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

2-05
23 March 2005

DRAFT ASSESSMENT REPORT

APPLICATION A543

FOOD DERIVED FROM INSECT-PROTECTED, GLUFOSINATE AMMONIUM-TOLERANT CORN LINE 59122-7

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 4 May 2005
SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

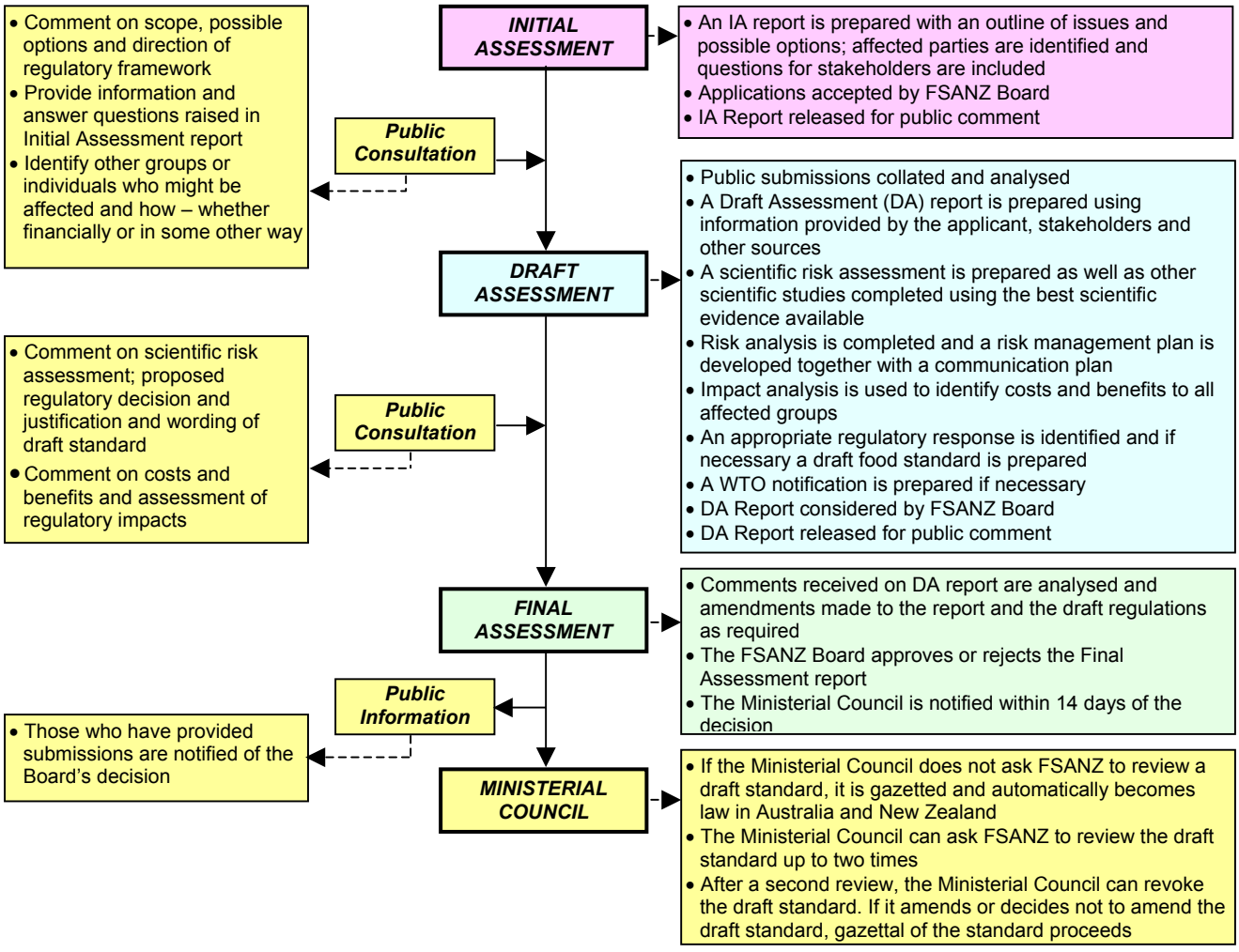
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

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



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A543; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

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

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

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

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

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

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
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Submissions need to be received by FSANZ by 6pm (Canberra time) 4 May 2005.

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

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

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

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Executive Summary and Statement of Reasons

An Application has been received from Dow AgroSciences to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from a genetically modified (GM), insect-protected, herbicide-tolerant corn, (line DAS-59122-7). Standard 1.5.2 – Food Produced using Gene Technology, requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

Corn line DAS-59122-7 has been genetically modified for protection from three significant pests of corn: Western, Northern and Mexican corn rootworms. Protection is conferred by the expression in the plant of bacterially derived protein toxins (*Bt*-δ endotoxins) that are specific for these insects. Corn line DAS-59122-7 is also tolerant to the herbicide glufosinate-ammonium due to the expression in the plant of a bacterially derived enzyme PAT (phosphinothricin acetyl transferase). Corn line DAS-59122-7 does not contain any additional novel genes.

If approved, food from corn line DAS-59122-7 will be entering Australia and New Zealand as imported products. There is no intention to grow this product in Australia or New Zealand.

Public submissions are now invited on this Draft Assessment Report. Comments are specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of food from corn line DAS-59122-7.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from corn line DAS-59122-7 as required under the *Food Standards Australia New Zealand Act 1999* (the FSANZ Act). The assessment included consideration of: (i) the genetic modification to the plant; (ii) the safety of any transferred antibiotic resistance genes; (iii) the potential toxicity and allergenicity of any new proteins; and (iv) the composition and nutritional adequacy of the food, including whether there had been any unintended changes.

No potential public health and safety concerns were identified in the assessment of food derived from corn line DAS-59122-7. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line DAS-59122-7 is as safe and wholesome as food derived from other corn varieties.

Labelling

If approved, food derived from corn line DAS-59122-7 will be required to be labelled as genetically modified if it contains novel DNA and/or protein. Information from the Applicant shows that novel proteins are present in the corn grain, however, most corn products imported into Australia and New Zealand are highly processed and contain no novel protein or DNA. These processed food products (processed such that no novel DNA and/or protein is present in the final food) would therefore not be required to be labelled.

Labelling addresses the requirement of section 10(1)(b) of the Act; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: either (1) no approval; or (2) approval of food derived from corn line DAS-59122-7 based on the conclusions of the safety assessment. Following cost and benefit analysis of the potential impact of each of the options on the affected parties (consumers, the food industry and government), Option 2 is the preferred option as it potentially offers significant benefits to all sectors with very little associated cost. The proposed amendment to the Code, giving approval to food from corn line DAS-59122-7, is therefore considered of net benefit to both food producers and consumers.

Consultation

FSANZ made an Initial Assessment of this Application and called for submissions on 20 October 2004. The closing date for submissions was 1 December 2004. Six submissions were received. A summary of submissions is at Attachment 3. Three submitters support the Application, one objects to the Application and the remaining two submitters are reserving their opinion until after the Draft Assessment Report has been released. Issues raised by the submitters in relation to this application are discussed in Section 5.3.

Statement of Reasons

An amendment to the Code to give approval to the sale and use of food derived from corn line DAS-59122-7 in Australia and New Zealand is recommended on the basis of the available scientific evidence for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce corn line DAS-59122-7;

- food derived from corn line DAS-59122-7 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the amendment to the Code is of net benefit to both food producers and consumers; and

- the proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

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

1. Introduction

An application was received from Dow AgroSciences on 8 July 2004 seeking approval for food derived from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7 under Standard 1.5.2 – Food Produced Using Gene Technology in the *Australia New Zealand Food Standards Code* (the Code).

The genetic modification involved the transfer of the following synthetic genes derived from bacterial genes into the corn plant:

- the maize-optimised synthetic *cry34Ab1* and *cry35Ab1* genes derived from *Bacillus thuringiensis*, which express the insect-specific protein δ endotoxins Cry34Ab1 and Cry35Ab1; and

- the plant optimised synthetic phosphinothricin-acetyltransferase gene, *pat,* derived from *Streptomyces viridochromogenes*, which expresses the enzyme phosphinothricin-acetyltransferase (PAT), conferring tolerance to the herbicide glufosinate ammonium.

A Draft Assessment of the Application, including a detailed safety assessment of food derived from corn line DAS-59122-7, has been completed and FSANZ has prepared a draft variation to Standard 1.5.2 of the Code (see Attachment 1). Public comment is now being sought to assist in the Final Assessment of the Application.

2. Regulatory Problem

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

Dow AgroSciences Australia Pty Ltd has developed a new variety of insect-protected corn, known as line DAS-59122-7, primarily for agronomic purposes. Before food derived from this corn can enter the food supply in Australia and New Zealand, it must first be assessed for safety and an amendment to the Code must be approved by the FSANZ Board, and subsequently be notified to the Australia New Zealand Food Regulation Ministerial Council (ANZFRMC). An amendment to the Code may only be gazetted, once the Ministerial Council process has been finalised.

Dow AgroSciences Australia Pty Ltd has therefore applied to have Standard 1.5.2 amended to include food derived from corn line DAS-59122-7 in the Table to clause 2.

