vitamins (B_1, B_2, B_6, E, niacin and folic acid), anti-nutrients (phytic acid and raffinose), secondary plant metabolites (furfural, ferulic acid and p-coumaric acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), and carbohydrates by calculation. Methods of analysis were based on internationally recognised procedures (e.g., AOAC International methods) or other published methods.

[14] H8751 and H9231 (Golden Harvest); N60-N2 (Northrup King); 590 (Burrus), 2784, 2E685, 2P682 and 2A791 (Mycogen); DKC62-15, DKC61-42 and DKC60-15 (Dekalb); 2730 (Pfister); SC1124A (Seed Consultants); 4908 (Crow's); RX708 (Asgrow).

[15] The sites used were: Jefferson County, Iowa; Jersey County, Illinois; Warren County, Illinois; York County, Nebraska; Fayette County, Ohio.

Of the 77 components measured, 16 in grain had greater than 50 % of the analytical values that were below the limit of quantitation, and therefore, were not included in the statistical analyses. The components not included in the statistical analysis were: sodium, furfural, raffinose, and a number of fatty acids (8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecanoic acid, 17:0 heptadecanoic acid, 17;1 heptadecanoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid).

Statistically significant differences were determined at the 5 % level of significance ($p<0.05$). There were 366 statistical comparisons conducted between MON 89034 and the conventional counterpart (61 comparisons in the combined site and 305 comparisons in the individual sites). Using the data for each component obtained from the reference lines, a tolerance interval was calculated to contain, with 95 % confidence, 99 % of the values expressed in the population of commercial hybrids. For those comparisons between MON 89034 and the conventional counterpart for which there was a statistically significant difference ($p<0.05$), the range of values for MON 89034 was then compared to the 99 % tolerance interval to determine if it was within the range of values for conventional corn hybrids.

The results of the combined site comparisons are presented in Tables 8 – 14. The results from individual trial sites were also evaluated but are not presented in this report. A summary of the statistically significant differences between MON 89034 and the conventional counterpart, from both combined and individual sites, is presented in Table 15.

5.2 Nutrients

Statistically significant differences between MON 89034 and the control hybrid were observed for three nutrients for the combined-site analyses. These were: phosphorus in forage (Table 8), and 18:0 stearic and 20:0 arachidic acids in grain (Table 10). The magnitude of the differences observed were relatively small (3.43 – 19.24 %) compared to the natural variation, with both the mean levels and ranges for MON 89034 corn being well within the 99 % tolerance interval for the commercial corn hybrids. The levels for these nutrients were also well within the ranges in the ILSI-CCD as well as published literature ranges (See Table 16).

At individual sites, 44 statistically significant differences were observed, although 33 of these were only observed at the one site. There were no consistent trends observed and, except in two cases, the mean and range of the nutrients measured in MON 89034 corn were well within the tolerance interval for the commercial hybrids. The mean levels and ranges for calcium and methionine in grain were just outside the tolerance interval but were within the ranges in the ILSI-CCD. Of the remaining differences observed at more than one site, there were no nutrients that were consistently and statistically different across all sites. In addition, there were no nutrients that showed statistically significant differences across three sites that had not been found to be different in the combined-site analysis. Statistically significant differences were observed in as many as two sites for three nutrients (carbohydrate, copper and iron), which had not been found to be different in the combined-site analysis. The observed differences were all small in magnitude and the mean levels and ranges are all within the tolerance interval for commercial corn hybrids.

Overall, these results show that the nutrient composition of MON 89034 corn is equivalent to that of conventional corn hybrids. The few statistically significant differences between MON 89034 corn and the conventional counterpart likely reflect the natural variability of the individual components since the mean levels of the specific nutrients in question are well within the tolerance intervals for commercial corn hybrids as well as the ranges in the ILSI-CCD, as well as the scientific literature.

5.3 Anti-nutrients and secondary plant metabolites

Corn contains a number of anti-nutrients: phytic acid, raffinose, 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and trypsin and chymotrypsin inhibitor. Only phytic acid and raffinose are considered to be biologically relevant (OECD 2002). DIMBOA is present at highly variable levels in corn hybrids and little evidence is available on either its toxicity or anti-nutritional effects and corn contains only low levels of trypsin and chymotrypsin inhibitor, neither of which is considered nutritionally significant.

Phytic acid is considered an important anti-nutrient for animals, especially non-ruminants, since it can significantly reduce the bioavailability of phosphorus in the plant. Feed formulators add the enzyme phytase to pig and poultry diets to improve the utilisation of phosphorus. Raffinose is a non-digestible oligosaccharide and is considered an anti-nutrient because of its gastrointestinal effects (flatulence). Raffinose was excluded from the statistical analyses because greater than 50 % of the analytical values were below the limit of quantitation.

Compositional analysis of the grain indicate that phytic acid was present at similar levels in MON 89034 and the conventional counterpart, and no statistically significant differences were observed in any of the comparisons at either the single site or the combined-site analyses (Table 14).

Secondary plant metabolites are neither nutrients nor anti-nutrients but are sometimes analysed as further indicators of the absence of unintended effects of the genetic modification on metabolism (OECD 2002). Characteristic metabolites in corn are furfural and the phenolic acids, ferulic acid and p-coumaric acid. Furfural was excluded from the statistical analyses because greater than 50 % of the analytical values were below the limit of quantitation.

Compositional analysis of the grain demonstrated that ferulic acid was present at similar levels in MON 89034 and the conventional counterpart, and no statistical differences were observed for any of the comparisons. A statistically significant difference was observed for p-coumaric acid at one site but not at any of the other sites (see Tables 14 and 15). As there was no consistent trend across sites, this result is not considered biologically meaningful.

Based on these results, the levels of anti-nutrients and secondary plant metabolites in MON 89034 corn are comparable to those found in conventional corn hybrids.

