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

06/03
18 December 2002

DRAFT ASSESSMENT REPORT

APPLICATION A446

FOOD DERIVED FROM INSECT-PROTECTED AND GLUFOSINATE-AMMONIUM TOLERANT CORN LINE 1507

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:
29 January 2003
(See "Invitation for Public Submissions" for details)

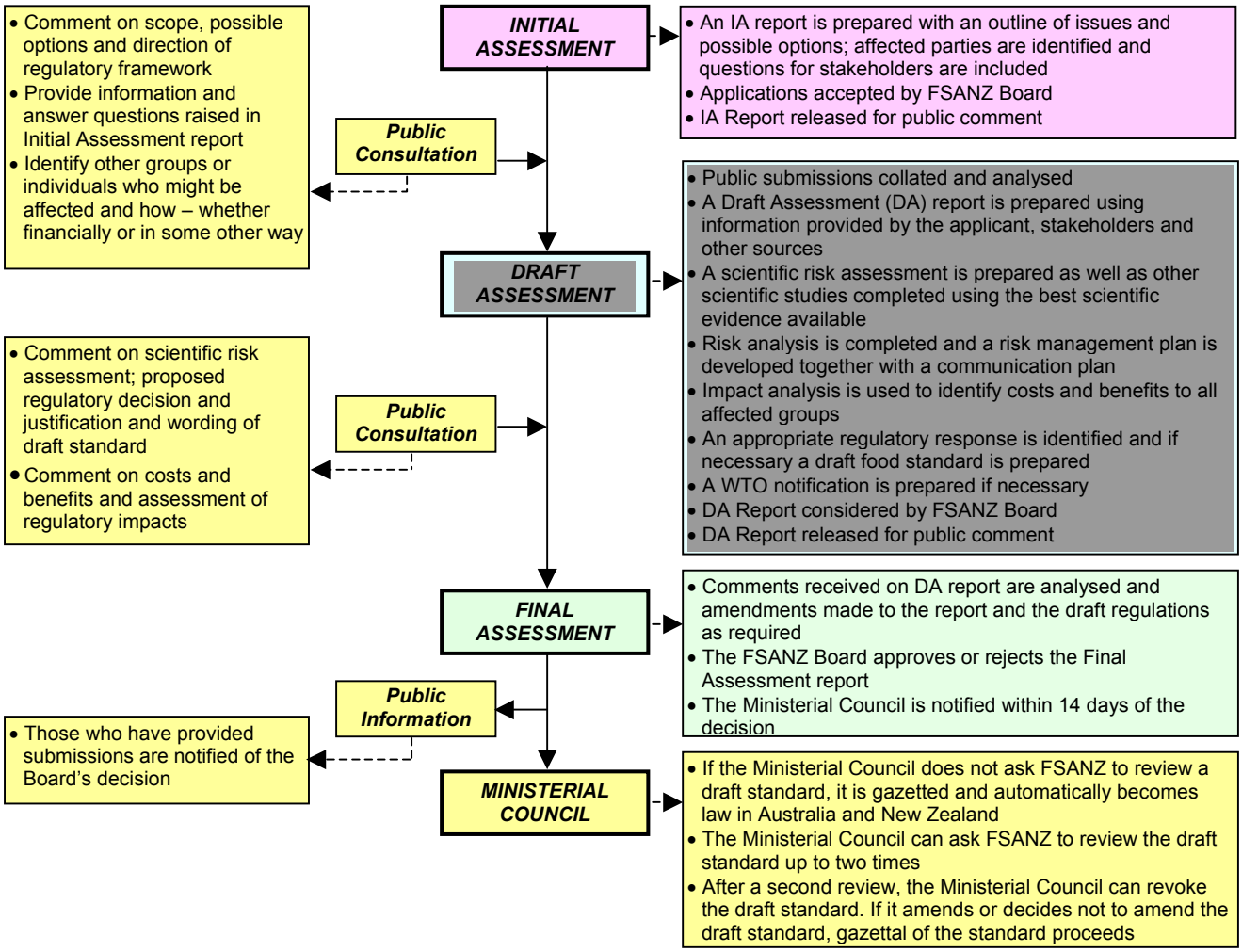
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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 Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

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

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

The process for amending the *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

The Authority has prepared a Draft Assessment Report of Application A446, and prepared a draft variation to Volume 2 of the *Food Standards Code.*

The Authority invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to Volume 2 of the *Food Standards Code* for the purpose of preparing an amendment to the *Food Standards Code* for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist the Authority in preparing the Final Assessment for this Application. Submissions should, where possible, address the objectives of the Authority as set out in section 10 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Information providing details of potential costs and benefits of the proposed change to the *Food Standards Code* (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 the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires the Authority to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

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

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

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

Submissions should be received by the Authority **by 29 January 2003**. Submissions received after this date may not be considered unless the Project Manager has given prior agreement for an extension. Submissions may also be sent 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 Liaison Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website or alternatively paper copies of reports can be requested from the Authority's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au including other general enquiries and requests for information.