3. Objective

The objective of this Application is to determine whether it is appropriate to amend the Code to approve the use of food derived from corn line DAS-59122-7. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;

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

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

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

4. Background

Corn plants have been developed by the Applicant that are genetically modified to be resistant to insect attack and to be tolerant to the broad-spectrum herbicide glufosinate-ammonium. These corn plants are referred to as corn line DAS-59122-7. The purpose of the modification is to provide growers with an effective method for controlling certain insect pests of corn and for control of weeds.

Corn line DAS-59122-7 contains two insecticidal genes (*cry34Ab1* and *cry35Ab1*), derived from the common soil bacterium *Bacillus thuringiensis* (*Bt*). These genes express insecticidal proteins (Cry34Ab1 and Cry35Ab1) that are toxic to specific insects, including three significant pests of corn: Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberi*) and Mexican corn rootworm (*Diabrotica vigifera zeae*).

In addition, corn line DAS-59122-7 contains a gene (*pat*) from the bacterium *Streptomyces viridochromogenes*, which produces an enzyme (phosphinothricin acetyltransferase, PAT) that detoxifies the herbicide glufosinate ammonium.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAOSTAT Database 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used in industrial products, such as ethyl alcohol by fermentation and highly refined starch by wet-milling.

Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other corn products such as cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

Corn line DAS-59122-7 is permitted for food and feed use in the United States. Applications to permit the use of corn line DAS-59122-7 for food and feed in Canada and Japan were made in 2003 and early 2004. The Applicant intends to submit applications to Taiwan, South Korea, Mexico, Switzerland and the European Union.

5. Relevant Issues

5.1 Safety assessment of food from corn line DAS-59122-7

Food from corn line DAS-59122-7 will be evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The safety assessment will include the following:

- a characterisation of the genetic modification to the plant;
- characterisation of any novel proteins, including their potential toxicity and allergenicity;
- a comparative analysis of the key constituents of corn line DAS-59122-7.

The Applicant submitted a comprehensive data package in support of their application and has provided studies on the molecular characterisation of the insert in line DAS-59122-7, the toxicity and potential allergenicity of Cry34Ab1, Cry35Ab1, and PAT, and compositional analyses of food derived from corn line DAS-59122-7. In addition to information supplied by the Applicant, FSANZ also has regard to other available information, including from the scientific literature, general technical information, independent scientists, other regulatory agencies and international bodies, and the general community.

No potential public health and safety concerns were identified in the assessment of food derived from corn line DAS-59122-7. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line DAS-59122-7 is as safe and wholesome as food derived from other corn varieties. The full safety assessment report is at **Attachment 2** to this document.

5.2 Labelling

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

Cry34Ab1 and Cry35Ab1 are present at very low levels in the corn kernels, which would therefore have to be labelled as genetically modified. More highly processed products that contain no novel DNA or protein would not be required to be labelled.

5.3 Issues arising from public submissions

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

[1] FSANZ (2003) Information for Applicants – Format for applying to amend the Australian New Zealand Food Standards Code – Food Produced Using Gene Technology.
[2] www.foodstandards.gov.au/mediareleasespublications/factsheets/factsheets2002/index.cfm

5.3.1 Data on glufosinate ammonium residues in corn line DAS-59122-7

The New Zealand Food Safety Authority (NZFSA) questions whether the use of corn line DAS-59122-7 may cause a change in herbicide application practices, which may lead to a change in residue levels. They suggest that the applicant be required to provide information on glufosinate ammonium residue levels to allow estimates of intake and a comparison with the ADI, to be included in the Draft Assessment Report.

5.3.1.1 Response

There is no MRL for glufosinate ammonium use on corn in Australia, therefore corn products containing residues of this herbicide cannot be sold as food. In New Zealand, the Codex Alimentarius Commission MRL of 0.1 mg/kg for glufosinate ammonium use on corn applies in the case of imported food.

5.3.2 Data on toxicological characteristics of the herbicide deactivation by-product, acetylated glufosinate ammonium

The New Zealand Food Safety Authority (NZFSA) suggests that the level of the herbicide deactivation by-product (acetylated glufosinate ammonium) in corn products should be examined as part of the Draft Assessment.

5.3.2.1 Response

The safety of the major glufosinate-ammonium metabolite (N-acetyl-L-glufosinate) has been addressed in Section 4.3 of the safety assessment report (Attachment 2).

Food products derived from corn line DAS-59122-7 must comply with the respective MRL for glufosinate ammonium (and metabolites) applicable in New Zealand and Australia.

5.3.3 Increase in costs for compliance testing

The South Australian Department of Health has raised the concern that the approval of corn line DAS-59122-7 will potentially add to compliance testing since GM proteins are likely to be present in the final foods which will therefore require labelling. Analysis costs may also rise if labs are required to purchase new testing kits.

5.3.3.1 Response

The approval of corn line DAS-59122-7 may lead to an increase in costs for compliance testing for product labelling. This is recognised in the impact analysis. However, this cost would not necessarily be avoided if this corn was not approved, as it could be necessary to test imported corn products to ensure an unapproved corn line was not being sold in Australia and New Zealand.

Moreover, FSANZ is required to assess GM foods on the basis of their risk to public health and safety. Labelling requirements for approved GM foods are not to address any safety concerns, but to allow consumers choice in the products they purchase and are generally thought to be desirable and worthwhile despite the potentially significant costs compliance may incur.

5.3.4 Cost/benefit analysis correction

The South Australian Department of Health suggested that the evidence for a possible benefit arising from option 2 of "lower production costs and reduced exposure to agricultural chemicals used to manage pests" may be questionable. Whether GM crops result in lower production costs and less chemical usage seems to depend on the crop, where it is being grown and who is collecting the information. However, as this corn will not be grown in Australia or New Zealand this issue may not be relevant to this application.

5.3.4.1 Response

The statement 'possible benefit to growers in lower production costs and reduced exposure to agricultural chemicals used to manage insect pests' in the impact analysis has been removed.

6. Regulatory Options

6.1 Option 1 – prohibit food from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7

Maintain the *status quo* by not amending the Code to approve the sale and use of food derived from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7.

6.2 Option 2 – approve food from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7

Amend the Code to permit the sale and use of food derived from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

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

7.2 Impact 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.

The following is a draft assessment by FSANZ of the costs and benefits of the two regulatory options identified so far. This is based on information supplied by the applicant and experience FSANZ has gained from consideration of previous Applications relating to GM foods. Your comments are also invited on the costs and benefits identified for the options below.

7.2.1 Option 1

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

Cost associated with higher retail prices for segregated foods.

No impact on consumers wishing to avoid GM foods, as food from corn line DAS-59122-7 is not currently permitted in the food supply.

Government: No immediate impact.

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

Industry: Cost in terms of restricting innovation in food/crop production for both growers and other sectors of the food industry. Cost to the food industry to source either segregated or non-GM supplies.

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

7.2.2 Option 2

Consumers: Possible benefit of lower prices, to the extent that savings from production efficiencies are passed on.

Benefit of access to a greater range of products including imported food products containing ingredients derived from corn line DAS-59122-7.

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

Government: No direct impact.

This decision may impact on monitoring resources as food derived from corn line DAS-59122-7 will be required to be labelled as GM.

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

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

Benefit to food retailers in an increased product range.

Possible cost to food industry as food derived from corn line DAS-59122-7 will be required to be labelled as genetically modified.

8. Consultation

8.1 Public Consultation

The Initial Assessment of this Application was advertised for public comment between 20 October 2004 and 1 December 2004. A total of six submissions were received during this period and a summary of these is included in **Attachment 3** to this Report. Three submitters support the approval of corn line DAS-59122-7, one submitter objected to approval of corn line DAS-59122-7. The remaining two submitters did not express a preference at this stage.

FSANZ carried out an assessment of the Application, including a safety assessment of the food, taking into account the comments received in the first round of consultation. These issues have been addressed in section 5.3 above.

8.2 World Trade Organization (WTO)

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

There are no relevant international standards for GM foods, however, the proposed amendment to the Code to allow food derived from corn line DAS-59122-7 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade.

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

9. Conclusion and Recommendation

An amendment to the Code to give approval to the sale and use of food derived from corn line DAS-59122-7 in Australia and New Zealand is recommended on the basis of the available scientific information for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce corn line DAS-59122-7;

- food derived from corn line DAS-59122-7 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the amendment to the Code is necessary, cost effective and of net benefit to both food producers and consumers; and

- the proposed draft amendment to the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

The proposed draft variation is provided in **Attachment 1**.

10. Implementation and review

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

ATTACHMENTS

Attachments to the Assessment Report could include:

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Draft safety assessment report
3. Submission summary

Attachment 1

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

To commence: On gazettal

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

Food derived from insect-protected, glufosinate ammonium-tolerant corn line DAS-59122-7

Attachment 2

DRAFT SAFETY ASSESSMENT REPORT

APPLICATION A543 – FOOD DERIVED FROM INSECT-PROTECTED, GLUFOSINATE AMMONIUM-TOLERANT CORN LINE DAS-59122-7.

SUMMARY AND CONCLUSIONS

Background

Food derived from genetically modified (GM) corn line DAS-59122-7 has been assessed for its safety for human consumption. This corn line has been genetically modified to be resistant to insect attack and herbicide tolerant and has been developed for cultivation in North America. Therefore, if approved, food derived from corn line DAS-59122-7 may enter the Australian and New Zealand food supply as imported food products.