Table 8: Combined site compositional analysis of forage for MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Component [1]	MON 89034 [2] (Range)	Control [2] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95% CI [1] (Lower, Upper)	p-Value	Reference range (99% Tolerance Interval)
Fibre						
Acid Detergent Fibre (%DW)	28.95 ± 1.69 (22.60 – 35.85)	27.26 ± 1.69 (19.93 – 35.59)	1.69 ± 1.18 (-6.22 – 10.45)	-0.81, 4.19	0.17	26.72 – 38.94 (16.76, 43.76)
Neutral Detergent Fibre (%DW)	39.69 ± 1.32 (33.99 – 46.82)	37.60 ± 1.32 (31.44 – 43.96)	2.09 ± 1.40 (-3.47 – 7.47)	-0.88, 5.05	0.155	33.70 – 46.74 (25.94, 55.67)
Minerals						
Calcium (%DW)	0.20 ± 0.019 (0.16 – 0.24)	0.19 ± 0.019 (0.13 – 0.28)	0.0066 ± 0.011 (-0.036 – 0.063)	-0.017, 0.031	0.569	0.11 – 0.29 (0.016, 0.38)
Phosphorus (%DW)	**0.25 ± 0.011 (0.22 – 0.32)**	**0.21 ± 0.011 (0.15 – 0.25)**	**0.040 ± 0.014 (-0.0019 – 0.13)**	**0.011, 0.069**	**0.010**	**0.14 – 0.25 (0.071, 0.32)**
Proximates						
Ash (%DW)	3.70 ± 0.27 (2.51 – 4.67)	3.90 ± 0.27 (2.59 – 5.10)	-0.20 ± 0.21 (-1.72 – 0.97)	-0.65, 0.25	0.356	3.40 – 5.45 (1.93, 6.31)
Carbohydrates (%DW)	86.90 ± 0.43 (84.93 – 89.13)	86.69 ± 0.43 (84.36 – 89.57)	0.21 ± 0.53 (-4.23 – 4.41)	-0.91, 1.33	0.697	84.88 – 88.39 (83.05, 90.74
Moisture (%FW)	72.20 ± 1.35 (68.50 – 75.40)	71.53 ± 1.35 (65.90 – 76.80)	0.67 ± 0.52 (-3.50 – 4.20)	-0.44, 1.77	0.220	64.90 – 77.40 (57.62, 86.45)
Protein (%DW)	7.82 ± 0.27 (6.34 – 8.98)	7.70 ± 0.27 (6.06 – 8.87)	0.13 ± 0.26 (-2.32 – 2.35)	-0.43, 0.68	0.635	6.58 – 8.82 (4.78, 10.38)
Total Fat (%DW)	1.57 ± 0.24 (0.63 – 3.17)	1.71 ± 0.24 (0.77 – 2.91)	-0.13 ± 0.23 (-2.28 – 1.95)	-0.59, 0.32	0.558	0.58 – 3.11 (0, 4.54)

[1] DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval
[2] Values are mean ± S.E.

Table 9: Combined site amino acid analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Amino Acid (% Dry Weight)	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Alanine	0.77 ± 0.039 (0.64 – 0.89)	0.78 ± 0.039 (0.67 – 0.89)	-0.0070 ± 0.019 (-0.13 – 0.089)	-0.046, 0.032	0.709	0.67 – 0.96 (0.48, 1.08)
Arginine	0.48 ± 0.013 (0.38 0 0.52)	0.47 ± 0.013 (0.41 – 0.51)	0.011 ± 0.012 (-0.090 – 0.062)	-0.014, 0.036	0.361	0.37 – 0.49 (0.33, 0.56)
Aspartic Acid	0.68 ± 0.029 (0.56 – 0.78)	0.67 ± 0.029 (0.60 – 0.76)	0.0038 ± 0.015 (-0.11 – 0.078)	-0.028, 0.036	0.804	0.57 – 0.77 (0.43, 0.90)
Cysteine	0.23 ± 0.0057 (0.20 – 0.26)	0.23 ± 0.0057 (0.21 – 0.25)	0.0023 ± 0.0038 (-0.022 – 0.023)	-0.0057, 0.010	0.554	0.20 – 0.24 (0.18, 0.27)
Glutamic Acid	1.97 ± 0.097 (1.63 – 2.29)	1.99 ± 0.097 (1.70 – 2.26)	-0.012 ± 0.049 (-0.33 – 0.24)	-0.11, 0.091	0.809	1.71 – 2.41 (1.25, 2.75)
Glycine	0.38 ± 0.0087 (0.32 – 0.41)	0.38 ± 0.0087 (0.36 – 0.41)	0.0042 ± 0.0071 (-0.067 – 0.035)	-0.011, 0.019	0.566	0.32 – 0.40 (0.28, 0.46)
Histidine	0.31 ± 0.011 (0.25 – 0.35)	0.31 ± 0.011 (0.28 – 0.34)	0.0027 ± 0.0055 (-0.050 – 0.030)	-0.0090, 0.014	0.632	0.26 – 0.33 (0.22, 0.38)
Isoleucine	0.36 ± 0.018 (0.30 – 0.43)	0.36 ± 0.018 (0.30 – 0.42)	-0.00003 ± 0.0088 (-0.056 – 0.041)	-0.019, 0.019	0.997	0.32 – 0.45 (0.23, 0.51)
Leucine	1.31 ± 0.077 (1.09 – 1.57)	1.32 ± 0.077 (1.08 – 1.55)	-0.014 ± 0.036 (-0.21 – 0.16)	-0.089, 0.062	0.700	1.14 – 1.68 (0.77, 1.92)
Lysine	0.33 ± 0.0097 (0.26 – 0.36)	0.32 ± 0.0097 (0.29 – 0.36)	0.0088 ± 0.0078 (-0.056 – 0.033)	-0.0077, 0.025	0.273	0.24 – 0.34 (0.20, 0.40)
Methionine	0.23 ± 0.0064 (0.20 – 0.27)	0.22 ± 0.0064 (0.20 – 0.24)	0.0038 ± 0.0047 (-0.017 – 0.028)	-0.0061, 0.014	0.427	0.17 – 0.22 (0.14, 0.25)

Table 9 (continued)