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Executive Summary and Statement of Reasons

FSANZ began assessment of a new genetically modified corn on 1 August 2001, on receipt of an Application from Dow AgroSciences Australia Pty Limited. The Application seeks approval for food derived from insect-protected and glufosinate-ammonium tolerant corn line 1507 under Standard 1.5.2 – Food Produced Using Gene Technology. A mandatory pre-market safety assessment is required under this Standard.

The new genetic traits confer (a) protection against certain insect pests and (b) tolerance to glufosinate-ammonium herbicide. Corn line 1507 has been developed primarily for cultivation in the Northern Hemisphere, but food derived from this line could enter the market in Australia and New Zealand via imported products, once the line is grown on a commercial scale.

Safety assessment

FSANZ has completed a comprehensive safety assessment of corn line 1507 as required under the standard. Corn line 1507 contains two new genes, *cry1F* and *pat*, each derived from soil bacteria. The *cry1F* gene encodes an insecticidal protein that, like other *Bt* proteins, is highly selective in controlling Lepidopteran insects. The *pat* gene encodes an enzyme that inactivates the herbicide, allowing the plant to grow in the presence of the herbicide. The herbicide tolerance trait was also used to identify appropriate plants during development and therefore antibiotic resistance marker genes were not required in this case.

Food derived from corn line 1507 has been evaluated according to the safety assessment guidelines prepared by FSANZ. The assessment considered the following aspects of the food: (1) the nature of the genetic modification; (2) general safety issues such as history of use and the potential for transfer of antibiotic resistance genes to microorganisms in the human digestive tract; (3) characterisation of novel proteins including toxicological and allergenicity issues; and (4) comparative analyses and nutritional impact of the food. On the basis of an assessment of the available information, it is concluded that food from corn line 1507 is as safe and wholesome as food produced from other commercial corn varieties

Under the revised labelling requirements of Standard 1.5.2 which came into effect on 7 December 2001, certain food fractions derived from corn line 1507 will require labelling where novel DNA and/or protein is present in the final food. Highly processed products such as corn oil or maize starch are not expected to contain protein or DNA and therefore are unlikely to require a label. No additional labelling subject to clause 7 is required.

Impact of regulatory options

Two regulatory options were considered: either (1) no approval, or (2) approval of corn line 1507 based on the conclusions of the safety assessment. The regulatory decision was considered to have a potential impact on consumers, government and various sectors of the food industry. Following an assessment of the potential impact of each of the options on the major parties, the conclusion was that the potential benefits to industry and consumers in approving corn line 1507, outweighed the costs to all parties. Option 2, to approve corn line 1507, was therefore identified as the preferred option.

Consultation

In response to the invitation to comment on the Initial Assessment Report, 38 submissions were received from the public. The majority of these were opposed to the application primarily because of concerns about the safety of the food and the potential impact of the GM crop on the environment. Several submissions expressed the concern that GM crops in general could potentially lead to a rise in the use of herbicides. There was also criticism of the labelling regime for GM foods, which does not require labelling on some highly processed foods.

The food safety concerns raised in submissions have been addressed in this Draft Assessment Report. Where appropriate, reference to other government agencies has been provided in relation to issues beyond the legal responsibilities of FSANZ.

Conclusions / Statement of Reasons

In relation to food derived from corn line 1507, FSANZ recommends the adoption of the draft variation for the following reasons:

- based on the available information, there are no public health and safety concerns associated with the genetic modification used to produce insect-protected and glufosinate-ammonium tolerant corn line 1507;

- food derived from corn line 1507 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from corn line 1507 will be required if novel DNA and/or protein is present in the final food;

- the benefits of permitting food derived from corn line 1507 in Australia and New Zealand primarily accrue to the food industry and to consumers, and are considered to outweigh the costs to government, consumers and industry, since the safety assessment has not identified any public health and safety concerns; and

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

The commencement date of the draft variation will be the date of gazettal.