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

History of Use

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Products derived from DAS-59122-7 corn may include flour, breakfast cereals, high fructose corn syrup and other starch products.

Description of the Genetic Modification

Corn line DAS-59122-7 contains two novel genes, *cry34Ab1* and *cry35Ab1,* encoding the insecticidal proteins Cry34Ab1 and Cry35Ab1. These two genes were derived from the soil bacterium *Bacillus thuringiensis* and are selectively toxic to certain insect pests of corn. Corn line DAS-59122-7 also contains a copy of the *pat* gene, encoding the enzyme phosphinothricin acetyl transferase (PAT), which confers tolerance to the herbicide glufosinate ammonium.

Detailed molecular and genetic analyses of corn line DAS-59122-7 indicate that the transferred *cry34Ab1*, *cry35Ab1* and *pat* genes are stably integrated into the plant genome at one insertion site and are stably inherited from one generation to the next.

Characterisation of Novel Protein

Corn line DAS-59122-7 expresses three novel proteins – Cry34Ab1, Cry35Ab1, and PAT. In the corn grain, the PAT protein is undetectable. Cry34Ab1 is expressed at levels ranging from 28.9-117 ng/mg dry weight in DAS-59122-7 corn grain and Cry35Ab1 at levels ranging from not detectable to 1.83 ng/mg.

Acute oral toxicity studies have been conducted on the Cry34Ab1, Cry35Ab1, and PAT proteins – there was no evidence of toxicity in all cases. Potential allergenicity was assessed by sequence comparison to known allergens, simulated digestion studies and by determining thermolability – these data did not indicate any potential for allergenicity.

Comparative Analyses

Compositional analyses were done to establish the nutritional adequacy of grain from corn line DAS-59122-7, and to compare it to a non-transgenic control line and commercial varieties of corn. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites and anti-nutrients.

No differences of biological significance were observed between the transgenic corn grain and its non-GM counterpart. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very small differences and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it was concluded that food from corn line DAS-59122-7 is equivalent in composition to that from other commercial corn varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that food derived from corn line DAS-59122-7 is equivalent in composition to food from non-GM corn varieties. The introduction of food produced from corn line DAS-59122-7 into the food supply is therefore expected to have minimal nutritional impact.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food produced from corn line DAS-59122-7. On the basis of the data provided in the present application, and other available information, food produced from corn line DAS-59122-7 can be considered as safe and as wholesome as food produced from other corn varieties.

1. INTRODUCTION

Dow AgroSciences Pty. Ltd. has submitted an application to Food Standards Australia New Zealand (FSANZ) to vary Standard 1.5.2 – Food Produced Using Gene Technology – in the *Australia New Zealand Food Standards Code*, to include food from a new genetically modified (GM) corn variety. The GM corn variety is known commercially as DAS-59122-7 corn.

Corn line DAS-59122-7 has been genetically modified for protection against the Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberi*), and Mexican corn rootworm (*Diabrotica vigifera zeae*). These species are serious insect pests of dent corn in the major corn-producing states of the north-central United States and Canada. Protection is conferred by the expression in the plant of bacterially derived protein toxins (*Bt*-δ-endotoxins) that are specific for these insects. Corn line DAS-59122-7 also contains a gene encoding resistance to the herbicide glufosinate ammonium.

Corn line DAS-59122-7 contains three novel genes, *cry34Ab1*, *cry35Ab1*, and *pat*. The two *cry* genes express insecticidal crystal proteins and the *pat* gene expresses the enzyme phosphinothricin acetyltransferase (PAT) which confers tolerance to the herbicide glufosinate ammonium.

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

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAO, 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used in industrial products, such as ethyl alcohol by fermentation and highly refined starch by wet-milling.

Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other corn products such as cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

Applications to permit the use of corn line DAS-59122-7 for food and feed use in the United States, Canada and Japan were made in 2003 and early 2004. No approvals have been granted to date. The Applicant intends to submit applications to Taiwan, South Korea, Mexico, Switzerland and the European Union. Corn line DAS-59122-7 is not being developed for cultivation in Australia. Therefore, if approved, food from corn line DAS-59122-7 may therefore enter the Australian and New Zealand food supply as imported food products.

2. HISTORY OF USE

2.1 Donor Organisms

Bacillus thuringiensis

The source of the *cry34Ab1* and *cry35Ab1* genes used in this GM corn is the ubiquitous soil and plant bacterium *Bacillus thuringiensis* (*Bt*). Both *cry* genes are synthetic versions of genes from the non-motile strain of *Bt*, PS149B1.

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

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

Insecticidal products using *Bt* were first commercialised in France in the late 1930s (Nester et al 2002) and were first registered for use in the United States by the Environment Protection Agency (EPA) in 1961 (EPA, 1998). The EPA thus has a vast historical toxicological database for *B. thuringiensis*, which indicates that no adverse health effects have been demonstrated in mammals in any infectivity/ pathogenicity/ toxicity study (McClintock *et al*., 1995; EPA, 1998; Betz *et al*., 2000). This confirms the long history of safe use of *Bt* formulations in general, and the safety of *B. thuringiensis* as a donor organism.

Streptomyces viridochromogenes

Streptomyces viridochromogenes is a ubiquitous soil fungus and was the source of the PAT encoding gene that is present in corn line DAS-59122-7. *S. viridochromogenes* is a gram positive sporulating soil bacteria. Few *Streptomyces* have been isolated from animal or human sources and pathogenicity is not a typical property of these organisms. *S. viridochromogenes* is itself not known to be a human pathogen and nor has it been associated with other properties (e.g. production of toxins) known to affect human health.

Agrobacterium tumefaciens

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

Agrobacterium naturally contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its genome into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing root deformation mainly with sugar beets, pome fruit and viniculture crops. However, adaptation of this natural process has now resulted in the ability to transform a broad range of plant species without causing adverse effects in the host plant.

Other donor organisms

The regulatory elements that were used in the gene construct were derived from *Solanum tuberosum* (potato), *Triticum aestivum* (wheat) and *Zea mays* (corn), plants that are widely consumed and generally recognised as safe. CaMV 35S promoter and terminator sequences are frequently used in transgenic plants and have no pathological characteristics (USDA, 1995).

2.2 Host Organism

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide (OECD, 2002b). Worldwide production of maize is 500 million tons a year, with the United States and China being the major producers.

The majority of grain and forage derived from maize is used as animal feed, however maize also has a long history of safe use as food for human consumption. The grain can be processed into industrial products such as ethyl alcohol (by fermentation), and highly refined starch (by wet-milling) to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain corn oil and numerous other products (White and Pollak, 1995).

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

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

The corn recipient line was the public line designated Hi-II. Hi-II is a derivative of the A188 and B73 inbred lines of corn which are publicly available inbred lines from the University of Minnesota and Iowa State University, respectively. Hi-II is approximately 50:50 of the two lines (Armstrong *et al.*, 1991).

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the genetic modification

Studies submitted
Coats, I. and Herman, R. (2002) Product Characterisation Data for *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins Expressed in Transgenic Maize Plants (PHP17662). Pioneer Hi-bred International, Johnston, Iowa. Study ID: PHI-2002-046

Coats, I. and Herman, R. (2003) Addendum to MRID#45790601: Product Characterisation Data for *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins Expressed in Transgenic Maize Plants (PHP17662). Pioneer Hi-bred International, Johnston, Iowa. Study ID: PHI-2002-046

Corn line DAS-59122-7 was produced by *Agrobacterium*-mediated transformation of *Zea mays line* Hi-II, using the transformation vector PHP17662. The plasmid contains the *cry34Ab1*, *cry35Ab1*, and *pat* genes and regulatory elements as shown in Table 1.

Immature embryos of corn were treated with *Agrobacterium tumefaciens* strain LBA4404 containing plasmid PHP17662. After a period of embryo and *Agrobacterium* co-cultivation on solid culture medium, the embryos were transferred to fresh culture medium that contained the herbicide glufosinate ammonium. The culture medium was stimulatory to the maize somatic embryogenesis and was selective for those cells that contain the integrated *pat* gene. The embryonic tissue was then regenerated into whole transgenic plants, which were transferred to the greenhouse.

Leaf samples were taken for molecular analysis to verify the presence of the transgenes by PCR and to confirm the expression of the *cry* proteins by ELISA. Plants were also subjected to a whole plant bioassay using corn rootworm. Positive plants were crossed with an inbred line to obtain seed from the initially transformed plants. A number of lines were evaluated in the field which resulted in the selection of line DAS-59122-7, based on its good agronomic characteristics and excellent resistance to corn rootworm.