Amino Acid (% Dry Weight)	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Phenylalanine	0.51 ± 0.028 (0.43 – 0.61)	0.52 ± 0.028 (0.43 – 0.60)	-0.0012 ± 0.013 (-0.080 – 0.067)	-0.029, 0.026	0.925	0.45 – 0.65 (0.32, 0.73)
Proline	0.93 ± 0.030 (0.79 – 1.05)	0.93 ± 0.030 (0.83 – 1.01)	0.0034 ± 0.019 (-0.15 – 0.10)	-0.037, 0.044	0.861	0.83 – 1.11 (0.68, 1.21)
Serine	0.52 ± 0.022 (0.44 – 0.61)	0.52 ± 0.022 (0.46 – 0.60)	-0.0046 ± 0.012 (-0.087 – 0.058)	-0.030, 0.021	0.703	0.45 – 0.62 (0.34, 0.71)
Threonine	0.33 ± 0.010 (0.27 – 0.37)	0.33 ± 0.010 (0.29 – 0.36)	0.00063 ± 0.0074 (-0.052 – 0.039)	-0.015, 0.016	0.933	0.29 – 0.37 (0.24, 0.41)
Tryptophan	0.056 ± 0.0018 (0.048 – 0.064)	0.056 ± 0.0018 (0.045 – 0.063)	0.00031 ± 0.0013 (-0.0055 – 0.0072)	-0.0025, 0.0031	0.817	0.043 – 0.059 (0.032, 0.072)
Tyrosine	0.37 ± 0.015 (0.22 – 0.43)	0.36 ± 0.015 (0.24 – 0.42)	0.0088 ± 0.016 (-0.21 – 0.14)	-0.026, 0.043	0.596	0.25 – 0.40 (0.17, 0.52)
Valine	0.49 ± 0.020 (0.40 – 0.55)	0.49 ± 0.020 (0.43 – 0.55)	0.0034 ± 0.010 (-0.084 – 0.055)	-0.019, 0.026	0.748	0.42 – 0.55 (0.35, 0.62

[1] Values are mean ± standard error (S.E.).
[2] CI = confidence interval

Table 10: Combined site fatty acid analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Fatty Acid (% Total Fatty Acid)	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
16:0 Palmitic	9.19 ± 0.060 (8.98 – 9.46)	9.12 ± 0.060 (8.91 – 9.34)	0.071 ± 0.049 (-0.14 – 0.33)	-0.034, 0.18	0.171	9.10 – 12.55 (6.12, 15.67)
16:1 Palmitoleic	0.13 ± 0.0058 (0.11 – 0.14)	0.12 ± 0.0058 (0.048 – 0.14)	0.0022 ± 0.0054 (-0.012 – 0.079)	-0.0093, 0.014	0.696	0.050 – 0.19 (0, 0.28)
18:0 Stearic	**1.89 ± 0.021 (1.79 – 2.03)**	**1.82 ± 0.021 (1.76 – 1.87)**	**0.072 ± 0.021 (-0.055 – 0.18)**	**0.028, 0.12**	**0.002**	**1.57 – 2.45 (0.86, 2.98)**
18:1 Oleic	24.96 ± 0.34 (23.38 – 25.75)	24.84 ± 0.34 (23.62 – 26.66)	0.12 ± 0.20 (-1.48, 1.15)	-0.32 – 0.55	0.574	21.17 – 35.33 (7.51, 46.46)
18:2 Linoleic	61.82 ± 0.40 (60.85 – 63.61)	62.07 ± 0.40 (60.51 – 63.41)	-0.25 ± 0.23 (-1.62 – 1.24)	-0.73, 0.24	0.292	50.33 – 63.59 (39.41, 76.74)
18:3 Linolenic	1.19 ± 0.027 (1.12 – 1.23)	1.22 ± 0.027 (1.15 – 1.43)	-0.028 ± 0.016 (-0.23 – 0.036)	-0.063, 0.0061	0.099	0.93 – 1.52 (0.63, 1.77)
20:0 Arachidic	**0.39 ± 0.0062 (0.36 – 0.42)**	**0.38 ± 0.0062 (0.36 – 0.40)**	**0.013 ± 0.0031 (-0.019, 0.032)**	**0.0063, 0.019**	**<0.001**	**0.32 – 0.47 (0.23, 0.54)**
20:1 Eicosenoic	0.28 ± 0.0040 (0.26 – 0.29)	0.28 ± 0.0040 (0.25 – 0.29)	0 ± 0.0024 (-0.014 – 0.011)	-0.0051, 0.0051	0.999	0.23 – 0.32 (0.15, 0.39)
22:0 Behenic	0.16 ± 0.0050 (0.13 – 0.20)	0.15 ± 0.0050 (0.13 – 0.18)	0.0027 ± 0.0062 (-0.019 – 0.029)	-0.010, 0.016	0.665	0.12 – 0.19 (0.081, 0.23

[1] Values are mean ± standard error (S.E.).
[2] CI = confidence interval

Table 11: Combined site mineral analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Mineral	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Calcium (% DW)	0.0050 ± 0.00034 (0.0038 – 0.0066)	0.0049 ± 0.00034 (0.0040 – 0.0059)	0.00016 ± 0.00011 (-0.00027 – 0.00090)	-0.00008, 0.00040	0.180	0.0031 – 0.0049 (0.0016, 0.0059)
Copper (mg/kg DW)	1.74 ± 0.38 (1.33 – 2.38)	2.07 ± 0.37 (1.26 – 4.54)	-0.33 – 0.53 (-2.96 – 0.78)	-1.45, 0.79	0.547	1.15 – 3.56 (0, 4.20)
Iron (mg/kg DW)	21.40 ± 1.00 (19.23 – 25.23)	22.20 ± 0.99 (19.03 – 28.26)	-0.80 ± 0.67 (-6.50 – 5.90)	-2.22, 0.62	0.250	18.04 – 29.22 (8.88, 34.51)
Magnesium (% DW)	0.12 ± 0.0043 (0.10 – 0.14)	0.12 ± 0.0043 (0.11 – 0.14)	-0.00028 ± 0.0021 (-0.018 – 0.011)	-0.0047, 0.0041	0.893	0.099 – 0.14 (0.075, 0.17)
Manganese (mg/kg DW)	6.79 ± 0.29 (5.43 – 9.32)	6.51 ± 0.29 (5.57 – 8.00)	0.28 ± 0.21 (-1.54 – 2.36)	-0.18, 0.73	0.213	5.56 – 8.64 (3.17, 9.99)
Phosphorus (% DW)	0.33 ± 0.0095 (0.27 – 0.36)	0.33 ± 0.0095 (0.29 – 0.36)	0.00039 ± 0.0043 (-0.038 – 0.026)	-0.0087, 0.0095	0.929	0.25 – 0.37 (0.18, 0.45)
Potassium (% DW)	0.36 ± 0.0065 (0.32 – 0.40)	0.36 ± 0.0065 (0.34 – 0.40)	0.0032 ± 0.0042 (-0.030 – 0.035)	-0.0052, 0.012	0.450	0.32 – 0.40 (0.26, 0.46)
Zinc (mg/kg DW)	22.05 ± 1.14 (18.91 – 26.89)	21.91 ± 1.14 (18.81 – 26.04)	0.14 ± 0.51 (-3.37 – 3.19)	-0.94, 1.22	0.788	16.72 – 34.04 (7.16, 38.55)