1. Introduction

1.1 Nature of Application

An Application was received from Dow AgroSciences Australia Pty Ltd on 1 August 2001 seeking approval for food derived from insect-protected and glufosinate-ammonium tolerant corn line 1507 under Standard 1.5.2 – Food Produced Using Gene Technology in the *Australia New Zealand Food Standards Code*. Corn line 1507 has been genetically modified (GM) by incorporation of two bacterial genes:

- the *cry1F* gene (derived from the soil bacterium *Bacillus thuringensis* var. *aizawai* strain PS811) which expresses an insect-specific protein toxin, Cry1F, and

- the *pat* gene (derived from the soil bacterium *Streptomyces viridochromogenes*), which expresses the enzyme phosphinothricin acetyltransferase (PAT), conferring tolerance to glufosinate-ammonium herbicides.

1.2 Transitional Requirements

This Application reached Preliminary (Initial) Assessment under the operation of the *Australia New Zealand Food Authority Act 1991* (ANZFA Act), and will be finalised in accordance with the provisions of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Under the ANZFA to FSANZ transitional arrangements, this application is regarded as having reached Initial Assessment under the FSANZ Act.

2. Regulatory Problem

Standard 1.5.2 requires that a GM food undergo a pre-market risk assessment through the submission of an application to FSANZ, before it can be sold in Australia and New Zealand. Foods that have been assessed under the Standard, when fully approved, are listed in the Table to clause 2 of the Standard.

Dow AgroSciences Australia Ltd has developed a new GM variety of insect-protected and herbicide-tolerant corn, known as corn line 1507, primarily for agronomic purposes. Before food derived from this line can enter the food supply in Australia and New Zealand, it must first be assessed for safety and an amendment to the *Food Standards 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 *Food Standards Code* may only be gazetted, once the Ministerial Council process has been finalised.

Dow AgroSciences Australia Ltd therefore applied to have Standard 1.5.2 amended to include food derived from insect-protected and glufosinate-ammonium tolerant corn line 1507. The Initial Assessment for this Application was completed on 12 September 2001, and underwent a public consultation period until 31 October 2001. The Application is currently at Draft Assessment.

3. Objective

In addressing the issue of approving the sale of food derived from corn line 1507, FSANZ is required by its legislation to meet three primary objectives that are set out in section 10 of the FSANZ Act. These are (in descending priority order):

- 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 or varying a food standard, 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 line 1507 has been genetically modified to produce a naturally occurring insecticidal protein that is toxic to certain Lepidopteran insect pests in the larval stage. The insecticidal protein (Cry1F) is one from a family of proteins that are produced by the soil bacterium *Bacillus thuringiensis* (Bt). Cry 1F is derived from the subspecies *aizawai*. Bt formulations are widely used as biopesticides on a variety of cereal and vegetable crops grown organically or under conventional agricultural conditions.

Field research showed that the Cry1F protein, as expressed in corn line 1507, is effective in controlling European Corn Borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), black cutworm (*Agrotis ipsilon*) and armyworms (*Spodoptera* sp.) that are common insect pests of corn in the USA where this variety is intended to be primarily cultivated. An additional agronomic benefit that has been observed from the control of insect pests using this technology is a reduction in moulds and associated mycotoxins which are both directly and indirectly associated with large economic losses in the USA.

Corn line 1507 is also tolerant to glufosinate-ammonium herbicide through the expression of a bacterial gene from *Streptomyces viridochromogenes*, encoding an enzyme, phosphinothricin acetyltransferase (PAT). This enzyme is able to specifically break down the herbicide in the plant, converting it to an inactive form, thus allowing the plants to grow normally. The production of PAT in the plants allows selection of GM plants in the field as well as providing tolerance to the herbicide when used at agricultural levels.

It is intended that corn line 1507 will be used also in conventional plant breeding programs to express the insect and herbicide tolerance traits in other commercial corn varieties. The family of traits will be referred to as Herculex™ Insect Protection.

The majority of grain and forage from corn is used for animal feed, with less than 10% of grain processed into food products for human consumption. Corn grain is also 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, corn germ can be processed to obtain corn oil.

Domestic production of corn in Australia (ca. 340,000 t) and New Zealand is supplemented by 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 1507 is currently approved for feed and food use in the United States, Japan and Canada. Submissions have also been provided to Argentina, the European Union and Korea.