Table 1: Genetic elements of the plasmid PHP17662

Genetic element	Size (bp)	Function
Right border	25	T-DNA right border region
UBI1ZM PRO	1,986	Ubiquitin promoter (plus ubiquiting 5' untranslated region and intron) from Zea mays (Christensen *et al.*, 1992).
cry34Ab1	369	Synthetic version of the *cry34Ab1* gene encoding the 14 kDa delta-endotoxin parasporal crystal protein from Bt (maize optimised).
PINII TERM	1,299	Terminator sequence from Solanum tuberosum proteinase inhibitor II (An *et al.*, 1989).
TA PEROXIDASE	1,299	Root-preferred promoter from Triticum aestivum peroxidase (Hertig *et al.*, 1991).
cry35Ab1	1,152	Synthetic version of the *cry35Ab1* gene encoding a 44 kDa delta endotoxin parasporal crystal protein from Bt (maize optimised).
PINII TERM	318	Terminator sequence from Solanum tuberosum proteinase inhibitor II (An *et al.,* 1989).
CaMV 35S PRO	549	35S promoter from the cauliflower mosaic virus, Strasbourg strain (Hohn *et al.*, 1982).
pat	552	Synthetic, plant optimised phosphinothrycin acetyltransferase coding sequence from *Streptomyces viridochromogenes*
CaMV 35S TERM	199	35S terminator from cauliflower mosaic virus
LEFT BORDER	25	T-DNA left border region

3.2 Function and regulation of novel genes

cry34Ab1 and cry35Ab1

The maize optimised synthetic *cry34Ab1* and *cry35Ab1* genes encode proteins 123 and 383 amino acids in length respectively. Although these genes were originally isolated from *B. thuringiensis*, the DNA sequences of these two genes have been modified in order to alter the guanosine and cytosine codon bias to a level more typical for plant codons. The deduced amino acid sequences of these proteins expressed in the transgenic corn are identical to the native Cry34Ab1 and Cry35Ab1 protein sequences. The regulatory elements are described in Table 1. The *cry34Ab1* gene is regulated by the ubiquitin promoter from *Zea mays* and the *Solunum tuberosum* proteinase inhibitor terminator. The *cry35Ab1* gene is regulated by the wheat peroxidase gene promoter and the *Solunum tuberosum* proteinase inhibitor terminator.

The *cry34Ab1* and *cry35Ab1* genes confer protection against corn rootworm. This is described in more detail in section 4.1.

Pat

The *pat* gene encodes the PAT enzyme, which confers resistance to the herbicide glufosinate ammonium. This gene was introduced as a selectable marker for the identification of transformed plants. The *pat* gene was originally isolated from *Streptomyces viridochromogenes* Tu494, but as with the two *cry* genes, in this construct the codons have been optimised for plant expression. The deduced amino acid sequence is identical to the native bacterial PAT enzyme.

The cauliflower mosaic virus 35S promoter controls the transcription of the *pat* gene in corn line DAS-59122-7.

No other genes were transferred to corn line DAS-59122-7.

3.3 Characterisation of the genes in the plant

Studies submitted:
Cressman, R.F., Luckring, A.K., Sanders, C.D., Hunt, S.L. and Locke, M.E. (2004). Insert and Border Sequence Characterisation of *B.t.* Cry34/35Ab1 Event DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-037

Locke, M.E. and Igo, E. (2003). Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-038

Locke, M.E., Dietrich, N. and Weber, N. (2003). Detailed Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-041

Insert and copy number

Southern blot analysis was used to establish the integration pattern and determine copy number of the *cry34Ab1*, *cry35Ab1*, and *pat* genes and to confirm the absence of DNA sequence from outside the T-DNA borders of the transformation vector.

Southern blot analyses of four different generations (designated T_1S_1, T_1S_2, BC_1 and BC_2S_1; described in Table 2) of corn line DAS-59122-7 demonstrate that the insert in corn line DAS-59122-7 occurred as a simple integration of a single intact T-DNA from plasmid PHP17662. No plasmid backbone fragments were present as determined by Southern blot analyses. In addition, the results did not indicate that rearrangements of the T-DNA had occurred, as all internal restriction sites appeared to be intact and produced hybridising fragments of the expected size. Figure 1 shows the insert in DAS-59122-7 corn.

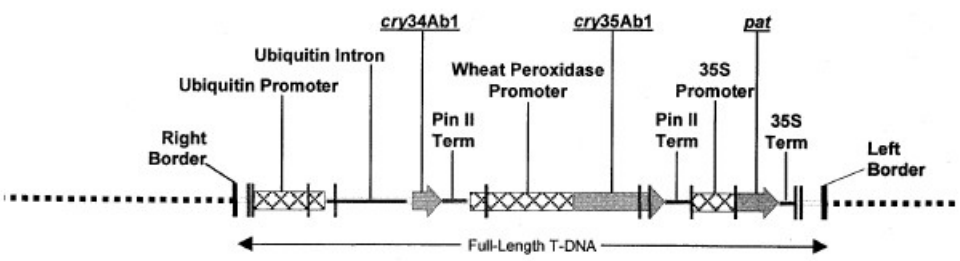

Figure 1: Schematic diagram of the DNA insert in corn line DAS-59122-7.

Table 2: Corn line DAS-59122-7 generations used in molecular characterisation studies

Generation	Description
T_0	Original Hi-II plant containing event DAS-59122-7
T_1S_1	T_0 generation corn plants were out-crossed for one generation to inbred line PH09B and selfed for one generation to produce the T_1S_1 seed
T_1S_2	T_0 generation corn plants were out-crossed for one generation to PH09B and selfed for two generations to produce the T_1S_2 seed
BC_1 hybrid	T_0 generation corn plants were out-crossed for one generation to inbred line PH09B. The resulting F_1 was crossed and then backcrossed to inbred 05F to make a BC_1. The BC_1 generation was then crossed to a second inbred 581 to produce the BC_1 hybrid seed
BC_2S_1	T_0 generation corn plants were out-crossed for one generation to PH09B, the resulting F_1 was crossed and then backcrossed twice to inbred 581 to make BC_2. The final generation represented here is a self-pollination (S_1) of the BC_2 creating a population that segregates at a ratio of 3:1.

PCR and sequence analysis

To further characterise the integrity of the inserted T-DNA and describe the genomic insertion site, the sequence of the T-DNA insert and flanking genomic DNA border regions of the insert in corn line DAS-59122-7 (T_1S_2) was determined. The entire insert was sequenced and this sequence compared to the DNA sequence of the transforming plasmid (PHP17662). In total, 7343 bp of T-DNA had become inserted into the corn genome. Twenty-two and 25 bp were found to be missing from the Right and Left border regions respectively. While T-DNA border sequences are known to play a critical role in T-DNA insertion into the genome, this result is not unexpected since insertions are often imperfect, particularly at the Left T-DNA border (Tinland and Hohn, 1995). Two nucleotide differences were observed in the non-translated wheat peroxidase promoter region of the T-DNA insert. Neither of these changes affected the open reading frame composition of the insert.

Flanking regions and putative Open Reading Frame analysis

The junctions between the insert and corn genomic regions were also sequenced. At the 5' end of the insert, 2593 bp of genomic DNA were sequenced, at the 3' end 1986 bp of genomic DNA were sequenced.

PCR amplification based on the insert and border sequences confirmed that the border regions were of maize origin. No further identification of the maize genomic border sequences was possible due to limited sequence homology with publicly available sequences in GenBank. Analysis of the sequence spanning the junction regions indicated that no novel open reading frame resulted from the insert in corn line DAS-59122-7.

Alignment of the entire transformation plasmid sequence with the border region sequences showed no significant homologies, indicating that the border regions do not contain fragments of the transforming plasmid.

The 5' and 3' junction regions between the corn genomic border sequence were analysed for the presence of novel open reading frames. No open reading frames of significant size (>100 amino acids) were identified in either region. The homology searches of these sequences with the known maize genomic sequences did not indicate the presence of endogenous maize open reading frames in the border regions that might have been disrupted by the insert in corn line DAS-59122-7.

Conclusion

Detailed molecular analyses have been performed on corn line DAS-59122-7 to characterise the novel genes present in the genome. Results indicate that there is one insertion site consisting of the entire T-DNA from plasmid PHP17662. The *cry34Ab1, cry35Ab1* and *pat* genes are intact.

Sequence analysis showed that two single nucleotide changes had occurred within the non-coding region of the insert. No novel ORFs (>100 amino acids) were created by the insertion of the novel genes and nor were any existing ORFs destroyed.

3.4 Stability of the genetic changes

Studies submitted:
Locke, M.E. and Igo, E. (2003). Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-038

Locke, M.E., Dietrich, N. and Weber, N. (2003). Detailed Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-041

Weber, N. and Igo E (2003) Characterisation of Transgenic Corn Event DAS-59122-7 to Investigate Genetic Equivalence of the Inserted DNA within a Single Generation. Pioneer Hi-Bred International, study ID: PHI-2003-012

Segregation analysis

Southern blot analysis was used to show that the insert is stably inherited within a single generation (Weber and Igo, 2003). Seventy-nine corn plants were grown from BC_2S_1 seed and were analysed for expression of the PAT (by leaf painting with glufosinate ammonium) and Cry34Ab1 (by lateral flow immunoassay) proteins. Of the 79 plants, 55 were positive for both PAT and Cry34Ab1 expression. The remaining 24 plants were negative for expression of both proteins (null segregants).

Genomic DNA was extracted from all 55 of the transgenic plants and 23 of the null segregants and used in Southern blotting to determine if the insert in each of the 55 plants was stably integrated. Southern blots were hybridised with probes specific to the *Cry34Ab1* gene, the *Cry35Ab1* gene, and the *pat* gene. The 23 null segregants showed no hybridisation with any of the three probes. The 55 transgenic plants all displayed a consistent hybridisation pattern with each of the probes, indicating the insert is the same in all individuals within the generation.