[1] Values are mean ± standard error (S.E.).
[2] CI = confidence interval

Table 12: Combined site proximate and fibre analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Component [1]	MON 89034 [2] (Range)	Control [2] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [1] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Proximate						
Ash (% DW)	1.41 ± 0.036 (1.25 – 1.56)	1.39 ± 0.036 (1.28 – 1.51)	0.014 ± 0.041 (-0.11 – 0.13)	-0.072, 0.10	0.734	1.12 – 1.62 (0.74, 1.96)
Carbohydrates (% DW)	84.85 ± 0.42 (83.29 – 86.52)	84.96 ± 0.42 (83.58 – 86.22)	-0.11 ± 0.18 (-1.42 – 0.84)	-0.50, 0.28	0.562	82.91 – 86.78 (81.08, 88.80)
Moisture (% FW)	9.52 ± 0.77 (7.89 – 12.80)	9.50 ± 0.77 (7.86 – 13.10)	0.021 ± 0.22 (-1.00 – 0.87)	-0.44, 0.48	0.923	7.60 – 15.30 (0.45, 19.52)
Protein (% DW)	10.43 ± 0.42 (8.54 – 11.98)	10.36 ± 0.42 (9.22 – 11.52)	0.070 ± 0.19 (-1.26 – 1.28)	-0.34, 0.48	0.725	9.33 – 11.82 (7.54, 13.13)
Total Fat (% DW)	3.32 ± 0.069 (3.05 – 3.89)	3.29 ± 0.069 (3.05 – 3.75)	0.025 ± 0.089 (-0.50 – 0.29)	-0.16, 0.21	0.784	2.66 – 3.71 (2.20, 4.55)
Fibre						
Acid Detergent Fibre (% DW)	5.48 ± 0.19 (3.82 – 7.24)	5.27 ± 0.19 (4.17 – 7.00)	0.21 ± 0.25 (-3.18 – 3.07)	-0.30, 0.72	0.410	4.11 – 6.33 (2.77, 7.56)
Neutral Detergent Fibre (% DW)	10.06 ± 0.37 (8.59 – 12.08)	9.75 ± 0.37 (8.48 – 11.75)	0.31 ± 0.34 (-2.26 – 2.05)	-0.41, 1.03	0.370	8.20 – 11.30 (5.93, 13.63)
Total Dietary Fibre (% DW)	15.17 ± 0.47 (13.39 – 17.02)	14.67 ± 0.47 (12.82 – 17.62)	0.50 ± 0.54 (-3.61 – 4.20)	-0.66, 1.65	0.375	12.99 – 18.03 (9.20, 20.27)

[1] DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval
[2] Values are mean ± S.E.

Table 13: Combined site vitamin analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Vitamin (mg/kg Dry Weight)	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Folic acid	0.35 ± 0.037 (0.26 – 0.48)	0.36 ± 0.037 (0.23 – 0.53)	-0.0080 ± 0.022 (-0.11 – 0.11)	-0.054, 0.038	0.717	0.13 – 0.45 (0.012, 0.69)
Niacin	30.08 ± 1.11 (25.72 – 34.84)	29.59 ± 1.11 (24.93 – 35.75)	0.48 ± 0.65 (-4.44 – 5.64)	-0.82, 1.79	0.461	16.17 – 29.19 (6.97, 37.83)
Vitamin B1	3.07 ± 0.13 (2.39 – 3.44)	2.94 ± 0.13 (2.39 – 3.36)	0.13 ± 0.17 (-0.66 – 0.68)	-0.24, 0.49	0.474	2.19 – 5.60 (0.37, 6.35)
Vitamin B2	1.42 ± 0.046 (1.24 – 1.65)	1.42 ± 0.046 (1.16 – 1.61)	0.0015 ± 0.050 (-0.30 – 0.45)	-0.099, 0.10	0.976	1.34 – 1.91 (0.91, 2.30)
Vitamin B6	6.22 ± 0.23 (5.28 – 6.99)	6.26 ± 0.23 (5.37 – 6.80)	-0.036 ± 0.18 (-0.72 – 1.10)	-0.41, 0.34	0.838	5.08 – 7.47 (3.12, 9.30)
Vitamin E	6.77 ± 0.42 (5.55 – 8.62)	6.63 ± 0.42 (2.72 – 9.02)	0.14 ± 0.36 (-2.35, -3.83)	-0.64, 0.91	0.714	2.71 – 13.94 (0, 20.49)

[1] Values are mean ± standard error (S.E.).
[2] CI = confidence interval

Table 14: Combined site anti-nutrient and secondary metabolite analysis of grain from MON 89034 corn compared to the conventional counterpart (LH198 x LH172)

Component	MON 89034 [1] (Range)	Control [1] (Range)	Difference (MON 89034 minus Control) Mean ± S.E. (Range)	95 % CI [2] (Lower, Upper)	p-Value	Reference Range (99% Tolerance Interval)
Anti-nutrients						
Phytic acid (% DW)	0.75 ± 0.050 (0.53 – 0.87)	0.73 ± 0.050 (0.56 – 0.88)	0.016 ± 0.027 (-0.15 – 0.18)	-0.037, 0.069	0.537	0.50 – 0.94 (0.21, 1.22)
Secondary Metabolites						
Ferulic Acid (µg/g DW)	2131.38 ± 108.09 (1790.25 – 2525.31)	2148.05 ± 108.09 (1878.66 – 2669.85)	-16.67 ± 50.08 (-330.17 – 264.79)	-116.98, 83.65	0.740	1412.68 – 2297.36 (1136.69, 2806.24)
p-Coumaric acid (µg/g DW)	194.25 ± 7.12 (166.11 – 253.04)	183.96 ± 7.12 (167.76 – 210.13)	10.28 ± 7.08 (-24.37 – 70.84)	-4.73, 25.30	0.165	99.30 – 285.75 (0, 378.57)

[1] Values are mean ± standard error (S.E.).
[2] CI = confidence interval

Table 15: Summary of the statistically significant differences between MON 89034 corn and the conventional counterpart (LH198 x LH172)

Component (Units) [1]	MON 89034 Mean	LH198 x LH172 Mean	Mean Difference (MON 89034 minus Control) % of LH198 x LH172	Mean Difference (MON 89034 minus Control) Significance (p-Value)	MON 89034 Range	Commercial Tolerance Interval
COMBINED SITE						
Forage Phosphorus (% DW)	0.25	0.21	19.24	0.010	0.22 – 0.32	0.071, 0.32
Grain 18:0 Stearic (% Total FA)	1.89	1.82	3.97	0.002	1.79 – 2.03	0.86, 2.98
Grain 20:0 Arachidic (% Total FA)	0.39	0.38	3.43	<0.001	0.36 – 0.42	0.23, 0.54
MORE THAN ONE SITE						
Site IA Grain Carbohydrates (% DW)	83.38	84.52	-1.34	0.008	83.29 – 83.55	81.08, 88.80
Site OH Grain Carbohydrates (% DW)	84.26	83.80	0.55	0.009	83.99 – 84.59	81.08, 88.80
Site IL-1 Grain Copper (mg/kg DW)	1.76	1.36	29.35	0.023	1.51 – 2.21	0, 4.20
Site NE Grain Copper (mg/kg DW)	2.15	1.67	28.66	0.023	1.92 – 2.38	0, 4.20
Site IL-1 Grain Iron (mg/kg DW)	20.86	19.48	7.11	0.048	19.23 – 21.79	8.88, 34.51
Site OH Grain Iron (mg/kg DW)	21.37	25.74	-17.00	0.006	20.59 – 21.76	8.88, 34.51
Site IL-1 Grain 18:0 Stearic (% Total FA)	1.96	1.82	7.94	<0.001	1.89 – 2.02	0.86, 2.98
Site IL-2 Grain 18:0 Stearic (% Total FA)	1.98	1.82	9.05	<0.001	1.93 – 2.03	0.86, 2.98
Site IL-1 Grain Arachidic (% Total FA)	0.41	0.39	5.23	0.007	0.40 – 0.42	0.23, 0.54
Site IL-2 Grain 20:0 Arachidic (% Total FA)	0.39	0.37	6.83	0.021	0.38 – 0.40	0.23, 0.54
Site OH Grain 20:0 Arachidic (% Total FA)	0.38	0.37	3.12	0.035	0.38 – 0.39	0.23, 0.54
ONE SITE ONLY						
Site IA Grain Alanine (% DW)	0.88	0.81	7.83	0.030	0.87 – 0.88	0.48, 1.08
Site IA Grain Arginine (% DW)	0.51	0.46	10.83	0.005	0.50 – 0.52	0.33, 0.56
Site IA Grain Aspartic Acid (% DW)	0.77	0.71	8.66	0.003	0.77 – 0.78	0.43, 0.90
Site IA Grain Cysteine (% DW)	0.25	0.23	7.54	0.014	0.24 – 0.26	0.18, 0.27
Site IA Grain Glutamic acid (% DW)	2.27	2.09	8.66	0.011	2.26 – 2.28	1.25, 2.75
Site IA Grain Glycine (% DW)	0.41	0.38	6.94	0.020	0.40 – 0.41	0.28, 0.46
Site IA Grain Histidine (% DW)	0.34	0.32	7.16	0.022	0.34 – 0.34	0.22, 0.38
Site IA Grain Leucine (% DW)	1.49	1.37	8.96	0.032	1.48 – 1.51	0.77, 1.92
Site IA Grain Lysine (% DW)	0.35	0.32	6.66	0.028	0.33 – 0.36	0.20, 0.40
Site IA Grain Methionine (% DW)	0.25	0.23	11.20	0.003	0.25 – 0.27	0.14, 0.25
Site IA Grain Phenylalanine (% DW)	0.58	0.53	9.45	0.028	0.57 – 0.59	0.32, 0.73
Site IA Grain Proline (% DW)	1.05	0.98	7.29	0.028	1.04 – 1.05	0.68, 1.21
Site IA Grain Serine (% DW)	0.60	0.56	8.28	0.004	0.60 – 0.61	0.34, 0.71

Table 15 (continued)

Component (Units) [1]	MON 89034 Mean	LH198 x LH172 Mean	Mean Difference (MON 89034 minus Control) % of LH198 x LH172	Mean Difference (MON 89034 minus Control) Significance (p-Value)	MON 89034 Range	Commercial Tolerance Interval
ONE SITE ONLY (CONT)						
Site IA Grain Threonine (% DW)	0.37	0.34	8.45	0.004	0.37 – 0.37	0.24, 0.41
Site IA Grain Tyrosine (% DW)	0.43	0.36	17.50	0.006	0.42 – 0.43	0.17, 0.52
Site IA Grain Protein (% DW)	11.89	10.85	9.59	0.005	11.73 – 11.98	7.54, 13.3
Site IL-1 Forage Moisture (% FW)	69.03	66.53	3.76	0.031	68.50 – 69.40	57.62, 86.45
Site NE Forage Ash (% DW)	3.20	4.39	-27.12	0.021	2.93 – 3.38	1.93, 6.31
Site NE Forage Carbohydrates (% DW)	88.16	84.98	3.74	0.004	86.86 – 88.84	83.05, 90.74
Site NE Grain Neutral Detergent Fibre (% DW)	10.52	9.05	16.27	0.028	10.43 – 10.69	5.93, 13.63
Site OH Forage Acid Detergent Fibre (% DW)	31.31	23.58	32.78	0.012	26.92 – 46.82	16.76, 43.76
Site OH Forage Neutral Detergent Fibre (% DW)	43.21	37.87	14.11	0.027	40.07 – 46.82	25.94, 55.67
Site IA Grain 18:3 Linolenic (% Total FA)	1.21	1.34	-9.40	0.009	1.20 – 1.23	0.63, 1.77
Site IL-1 Grain 16:1 Palmitoleic (% Total FA)	0.13	0.14	-6.87	0.012	0.12 – 0.13	0, 0.28
Site IL-2 Grain 18:1 Oleic (% Total FA)	24.75	23.82	3.93	0.003	24.14 – 25.25	7.51, 46.46
Site IL-2 Grain 18:2 Linoleic (% Total FA)	61.87	63.17	-2.07	0.001	61.19 – 62.42	39.41, 76.74
Site NE Grain 20:1 Eicosenoic (% Total FA)	0.28	0.29	-1.50	0.030	0.28 – 0.28	0.15, 0.39
Site IA Grain Calcium (% DW)	0.0064	0.0058	10.96	0.012	0.0062 – 0.0066	0.0016, 0.0059
Site IA Grain Manganese (mg/kg DW)	8.34	6.99	19.32	0.017	7.62 – 9.32	3.17, 9.99
Site IA Forage Calcium (% DW)	0.24	0.26	-8.77	0.033	0.24 – 0.24	0.016, 0.38
Site NE Forage Phosphorus (% DW)	0.25	0.17	46.95	0.036	0.23 – 0.28	0.071, 0.32
Site IL-2 Grain Folic Acid (mg/kg DW)	0.37	0.32	13.81	<0.001	0.35 – 0.38	0.012, 0.69
Site OH Grain p-Coumaric Acid (µg/g DW)	218.38	185.63	17.64	0.032	187.79 – 253.04	0, 378.57