All results correlated with the previous Southern analyses on different generations of corn line DAS 59122-7 indicating that a single intact DNA insertion has integrated stably into the corn genome.

Chi squared analysis showed no significant difference between the observed ratio of 55 positive to 24 null plants in the BC_2S_1 generation to the expected segregation ratio of 3:1.

Another study analysed the Mendelian segregation of corn line DAS-59122-7 over eight generations. The T_0 generation corn plant was out-crossed for one generation to inbred line PH09B to produce T_1 generation plants which were either self pollinated to produce the T_1S_1 generation or out-crossed with Dow AgroSciences inbred lines designated inbred B (DAS male) or inbred C (DAS female) to produce a number of backcrosses. Since the insert in corn line DAS-59122-7 was expected to segregate as a single dominant gene, each generation was sprayed with glufosinate ammonium to eliminate susceptible plants to determine if the insert was segregating as expected.

All plants found to be herbicide tolerant were also tested with Cry34Ab1 immunoassay lateral flow devices. All of the plants determined to be herbicide tolerant were also positive for CryAb341. In five of the eight generations, no significant deviation from the expected segregation ratios was observed (Table 3).

Significant deviation from the expected segregation ratio occurred in the BC_1, BC_4 and BC_4S_1 generations in only one of two inbreds in each generation. A more consistent pattern of deviations from expected across generations and across inbred would be anticipated if the insert were responsible for these inconsistencies. The Applicant stated that the most likely explanation for the significant difference between the observed and expected segregation ratio in the BC_1 generation is the small sample size. A breeding error that allowed extra susceptible plants in the BC_4 and BC_2S_1 generations might also be an explanation. The deviation in the BC_4 S_1 generation occurred only in one inbred background and was not seen in either inbred in the BC_2S_1 generation.

Since a majority of the generations showed no significant deviations from the expected ratios, and the deviations that occurred were inconsistent across generations and inbreds, it was concluded that the significant differences observed were likely to be due to experimental error and that the insert in corn line DAS-59122-7 is inherited as a Mendelian dominant gene.

A more powerful Chi-square test across all generations with an expected ratio of 1:1 (2644:2750) resulted in no significant difference between expected and observed ratios, as did a test across all generations with an expected segregation ratio or 3:1 (1354:472).

Table 3: Mendelian segregation of corn line DAS-59122-7

Generation	Expected segregation	Inbred	Number resistant	Number susceptible	Chi-Sq significance
T_1S_1	3:1	Hi-II	34	10	NS
F_1	1:1	Inbred B	21	23	NS
	1:1	Inbred C	22	28	NS
BC_1	1:1	Inbred B	57	80	P<0.05
	1:1	Inbred C	66	78	NS
BC_2	1:1	Inbred B	466	466	NS
	1:1	Inbred C	517	471	NS
BC_2S_1	3:1	Inbred B	267	82	NS
	3:1	Inbred C	302	98	NS
BC_3	1:1	Inbred B	431	434	NS
	1:1	Inbred C	415	447	NS
BC_4	1:1	Inbred B	451	483	NS
	1:1	Inbred C	198	240	P<0.05
BC_4S_1	3:1	Inbred B	369	121	NS
	3:1	Inbred C	382	161	P<0.025

Data expressed as the number of plants expected to be resistant to glufosinate ammonium : the number of plants expected to be susceptible.

Conclusion

The studies show that the T-DNA insert is stably integrated into the corn genome in line DAS-59122-7 and segregates as expected over the generations that were examined.

3.5 Antibiotic resistance genes

No antibiotic resistance marker genes are present in corn line DAS-59122-7.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Biochemical function and phenotypic effects

Corn line DAS-59122-7 contains three novel proteins: Cry34Ab1; Cry35Ab1; and PAT.

Study submitted
Narva, K.E., Schnepf, H.E., Nygaard, L.R. and Wolt, J.D. (2003) Cry34/35 Protein Distribution and Familiarity. Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC Indiana. Study ID: GH-C 5702

Cry34Ab1 and Cry35Ab1

These proteins are insectidal δ-endotoxins derived from *B. thuringiensis* strain PS149B1. During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 μm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxin-containing crystals.

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

Corn line DAS-59122-7 contains two separate parasporal crystal proteins, Cry34Ab1 (123 amino acids) and Cry35Ab1 (383 amino acids), with respective molecular weights of 14 kDa and 44 kDa. The transgenes that encode these proteins were optimised for expression in corn plants. The proteins encoded by the synthetic transgenes are identical in sequence to the native *B.t* crystal proteins.

The Cry34Ab1 and Cry35Ab1 proteins do not have a high degree of sequence homology to other Cry proteins currently in commercial transgenic plant products for insect control, however, they are related to commercial microbial products and proteins that are ubiquitous in *B.t.* strains isolated from the environment. Genomic serotyping of total genomic DNA from *B.t.* strain collections identified 78 strains containing sequences related to *cry35Ab1*. Crude fermentation broth extracts taken from a subsample of these strains showed the presence of one or both Cry34/35Ab1 proteins in 37 of 42 samples. Analysis of nucleic acid and deduced polypeptide sequences reveals that Cry34/35Ab1 proteins comprise large families of related insecticidal proteins.

Both proteins are required together for mortality of the corn rootworm larvae. Although the Cry34Ab1 protein is active alone in corn rootworm larvae when applied at high concentrations in bioassays, the Applicant has stated that transgenic plants which expressed only the Cry34Ab1 protein do not control western corn rootworms. The activity of the Cry34Ab1 protein in bioassays is greatly potentiated by Cry35Ab1. The Cry35Ab1 protein alone is not active against corn rootworm. *In vivo*, only a small quantity of Cry35Ab1 is needed in the Cry34/35Ab1 insecticidal crystal protein (ICP). Therefore, the majority of the activity seen with mixtures of Cry34Ab1 and Cry35Ab1 may be explained by the concentration of the Cry34Ab1 protein.

The Cry34/35Ab1 ICP causes disruption of the western corn rootworm larval midgut membranes when ingested as shown by histology. In experiments using artificial membranes, the ICP produces ion channels or pores which is at least partially responsible for the disruption of the synthetic membranes (Masson *et al.*, 2004). The formation of ion channels in artificial membranes has also recently been reported for Cry34Ab1 (Baum *et al.*, 2004). Meaningful *in vivo* activity with the ICP has only been observed in a subset of coleopteran larvae (corn rootworm). *In vivo* activity has not been found in adult corn rootworms, a corn aphid species or certain lepidopteran pests, indicating selective activity for corn rootworm larvae. Cry34Ab1 and Cry35Ab1 have not been observed to associate to form a hetero-dimer.

PAT

The herbicide tolerant trait, which was used as a selectable marker following transformation, is conferred by the expression of the introduced *pat* gene, which encodes the phosphinothricin acetyltransferase (PAT) protein.

The PAT protein consists of 183 amino acids, has a molecular weight of 22 kDa, and exhibits a high degree of enzyme specificity; recognising only one substrate. PAT functions by detoxifying phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides. PPT acts by inhibiting the endogenous enzyme glutamine synthetase, an enzyme involved in amino acid biosynthesis in plant cells. By inhibiting this enzyme, PPT causes rapid accumulation of ammonia in the plant cell, leading to plant death. In transformed corn plants, the introduced PAT enzyme chemically inactivates the PPT by acetylation of the free ammonia group, giving rise to herbicide tolerance in the whole plant.

4.2 Protein expression analysis

In corn line DAS-59122-7, it is expected that three novel proteins will be expressed. These are the Cry34Ab1, Cry35Ab1 and PAT proteins. Expression levels of these proteins were determined using enzyme-linked immunosorbent assay (ELISA) and are reported below.

Study submitted:
Essner, R (2003) Agronomic Characteristics, Quantitative ELISA and Nutrient Composition Analysis of Hybrid Maize Lines Containing the *cry34Ab1, cry35Ab1*, and *pat* Genes: Chile Locations. Pioneer Hi-Bred International Inc. Study ID PHI-2002-050

Field trials of corn line DAS-59122-7 and control lines were conducted in Chile in 2002-2003. Six separate field locations each contained four blocks. Each block contained the corn line DAS-59122-7 hybrid[3] and a near isoline inbred control. Block 1 also contained the corn line DAS-59122-7 inbred[4]. Plots of the GM hybrids were either left untreated or received two sequential applications of a herbicide containing glufosinate ammonium as the active ingredient. Leaf, root, whole plant, pollen, stalk, forage, and grain samples were collected from the GM hybrid, GM inbred, and control lines and Cry34Ab1, Cry35Ab1 and PAT concentrations were measured using an ELISA.

No Cry34Ab1, Cry35Ab1 or PAT protein was detected in the control corn line. All matrices from DAS-59122-7 were found to express the Cry34Ab1 and Cry35Ab1 proteins at measurable levels. However, the PAT protein was undetectable in both the pollen and the grain of corn line DAS-59122-7. No PAT was detected in the forage samples from either of the hybrid DAS-59122-7 lines. The samples for the inbred DAS-59122-7 forage was pooled with other samples and not available for testing. All other matrices expressed PAT at detectable levels.