[1] DW – dry weight; FW = fresh weight; FA = fatty acids

Table 16: Ranges of components of corn forage and grain from the ILSI Crop Composition Database and the Scientific Literature

Tissue/Component [1]	Literature Range [2]	ILSI Range [3]
FORAGE		
Proximates (% DW)		
Ash	2.43 – 9.64 [a]; 2 – 6.6 [b]	1.527 – 9.638
Carbohydrates	83.2 – 91.6 [b]; 76.5 – 87.3 [a]	76.4 – 92.1
Total Fat	0.35 – 3.62 [b]; 1.42 – 4.57 [a]	0.296 – 4.570
Moisture (% FW)	56.5 – 80.4 [a]; 55.3 – 75.3 [b]	49.1 – 81.3
Protein	4.98 – 11.56	3.14 – 11.57
Fibre (% DW)		
Acid Detergent Fibre	18.3 – 41.0 [b] ; 17.5 – 38.3 [a]	16.13 – 47.39
Neutral Detergent Fibre	26.4 – 54.5 [b] ; 27.9 – 54.8 [a]	20.29 – 63.71
Minerals (% DW)		
Calcium	0.0969 – 0.3184 [b]	0.0714 – 0.5768
Phosphorous	0.1367 – 0.2914 [b]	0.0936 – 0.3704
GRAIN		
Proximates (% DW)		
Ash	1.1 – 3.9 [d] ; 0.89 – 6.28 [b]	0.616 – 6.282
Carbohydrates	77.4 – 87.2 [b] ; 82.2 – 88.1 [a]	77.4 – 89.5
Total Fat	3.1 – 5.7 [d] ; 2.48 – 4.81 [b]	1.742 – 5.823
Moisture (% FW)	7 – 23 [d] ; 8.18 – 26.2 [b]	6.1 – 40.5
Protein	6 – 12 [d] ; 9.7 – 16.1 [c]	6.15 – 17.26
Fibre (% DW)		
Acid Detergent Fibre	3.3 – 4.3 [d] ; 2.46 – 11.34 [a,b]	1.82 – 11.34
Neutral Detergent Fibre	8.3 – 11.9 [d] ; 7.58 – 15.91 [b]	6.1 – 40.5
Total Dietary Fibre	10.99 – 11.41 [h]	8.82 – 35.31
Minerals		
Calcium (% DW)	0.01 – 0.1 [d]	0.00127 – 0.02084
Copper (mg/kg DW)	0.9 – 10 [d]	0.73 – 18.50
Iron (mg/kg DW)	1 – 100 [d]	10.42 – 49.07
Magnesium (% DW)	0.09 – 1 [d]	0.0594 – 0.194
Manganese (mg/kg DW)	0.7 – 54 [d]	1.69 – 14.30
Phosphorus (% DW)	0.26 0 0.75 [d]	0.147 – 0.533
Potassium (% DW)	0.32 0 0.72 [d]	0.181 – 0.603
Zinc (mg/kg DW)	12 – 30 [d]	6.5 – 37.2
Amino Acids (% DW)		
Alanine	N/A	0.439 – 1.393
Arginine	N/A	0.119 – 0.639
Aspartic Acid	N/A	0.335 – 1.208
Cysteine	N/A	0.125 – 0.514
Glutamic Acid	N/A	0.965 – 3.536
Glycine	N/A	0.184 – 0.539
Histidine	N/A	0.137 – 0.434
Isoleucine	N/A	0.179 – 0.692
Leucine	N/A	0.642 – 2.492
Lysine	N/A	0.172 – 0.668
Methionine	N/A	0.124 – 0.468
Phenylalanine	N/A	0.244 – 0.930
Proline	N/A	0.462 – 1.632
Serine	N/A	0.235 – 0.769
Threonine	N/A	0.224 – 0.666
Tryptophan	N/A	0.0271 – 0.215
Tyrosine	N/A	0.103 – 0.642
Valine	N/A	0.266 – 0.855

Table 16 (continued)

Tissue/Component	Literature Range	ILSI Range
Fatty Acids	(% Total Fat)	(% Total Fatty Acid)
16:0 Palmitic	7-19 [e]	7.94 – 20.71
16:1 Palmitoleic	1 [e]	0.095 – 0.447
18:0 Stearic	1 – 3 [e]	1.02 – 3.40
18:1 Oleic	20 – 46 [e]	17.4 – 40.2
18:2 Linoleic	35 – 70 [e]	36.2 – 66.5
183 Linolenic	0.8 – 2 [e]	0.57 – 2.25
20 :0 Arachidic	0.1 – 2 [e]	0.279 – 0.965
20:1 Eicosenoic	N/A	0.170 – 1.917
22:0 Behenic	N/A	0.110 – 0.349
Vitamins (mg/kg DW)		
Folic Acid	0.3 [d]	0.147 – 1.464
Niacin	9.3 – 70 [d]	10.37 – 46.94
Vitamin B_1	3 – 8.6 [e]	1.26 – 40.00
Vitamin B_2	0.25 – 5.6 [e]	0.50 – 2.36
Vitamin B_6	5.3 [d] ; 9.6 [e]	3.68 – 11.32
Vitamin E	3 – 12.1 [e] ; 17 – 47 [d]	1.5 – 68.7
Anti-nutrients (% DW)		
Phytic Acid	0.48 – 1.12 [a]	0.111 – 1.570
Raffinose	0.08 – 0.30 [e]	0.020 – 0.320
Secondary Metabolites (µg/g DW)		
Ferulic Acid	113 – 1194 [f]; 3000 [g]	291.9 – 3885.8
p-Coumaric Acid	22 – 75 [f]	53.4 – 576.2

[1] FW = fresh weight; DW = dry weight; N/A = not available as percent dry weight
[2] Literature range references: [a] Ridley et al 2002; [b] Sidhu et al 2000; [c] Jugenheimer 1976; [d] Watson 1987; [e] Watson 1982; [f] Classen et al 1990; [g] Dowd & Vega 1996; [h] Choi et al 1999
[3] ILSI range is from ILSI Crop Composition Database (2006)

6. NUTRITIONAL IMPACT

In assessing the safety of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well being. 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.

Where a GM food has been shown to be compositionally equivalent to conventional varieties, the evidence to date indicates that feeding studies using target livestock species will add little to the safety assessment and generally are not warranted (OECD 2003).

If the compositional analysis indicates biologically significant changes to the levels of certain nutrients in the GM food, additional nutritional assessment should be undertaken to assess the consequences of the changes and determine whether nutrient intakes are likely to be altered by the introduction of such foods into the food supply. This assessment should include consideration of the bioavailability of the modified nutrient.

In this case, MON 89034 corn is the result of a simple genetic modification to confer insect protection with no intention to significantly alter nutritional parameters in the food.

In addition, extensive compositional analyses have been undertaken to demonstrate the nutritional adequacy of MON 89034 corn and these indicate it is equivalent in composition to grain from conventional corn hybrids. The Applicant has however submitted a feeding study comparing the nutritional performance of MON 89034 corn with conventional corn varieties. This study is evaluated below as additional supporting information.

6.1 Feeding study in broiler chickens

The purpose of the study was to compare the wholesomeness of MON 89034 corn to its conventional counterpart as well as to four commercial corn hybrids[16]. The study was conducted using rapidly growing broiler chicks (Ross x Ross 308), which are sensitive to changes in nutrient quality in their diets, and therefore are often used as a model to assess the wholesomeness of corn.

Diets were formulated on the basis of individual nutrient analyses of grain from the MON 89034, control and commercial lines tested. Each diet consisted predominantly of a mixture of either the MON 89034, control or commercial corn grain and soybean meal. The corn grain was ground prior to incorporating into the diets. Chicks were fed a starter diet from Day 0 – 21 and then switched to a grower/finisher diet for the remainder of the study (up to 42 days). Each diet type (starter, grower/finisher) was formulated to be isocaloric and contain approximately the same amount of corn. The maximum amount of corn possible (approximately 55 % for starter diets and 59 % for grower/finisher diets) was formulated into the diets. The sources of dietary protein in this study were primarily from corn and soybean meal. Water and feed were provided *ad libitum* throughout the study.

A randomised complete block design was used, consisting of six treatments corresponding to the six corn lines being tested. Treatments were assigned to pens with 50 males and 50 females per each of five blocks. All treatments were represented in each block consisting of 10 pens (five male and five female) with 10 broilers per pen for a total of 60 pens and 600 broilers. For each treatment group there were 100 broilers in 10 pens, 5 pens of males (10 broilers/pen) and 5 pens of females (10 broilers/pen). At the start of the study, two additional broilers were added to each pen to compensate for possible losses due to mortality from starve-outs (broilers refusing feed) and dehydration, which normally occurs in the first few days of a feeding study. On Day 7, the group size was reduced to 10 broilers/pen.

Birds were observed daily and were weighed by pen on study Day 0, at study end (Day 42), and individually immediately prior to slaughter for processing. Performance was determined by calculating the average weight gain per bird on Day 42. The average feed:gain ratio was calculated for Days 0 – 42 by dividing the total feed consumption by the total weight gain of surviving birds for each pen. The adjusted feed:gain ratio was calculated by dividing the total feed consumption by the weight gain of surviving birds plus weight gain of birds that died or were removed from the pen. All surviving birds in each pen were slaughtered then processed for determination of carcass and meat characteristics. Statistical analysis was conducted on performance, carcass yield and meat quality parameters.

[16] ASGROW RX690, ASGROW RX772, DKC60-15, and DKC57-01

Chick mortality (29 birds, representing 4.0 %) was observed during the first seven days of the study and related to bacterial infection, dehydration and starve-out. This mortality was random and was not treatment-related. The number of birds that died from Day 7 to study termination varied by treatment group, with an average across treatment groups of 4.5 % and range of 0 to 8 %. The MON 89034 treatment group had a slightly lower than average mortality rate of 3.0 % from Day 7 – 42. The mortality observed was not treatment-related.

Performance measurements of bodyweight at Day 0 (g/bird and kg/pen) and Day 42 (kg/bird and kg/pen), total feed intake (kg/bird and kg/pen), feed conversion (kg/kg) and carcass measurements of chill weight (kg and % live weight), fat pad (kg and % live weight), breast (kg and % chill weight), wing (kg and % chill weight), drum (kg and % chill weight) and thigh (kg and % chill weight) were similar ($P>0.05$) across treatments for the broilers fed diets contain MON 89034, the control or commercial corn grain. A significant difference ($P<0.05$) was noted for adjusted feed conversion between MON 89034 and control fed birds (1.593 kg/kg and 1.636 kg/kg, respectively), however individual treatment comparisons detected no difference between the MON 89034 and three of the four commercial corn diets. This small difference is not considered to be biologically meaningful. No differences among diets were observed in the percentage of moisture, protein, and fat in the thigh and breast meat of broilers.

In conclusion, no biologically relevant differences were observed in the parameters measured between broilers fed the MON 89034 diet and the control diet. For the individual treatment comparisons, broilers in general had similar performance values and carcass yield and meat composition, regardless of whether the diets contained grain from MON 89034, the conventional counterpart or commercial corn hybrids.