Expression levels of the three novel proteins in the corn grain are shown in Tables 5, 6, and 7. Mean expression levels of Cry34Ab1 in all matrices ranged from 29.2 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 stalk) to 232 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 leaf). Mean expression levels of Cry35Ab1 in all matrices ranged from 0.01 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 pollen) to 85.3 ng/mg tissue dry weight (in non-sprayed hybrid DAS-59122-7 leaf). Mean expression levels of PAT ranged from below the limit of quantitation in pollen, forage and grain to 11.2 ng/mg tissue dry weight (in non-sprayed hybrid DAS-59122-7 leaf). These are shown in Table 4.

[3] The hybrid DAS-59122-7 line consisted of backcross 1 (BC_1) generation seed, produced from crossing the DAS-59122-7 T_0 plants twice with a recurrent inbred line and then with a different inbred line.
[4] The inbred DAS-59122-7 line consisted of BC_1 generation seed, produced from backcrossing the 59122-7 T_0 plants twice with a recurrent inbred

Table 4: Maximum and minimum mean expression levels of novel proteins in DAS-59122-7 corn

	Minimum Mean*	Maximum Mean*
Cry34Ab1	29.2 (sprayed hybrid stalk)	232 (sprayed hybrid leaf)
Cry35Ab1	0.01 ng/mg (sprayed hybrid pollen)	85.3 (non-sprayed hybrid leaf)
PAT	<LOQ (pollen, forage and grain)	11.2 (non-sprayed hybrid).

*ng/mg tissue dry weight

Table 5: Summary of expression levels of Cry34Ab1 protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	49.7	16.2	28.9-84.8	30/0
GM hybrid sprayed	61.1	19.4	30.9-117	30/0
GM inbred	51.7	11.5	38.6-78.2	15/0

[1]The limit of quantitation (LOQ) for Cry34Ab1 for grain was 0.072 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Table 6: Summary of expression levels of Cry35Ab1 protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	0.99	0.33	0.48-1.58	30/0
GM hybrid sprayed	0.92	0.30	0.50-1.61	30/0
GM inbred	1.10	0.54	0-1.83	15/2

[1]The limit of quantitation (LOQ) for Cry35Ab1 for grain was 0.072 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Table 7: Summary of expression levels of PAT protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	0	0	0-0	30/30
GM hybrid sprayed	0	0	0-0	30/30
GM inbred	0	0	0-0	15/15

[1]The limit of quantitation (LOQ) for PAT for grain was 0.06 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Potential dietary exposure to novel proteins

The highest level of expression of Cry34Ab1 and Cry35Ab1 in the grain of DAS-59122-7 corn based on the expression data above was 117 ng/mg and 1.83 ng/mg dry weight respectively. The actual exposure to these two proteins in the diet is expected to be lower than this due to a number of factors including:

- protein degradation during the transport and storage of grain,
- grain containing these novel proteins is likely to be mixed with other non-GM and GM corn grain and thus dilute the novel proteins, and
- reductions in the protein concentrations during processing to produce high fructose corn syrup and vegetable oils (which contain negligible levels of protein).

Even at the highest levels of novel protein expression, and without accounting for the above factors, which are expected to lower the dietary exposure, the levels are extremely low i.e. 12 mg Cry34Ab1/100g corn and 0.2 mg Cry35Ab1/100g corn.

4.3 Potential toxicity of novel proteins

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

Studies submitted:
Schafer, BW, Collins, RA, Schwedler DA, and Xu X (2003) Characterisation of Cry34Ab1 and Cry35Ab1 Proteins derived from transgenic maize event E4497.59.1.22 (DAS-59122-7). Dow AgroSciences LLC, Indianapolis Indiana. Study ID: 030033

Korjagin, V.A. (2000) Characterisation of *Pseudomonas* produced and transgenic maize expressed phosphinothricin acetyltransferase (PAT) protein. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC, Indiana. Study ID: 000369.

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 14 kDa Protein: Acute Oral Toxicity Study in Cd-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001130

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 44 kDa Protein: Acute Oral Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001129

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 14 kDa and 44 kDa Proteins: Acute Oral Toxicity Study in CD-1 mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001128

Brooks, K.J. (2000) PAT Microbial Protein (FL): Acute Oral Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID:991249

The Applicant submitted three acute oral toxicity studies in mice to support the safety of the Cry34Ab1 and Cry35Ab1 proteins: Cry34Ab1 only; Cry35Ab1 only; and a mixture of both Cry34Ab1 and Cry35Ab1.

As it is very difficult to extract and purify sufficient quantities of the subject protein from transgenic corn plants for the acute oral toxicity studies, it has become standard practice to instead use equivalent proteins that have been produced using bacterial expression systems. Prior to use, the bacterially produced proteins are compared to the proteins produced *in planta* in order to establish their equivalence. Cry34Ab1 and Cry35Ab1 proteins were produced in recombinant *Pseudomonas fluorescens*.

The molecular identity and biochemical characteristics of the proteins expressed *in planta* and in the bacterial-expression systems were examined using various biochemical methods such as N-terminal sequencing, molecular weight determination, immunoreactivity, glycosylation analysis, peptide mass fingerprinting and matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry. These studies established that bacterially produced Cry proteins were equivalent to those proteins produced in corn line DAS-59122-7, thus the bacterial proteins were used in the toxicity testing.

Potential toxicity of Cry34Ab1 and Cry35Ab1 individually

The acute oral toxicity of the two Cry proteins, both individually and combined was studied using an acute oral toxicity study in mice. The Cry proteins were produced in *Pseudomonas fluorescens*.

Test material	PS149B1 14 kDa protein (54% Cry34Ab1) or PS149B1 44 kDa protein (37% Cry35Ab1)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male CD-1 mice for each of two test materials
Dose	5000 mg/kg body weight (2700 mg Cry34Ab1/kg body weight, or 1850 mg Cry35Ab1/kg body weight) in one gavage dose of 25 mL/kg
Control	No control was performed

The mice received a single dose of either 2700 mg/kg bw Cry34Ab1 or 1850 mg/kg bw Cry35Ab1 and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. No clinical signs were observed during the study. Three mice given Cry34Ab1 and two mice given Cry35Ab1 lost weight between days 1 and 2 but gained weight for the rest of the study period. One mouse given Cry35Ab1 had fluctuating body weight throughout the study. This was thought to be due to gavage with a maximum volume of methylcellulose. The remaining mice gained weight throughout the study. There were no treatment related gross pathological observations.

Therefore, under the conditions of this study, the acute oral LD_{50} of Cry34Ab1 in male mice is greater than 2700 mg/kg bw and of Cry35Ab1 is greater than 1850 mg/kg bw.

Potential toxicity of Cry34Ab1 and Cry35Ab1 combined

As Cry34Ab1 and Cry35Ab1 are present together in corn line DAS-59122-7 and are required to be expressed together to be effective in combating corn rootworm, the Applicant performed an acute oral toxicity study in mice using a combination of the two novel proteins.

Test material	a mixture of PS149B1 14 kDa protein and 44 kDa protein (at a 1:3 ratio of Cry34Ab1 to Cry35Ab1 to provide an equimolar mixture of the two proteins)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male and 5 female CD-1 mice
Dose	5000 mg/kg body weight (482 mg Cry34Ab1/kg bw and 1520 mg Cry35Ab1/kg bw) in one gavage dose of 25 mL/kg
Control	No control was performed

The mice received a single dose of 482 mg/kg bw Cry34Ab1 and 1520 mg/kg bw Cry35Ab1 and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. One female mouse had protruding or enlarged eyes on test days 6 and 7, however this was not considered to be treatment related. No other clinical signs were observed during the study. Two male mice lost weight between days 1 and 2 but gained weight over the rest of the study period. The remaining mice gained weight throughout the study. There were no treatment related gross pathological observations.

Therefore, under the conditions of this study, the acute oral LD_{50} of a 1:3 mixture of Cry34Ab1 and Cry35Ab1 in CD-1 mice is greater than 2000 mg /kg bw (482 mg Cry34Ab1 /kg bw and 1520 mg Cry35Ab1 /kg bw).

Potential toxicity of PAT

Extensive animal testing has shown that the PAT protein is non-toxic to humans and animals. The same gene has been expressed in other transgenic crops assessed by FSANZ (applications A372, A375, A386, A481, and A518) and is considered to pose no risks to human health and safety.

However, the Applicant submitted an acute oral toxicity study of the PAT protein in 5 male and 5 female CD-1 mice.

Test material	PAT protein produced in *Pseudomonas florescenes* (84% pure)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male and 5 female CD-1 mice
Dose	6000 mg/kg body weight (5000 mg/kg PAT) in two gavage doses of one hour apart
Control	No control was performed

Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. One female mouse had increased pupil size on test days –1 to 6, however this was not considered to be treatment related. No other clinical signs were observed during the study.

All mice had a decrease in body weight between days 1 and 2. This was minor, transient and typical of high volume gavage doses, and not attributed to the test material. All mice except one female gained weight over the rest of the study. One female lost 0.5 gm over the duration of the study. There were no gross pathological lesions on any animal in the study.

Therefore, under the conditions of this study, the acute oral LD_{50} of the PAT protein in CD-1 mice is greater than 5000 mg /kg bw.