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Attachment 3

Summary of submissions

Submitter	Comments
Australian Food & Grocery Council	• States its support for the Application contingent on a satisfactory safety assessment. • Notes that an earlier version of insect-protected corn, MON 810, is already approved and it therefore does not anticipate there would be any health or safety concerns with this Application.
Bayer CropScience	• States its support for the approval of food from corn line MON 89034. • Comments that approval of GM crops like MON 89034 is important to facilitate future and ongoing trade in corn and its derived food products throughout Australia and New Zealand. • Notes this Application has already progressed through several other regulatory bodies internationally and trusts the data presented supports a consistent decision in Australia and New Zealand.
Food Technology Association of Victoria	• Advises that the Technical Sub-Committee has accepted Option 2 – to approve food derived from corn line MON 89034.
Joanne Garcia	• Asserts that pig farmers who have been feeding their pigs GM corn have noticed their pigs have become sterile, contracted cancer or died. Wants to know why these findings have not been studied vigorously by the Australian and New Zealand Governments. • Cites statements made by Professor B. Franklin Pugh in the 29 March 2007 issue of Nature and asks if a noted expert in the field admits that there is still plenty we do not know about the regulation of genes, how can the Government say that GM foods that have passed regulatory scrutiny be truly safe to eat? • Urges FSANZ not to approve food from this corn line, but if it must be done then to stipulate that food derived from animals fed with the GM corn be labelled.
Janet Grogan	• Argues that the expression of two insecticidal proteins in MON 89034 corn should be enough to make the corn sufficiently different from the earlier version MON 810, to warrant the undertaking of animal feeding studies. • Claims animal feeding studies done with the previously approved corn line MON 863 show 'disturbing physiological results'. • States there should be more independent research and mandatory feeding and environmental impact trials over longer periods of time, which will allow potential allergens and or mutations to develop and be detected. • States all GM food products should be labelled as containing GM ingredients.

Submitter	Comments
Hayley Halliwell	• States that in the future, Australia will be able to possibly benefit from GM products but at present claims it is not possible to determine their sustainability or acceptance by the Australian community. • States that FSANZ is required and expected to undertake their own tests and investigations to determine the safety of the product and that the safety for human consumption of products is not something that should be taken for granted or by word of mouth. • Recommends that FSANZ undertakes an in depth investigation into GM foods, particularly corn, without actually approving it. Argues this will allow FSANZ to understand more thoroughly the effects GM foods will have on humans and the environment which will allow for future consideration of new applications for GM foods in Australia and New Zealand. • Recommends that FSANZ conducts a quantitative and qualitative survey of Australian citizens in favour or against GM foods being produced and sold in Australia.
Ivan Jeray	• States his strong opposition to the Application and requests that FSANZ refuses consent. • Claims the use of GM ingredients in foods has not been proven safe or economically viable in the medium to long term. • States that every Australian and New Zealand consumer has a right to know what they will eat. • Claims the notification on the FSANZ website is poor because it did not highlight that the application involved a GM food. States the Application title should include this information.
Maggie Lilith	• Submission virtually identical to that of Janet Grogan above.
New Zealand Food Safety Authority	• States it will comment on the Application at the Draft Assessment stage and have no information provide at the present time.
NSW Food Authority	• States it supports FSANZ in accepting the Application and proceeding to Draft Assessment. • Notes that the Director-General of the NSW Food Authority wrote to FSANZ in 2005 on the cost impact of GM food applications. While they do not oppose current GM food applications on this ground, they believe there is a need to consider a national enforcement strategy. • States the costs of monitoring (labour, reagent costs, methodology validation, methodology consistency, and competency maintenance) should be factored into the benefit cost analysis.
Queensland Health	• States its submission represents a whole of Government response and advises they will review their position contingent on the food safety assessment. • Notes that a decision to approve food derived from insect-protect corn MON 89034 will impact on monitoring resources for their jurisdiction. • Refers to the 2003 National Genetically Modified Food Labelling Pilot Survey, which it states clearly demonstrated that the costs incurred in GM testing is expensive and each new GM food adds to this cost as reference laboratories need to purchase marker genes for the new product and test accordingly. • States that a national enforcement strategy for GM food, which includes education, needs to be progressed without delay.

Submitter	Comments
Toni Reid	• Opposes the Application. • Claims that the decline in the bee population from the Collapsing Colony Syndrome) may be due to GM corn, especially Bt varieties. • Cites evidence which purports to implicate GM crops.
Daniel Rose	• States the costs, and potential costs of permitting the use of MON 89034 corn in Australia far outweigh the benefits. • Argues that, because Bt sprays are already available in Australia, this negates much of the benefit of MON 89034 as the product does not introduce any new protection. • States that although this product may be beneficial to corn producers, it is not worth the costs or potential costs in terms of public health and safety. • Concedes that Monsanto appear to have done a thorough job in testing the product but argues that independent, peer-reviewed data proving the modifications pose no public health threat are lacking. • Claims that modification of the DNA of any plant may cause many problems that are unable to be tested for in a laboratory. • Claims that other Monsanto products using *Bacillus thuringiensis* have been found to cause hepatorenal toxicity (cites Seralini et al 2007). • States that GM products are tested only to the extent required to obtain approval to distribute them and expresses concern for current and future generations who will deal with the problems caused by GM food items being used without definitive, verified data from an independent source that they are indeed safe for human consumption.
Soil & Health Association of New Zealand	▪ States its opposition to genetic engineering in food and the environment. ▪ Refers to animal feeding studies done with MON 863 corn and expresses concern that the feeding studies done with MON 89034 are inadequate. ▪ Refers to losses of bees in the USA and Europe and expresses concern that this may be due to Bt corn. ▪ Believes that FSANZ uses the precautionary principle in a less than rigorous way. ▪ States that long term feeding studies must be undertaken ahead of any decision by FSANZ to allow the release of further GM products into the food chain. ▪ Refers to a recent hearing conducted by the New Zealand Environmental Risk Management Authority for field trials of GM Brassicas and urges FSANZ to access the submissions supplied to that hearing to assist with consideration of this Application. ▪ States it does not want MON 89034 corn allowed into the food chain.