Potential toxicity of glufosinate ammonium metabolites

Glufosinate ammonium herbicide contains both the L-isomer and the D-isomer of glufosinate. Unlike the L-isomer, the D-isomer does not competitively inhibit the glutamine synthase enzyme in plants and is not herbicidally active. In plants expressing the *pat* gene, the herbicidally active component of glufosinate ammonium, the L-isomer, is rapidly metabolized by the action of the enzyme phosphinothricin acetyltransferase (PAT) into the non-phytotoxic stable metabolite N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinico-butanoic acid) (NAG). This metabolite does not inhibit glutamine synthetase, therefore the plants will survive applications of this herbicide (OECD, 2002a).

The toxicity of NAG and a second metabolite of glufosinate ammonium produced by both non-tolerant and tolerant plants, 3-[hydroxy(methyl) phosphinoyl]propionic acid was compared with that of glufosinate ammonium by the Joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR) in 1999. JMPR concluded that the toxicity of the metabolites was comparable to or less than that of the parent compound. An Acceptable Daily Intake (ADI) was established for this group of 0-0.02 mg/kg bw for glufosinate ammonium, NAG and 3-[hydroxy(methyl) phosphinoyl]propionic acid (alone or in combination). Due to the low acute toxicity of glufosinate-ammonium and its metabolites, it was considered unnecessary to establish an acute reference dose (JMPR, 1999).

Similarities with known protein toxins

Studies Submitted:
Cressman, R.F. (2003) Evaluation of the Sequence Similarities of the Cry34Ab1, Cry35Ab1, and PAT Proteins to the Public Protein Sequence Datasets. Pioneer Hi-Bred International Inc. Study ID: PHI-2003-046

Bioinformatic analyses were done to assess the Cry34Ab1, Cry35Ab1 and PAT proteins for any similarity with known protein toxins. The similarity search was conducted against the GenPept dataset using the BLASTP 2.2.6 algorithm with a cut-off expectation (E) value of 1.0.

Cry34Ab1

The Cry34Ab1 similarity search identified ten proteins. Five of these represent closely related or identical Cry proteins from *B. thuringiensis*. The other five represent putative microbial collagenases and hypothetical proteins from several genome sequencing projects. None of the similar proteins were identified as toxins or potential toxins.

Cry35Ab1

The results of the Cry35Ab1 protein search returned 22 protein accessions with E-values of less than 1. Seven of these were highly similar or identical Cry proteins from *B. thuringiensis*. Eleven were from a related species, *B. sphaericus*. Four represent conceptual or hypothetical proteins from genome sequencing projects. None were identified as toxins or potential toxins.

PAT

Searching the dataset with the PAT protein revealed 148 accessions, 18 of which represent accessions for PAT or other acetyltransferases. The remaining 130 proteins are unidentified proteins and / or hypothetical proteins translated from genome sequencing data. Again, none of the similar proteins returned by the search were identified as toxins or potential toxins.

Conclusion

The data from acute oral toxicity studies and bioinformatic analyses of the novel proteins indicate that none of the three proteins are toxic at high levels in mice, nor do they show any similarity with known protein toxins.

4.4 Potential allergenicity of novel proteins

A possible concern is that new proteins introduced into food will cause allergic reactions in some individuals. The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid similarity between the novel protein and that of known allergens, and the structural properties of the novel protein, including susceptibility to degradation in simulated digestion models. Applying such criteria systematically provides reasonable evidence about the potential of the newly introduced proteins to act as an allergen (Jones and Maryanski, 1991; Lehrer and Reese, 1998).

The two Cry proteins expressed in corn line DAS-59122-7 were assessed using these criteria for their potential allergenicity.

Similarity to known allergens

Studies submitted:
Song, P. (2003) Comparison of the Amino Acid Sequence of *Bacillus thuringiensis* Strain PS149B1 Cry34Ab1 and Cry35Ab1 Insecticidal Crystal Proteins as Expressed in Maize to Known Protein Allergens. Dow AgroSciences LLC, Indiana. Study ID: GH-C 5671

Stelman, S.J. (2000) Comparison of the Amino Acid Sequence of *Bacillus thuringiensis* Strain PS149B1 13.6 kDa and 43.8 kDa Insecticidal Crystal Proteins to Known Protein Allergens. Dow AgroSciences LLC, San Diego, California. Study ID: GH-C 5140

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

Sequence evaluation guidelines based on those formulated by Gendel (1998), by the Joint FAO/WHO Expert Consultation (2001) and by the Codex Alimentarius Commission (2001) were followed (Gendel, 1998; Joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology, 2001; Codex, 2001; Joint FAO/WHO expert consultation, 2001). An immunologically significant sequence identity requires a match of at least eight contiguous identical amino acids, or 35% identity over eighty amino acid residues. No such sequence identity was detected for either the Cry34Ab1 or Cry35Ab1 sequences. Therefore, based on homology of the amino acid sequences with known protein allergens, the Cry34Ab1 and Cry35Ab1 sequences are not predicted to have allergenic potential.

In vitro digestibility

Studies submitted
Korjagin,V.A. and Ernest, A.D. (2000) *In vitro* Digestibility of PS149B1 Proteins. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC, Indiana. Study ID: 000302

Herman, R.A., Schafer, B.W., Korjagin, V.A. and Ernest, A.D. (2003) Rapid Digestion of Cry34Ab1 and Cry35Ab1 in Simulated Gastric Fluid. *J. Agric. Food Chem*. 51:6823-6827.

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Astwood and Fuchs, 1996; Metcalfe *et al.,* 1996; Kimber *et al*., 1999). The Cry1Ac and Cry1F proteins were therefore investigated for their digestibility in simulated digestion models.

The Applicant submitted two studies showing the *in vitro* digestibility of the two Cry proteins. In the first study (Korjagin and Ernest, 2000), Cry34Ab1 was degraded in simulated gastric fluid (SGF) after 30 minutes incubation at 37ºC, as determined by Western blotting. Cry35Ab1 was degraded more quickly, with no visible hybridisation after just 5 minutes at 37ºC.

In the second study (Herman *et al*., 2003) a more quantitative approach was taken. More than 97% of the Cry35Ab1 was found to be degraded after 5 minutes incubation with SGF at 37ºC, based on a limit of detection of the SDS-PAGE of <15.6 ng/lane. In two experiments, the estimated half-life of Cry34Ab1 in SGF at 30ºC was 1.9 and 2.0 minutes. The time taken for 90% of the sample to be degraded under the same conditions was 6.3 and 6.8 minutes based on SDS-PAGE analysis. This is comparable to other *Bt* proteins that have been used in GM plants (Herman *et al*., 2003).

Thermolability

Studies submitted:
Herman, R.A. (2000) Thermolability of PS149B1 Binary Delta-Endotoxin. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC Indiana. Study ID: 001041

Herman, R.A. (2002) Heat Lability of Individual Proteins of the PS149B1 Binary ICP. Global Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC Indiana. Study ID: 010144

The Applicant submitted two studies assessing the thermolability of the Cry34Ab1 and Cry35Ab1 proteins.

A mixture of both Cry proteins (at a ratio of 1:1) were incubated for 30 minutes at 4ºC (control), 60ºC, 75ºC and 90ºC. These samples were then fed to Southern corn rootworm (SCR) neonate larvae as part of their standard feed. The variable measured was growth inhibition of SCR larvae. This is a qualitative assessment of heat lability as the rate of denaturation is not directly obtainable since a reduction in biological activity cannot be directly linked to protein concentration. Also, other factors, such as the properties of the buffer and concentration of the heated samples will affect the rate of denaturation. Therefore these studies can only give a qualitative statement of "heat stable" or "heat labile".

After 6 days on the treated diet, the weight of the larvae was measured and the growth inhibition was calculated based on comparison with negative controls. The results of this study indicated that the protein mixture was deactivated after exposure to 60ºC, 75ºC and 90ºC for 30 minutes.

Treatment		% Growth Inhibition
Cry34/35Ab1	4ºC	70
Cry34/35Ab1	60ºC	-3
Cry34/35Ab1	75ºC	-3
Cry34/35Ab1	90ºC	1

A second study was conducted to determine the heat lability of the individual component proteins by fortifying heated samples of the two proteins with non-heated samples of the individual proteins. This allowed the heat lability of the complementary protein to be measured since both proteins are required for maximum activity against corn rootworm. This study showed that the Cry35Ab1 protein is heat labile at 60ºC, 75ºC, and 90ºC. The Cry34Ab1 protein was also found to be heat labile at 90ºC, however, some Cry34Ab1 activity was observed at 60ºC and 75ºC.

4.5 Conclusion regarding characterisation of the novel proteins

Corn line DAS-59122-7 expresses three novel proteins – Cry34Ab1, Cry35Ab1, expressed at low levels in the corn grain, and PAT which is undetectable in the corn grain.

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

5. COMPARATIVE ANALYSES

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

A comparative approach focussing on the determination of similarities and differences between the GM food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods (WHO, 2000). The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO, 1996).

The key nutrients and toxicants/anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased). The key components of corn that should be considered in the comparison include protein, fat, carbohydrates, amino acids, fatty acids, vitamins, minerals, and phytic acid (OECD, 2002b).

5.1 Nutrient analysis

Study submitted
Essner R. (2003) Agronomic characteristics, quantitative ELISA and nutrient composition analysis of hybrid maize lines containing Cry34Ab1, Cry35Ab1 and PAT genes: Chile locations. Pioneer Hi-bred International, Inc. Study ID: PHI-2002-050

To determine whether unexpected changes had occurred in the nutrient composition of corn line DAS-59122-7 as a result of the genetic modification, and to assess the nutritional adequacy of this line, compositional analysis was done on whole corn grain from corn line DAS-59122-7 and from its non-transgenic counterpart. The non-transgenic counterpart used as a control was a near isoline corn, which has the same genetic background as corn line DAS-59122-7 without the insert.

Corn line DAS-59122-7 and the control corn line were grown at 6 different locations in 2002-2003. Plots of the transgenic corn were either left untreated or received two sequential applications of a herbicide containing the active ingredient glufosinate ammonium. Five grain samples (single ears of corn) were collected from each treatment group at each location. One sample was collected from the control group at each location.

A total of 51 components were analysed - these were proximate content (moisture, fat, protein, fibre, ash and carbohydrate), amino acids, fatty acids, minerals, vitamins, secondary metabolites, and antinutrients.

The results were compared within and across sites. Comparisons across all locations are shown in Tables 8-13 and discussed below to evaluate the overall equivalence of DAS-59122-7 corn grain with conventional corn. The results from individual trial sites were also evaluated but are not presented in this report.

Of the 102 comparisons across sites, 34 comparisons were found to be significantly different at the 5% level. Every single one of these differences, however, was within the literature range and represented only a small difference compared to the control value. Furthermore, there was no pattern of change within sites that might indicate that further investigation is necessary.

Beta-carotene levels in the GM corn grain (sprayed and unsprayed) were higher than reported averages, but were comparable to the control mean. This may be due to other xanthophylls or carotenoid pigments inadvertently being measured as beta-carotene. Levels of vitamin B2 were below the limit of quantitation for the assay used for this analysis and were not detected.

These minor differences are unlikely to be biologically meaningful, and the grain and forage from DAS-59122-7 corn can be considered to be compositionally equivalent to that of non-GM corn.

5.2 Conclusion

The comparative analyses do not indicate that there are any compositional differences of biological significance in corn grain from transgenic corn line DAS-59122-7, compared to the non-GM control. Several minor differences in key nutrients and other constituents were noted, however, the levels observed were generally within the range of natural variation for commercial corn lines and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it can be concluded that DAS-59122-7 corn grain is equivalent in composition to non-GM corn grain.

Table 8: Summary of proximate and fibre analysis in DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Crude protein	6-16.1	10.0*	10.3*	9.61
Crude fat	1.2-18.8	4.69	4.62	4.49
Crude fibre	1.6-5.5	2.3	2.2	2.3
ADF[4]	1.82-11.3	3.5	3.6	3.5
NDF[5]	3.0-22.6	10.8	11.2*	10.3
Ash	0.62-6.28	1.55*	1.6*	1.42
Carbohydrate[6]	63.3-89.8	83.8	83.5*	84.5

[1]Percent dry weight
[2]Watson, 1982 and 1987; Jugenheimer, 1976; OECD, 2002; ILSI, 2003; Essner, 2003
[3]Least square means
[4]Acid detergent fibre
[5]Neutral detergent fibre
[6]Carbohydrates are calculated using the following formula = 100% - % protein - % fat - % ash
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 9: Summary of mineral analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Calcium	0.002-0.1	0.00278*	0.00286*	0.00227
Phosphorus	0.21-0.75	0.299	0.308*	0.266
Copper	0.000085-0.001	0.000112	0.000104	0.000118
Iron	0.0001-0.01	0.00199	0.00225	0.00194
Magnesium	0.08-1.0	0.117	0.123	0.108
Manganese	0.00007-0.0054	0.000648	0.000686*	0.000577
Potassium	0.28-0.72	0.352	0.362	0.332
Sodium	0.0-0.15	0.000437	0.000367	0.000378
Zinc	0.00065-0.0037	0.00183	0.00179	0.00163

[1]Percent dry weight
[2]Watson, 1982 and 1987; OECD, 2002; ILSI, 2003.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 10: Summary of fatty acid analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Palmitic acid	6.51-19	11.5*	11.7	12.1
Stearic acid	0-4.17	1.39*	1.40*	1.57
Oleic acid	18.6-46	22.8	23.1	23.3
Linoleic acid	34-70	63.0*	62.4	61.7
Linolenic acid	0-2.0	1.14	1.15*	1.07

[1]Percent total fatty acids
[2]Watson, 1982; Iowa Gold Catalog, 1997; Essner, 2003; ILSI, 2003.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 11: Summary of amino acid analysis in DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Methionine	0.1-0.46	0.20	0.19	0.19
Cysteine	0.08-0.32	0.23	0.22	0.22
Lysine	0.05-0.55	0.28	0.29	0.28
Tryptophan	0.04-0.13	0.06*	0.06	0.06
Threonine	0.21-0.58	0.38	0.41*	0.37
Isoleucine	0.19-0.71	0.34*	0.35*	0.33
Histidine	0.15-0.40	0.26*	0.28*	0.25
Valine	0.21-0.85	0.46*	0.48*	0.45
Leucine	0.43-2.41	1.33*	1.38*	1.28
Arginine	0.22-0.64	0.29*	0.30*	0.28
Phenylalanine	0.04-0.83	0.56*	0.59*	0.54
Glycine	0.24-0.50	0.35	0.36*	0.33
Alanine	0.37-1.20	0.82	0.83*	0.80
Aspartic acid	0.37-0.95	0.69	0.70*	0.66
Glutamic acid	0.89-3.04	2.03	2.08*	1.97
Proline	0.43-1.46	0.96*	0.98*	0.91
Serine	0.24-0.91	0.51	0.54*	0.50
Tyrosine	0.11-0.79	0.24*	0.26*	0.21

[1]Percent dry weight
[2]Watson, 1982; Iowa Gold Catalog, 1994, 1997; OECD, 2002; Essner, 2003; ILSI, 2003; Pioneer Commercial Hybrids.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 12: Summary of vitamin analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Beta-carotene	1.0, 2.5[4]	7.62	7.74	6.87
Vitamin B1	1.0-8.6	5.45	5.93	5.77
Vitamin B2	0.25-16.5	ND	ND	ND
Folic acid	0.147 – 1.209[5]	0.593*	0.603	0.634
Vitamin E[6]	1.5-6.87	6.59*	6.60*	5.65

[1]parts per million on a dry weight basis
[2]Watson, 1982, 1987; OECD 2002; ILSI version 1 2003.
[3]Least square means
[4]ILSI version 1 – 1ppm, OECD – 2.5 ppm average
[5] ILSI version 2 2004.
[6]Measured as α-tocopherol
ND – not detected
*Statistically significant difference between DAS-59122-7 grain and control grain (P<0.05).

Table 13: Summary of secondary metabolites and anti-nutrients of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Secondary metabolites				
Inositol	NR	0.022	0.022	0.021
Raffinose	0.08-0.31	0.13	0.13	0.12
Furfural	NR	ND	ND	ND
P-Coumaric acid	0.003-0.058	0.014	0.014	0.015
Ferulic acid	0.02-0.37	0.177	0.176	0.182
Antinutrients				
Phytic acid	0.29-1.29	0.877	0.798	0.798
Trypsin inhibitor (TIU/g)	1.1-7.18	2.82	2.84	2.84

[1]Percent dry weight
[2]Watson, 1982; OECD, 2002; ILSI, 2003.
[3]Least square means
NR – Not reported
ND – Not detected
*Statistically significant difference between DAS-59122-7 grain and control grain (P<0.05).

6. NUTRITIONAL IMPACT

Study submitted
Smith, B. (2003) Nutritional Equivalency Study of Maize Containing Cry34Ab1 and Cry35Ab1: Poultry Feeding Study. Solution BioSciences Inc. Study ID: 2001-OPT-48-BB

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

To date, all approved GM plants with modified agronomic production traits have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies with feeds derived from the approved GM plants have shown equivalent animal performance to that observed with the non-GM feed. Thus the evidence to date is that for GM varieties shown to be compositionally equivalent to conventional varieties, feeding studies with target livestock species will add little to a safety assessment and generally are not warranted.

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

In the case of corn line DAS-59122-7, the extent of the compositional and other available data is considered to be adequate to establish the nutritional adequacy of the food. However, the Applicant supplied the results of a 42-day feeding study of a similar GM corn (containing the *cry34Ab1*, *cry35Ab1*, and *pat* genes) in commercial broiler chickens. No adverse effects were observed in this study.

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Attachment 3

Summary of Public Submissions

Submitter	Preferred Option	Comments
1. New Zealand Food Safety Authority	-	Raises the issue of herbicide residues, including the de-activated by-products, on the food.
2. GE Free New Zealand & Environment	1	Objects to all GMOs for a number of reasons, none specific to this application . Urges FSANZ to adopt the precautionary principal in relation to GM food.
3. Australian Food and Grocery Council	2	-
4. Department of Human Services, Victoria	2	-
5. Food Technology Association Victoria	2	-
6. Department of Health, South Australia	-	Comments on the added cost to government for each new GM approval as reference laboratories need to purchase marker genes for the new product and test accordingly. Queries whether the possible benefit to growers listed under Option 2 in the Initial Assessment Report of 'lower production costs and reduced exposure to agricultural chemicals' is relevant as this food will not be grown in Australia or New Zealand. This has been changed in the Draft Assessment Report.