5. Relevant Issues

5.1 Safety of corn line 1507

Food from corn line 1507 has been evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The assessment considered the following:

1. The nature of the genetic modification;

2. General safety issues such as the history of use of corn, the source of the new genes, and the potential for transfer of antibiotic resistance genes to microorganisms in the human digestive tract;

3. Characterisation of the novel proteins including toxicological and allergenicity issues; and

4. A comparative analysis of key components of corn and the nutritional impact of the GM corn.

On the basis of the submitted scientific data and other available information, FSANZ has concluded that food derived from corn line 1507 is as safe and wholesome as food from other commercial corn varieties. The full safety assessment report is at Attachment 2 to this document.

5.2 Labelling of food from corn line 1507

On 28 July 2000, the Ministerial Council agreed to a revised standard requiring labelling of GM food where novel DNA and/or protein is present in the final food, and also where the food has altered characteristics. The revised standard came into effect on 7 December 2001. An exemption for stock-in-trade products was applied for a further period of 12 months from that date.

[1] FSANZ (2001) Information for Applicants – Amending Standard A18/Standard 1.5.2 – Food Produced Using Gene Technology.

Under these new provisions, food derived from corn line 1507 will require labelling if novel protein or novel DNA is present in the final food at detectable levels, or if the food has altered characteristics. Corn grain is used as the source of a variety of processed corn products. Products such as corn oil and maize starch may be exempt from labelling due to the extensive refining processes used in their manufacture that remove plant proteins and DNA. Other products, such as corn flour or meal, are likely to contain plant proteins.

5.3 Issues arising from public submissions

The majority of public submissions expressed opposition to this application for a variety of reasons including concern for public health and safety and the environment. However the matters raised were of a general nature relating to gene technology, rather than being of specific relevance to corn line 1507. A discussion of the general issues raised in connection with GM foods, as a whole is included at **Attachment 4.**

In light of rapid developments in the field of biotechnology in food production, the discussion of the general issues at Attachment 4 has been updated. The revised paper reflects recent outcomes of discussions on gene technology issues in the international arena. This includes findings and recommendations of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on '*New Biotechnology Food and Crops: Science, Safety and Society*', and the deliberations of various international committees and taskforces including those of the Codex Alimentarius Commission, the OECD and FAO/WHO Expert Consultations.

5.3.1 Specific issues raised in public submissions

(i) Increase in the development of resistance to Bt

Several submitters including Janet Ablitt (Aus), Pam Bourne (Aus) and The National Council of Women of Australia raised the issue that insect-protected GM plants such as corn line 1507 may increase the possibility of target insects developing resistance to Bt proteins. They raise concerns that organic farmers could lose the use of Bt as a bioinsecticide due to the cultivation of GM crops expressing one or more Bt proteins.

Response

Insect resistance is a frequently raised environmental issue. For crops intended for cultivation in Australia, the assessment of risk of insect pest resistance due to the planting of Bt plants is carried out by the Office of the Gene Technology Regulator (OGTR), in addition to other government agencies such as the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) and Environment Australia. In New Zealand, the Environmental Risk Management Authority (ERMA) in conjunction with the Ministry of Agriculture and Forestry (MAF) is responsible for assessing the risks to the New Zealand environment from the cultivation of GM crops.

FSANZ has notified the OGTR of these environmental concerns relating to the use of Bt crops. However, currently there is no application to the relevant authorities for a risk analysis on corn line 1507. The current application seeking food approval is due to the possibility that, following commercial planting overseas, food from this line may enter the Australian and New Zealand food supply as imported products.

The development of insect resistance is an ongoing issue for all agricultural sectors using Bt products, including growers of organic, conventional and GM crops. In Australia, as well as in other countries, it is a requirement for insect resistance management plans to be implemented when Bt crops are grown. Such plans are designed specifically to minimise the build up of resistance in the pest population and take into consideration a range of factors (eg. buffer zones and insect refuges, pest biology, ecology data, monitoring and surveillance and remedial action).

(ii) Corn grown in Australia

The National Council of Women of Australia notes that cultivation of corn line 1507 is not approved in Australia and that there is no permission in Australia for the use of glufosinate-ammonium herbicide on corn.

Response

Corn line 1507 was developed for cultivation in the major corn-growing regions of the Northern Hemisphere including in the United States and Europe. There are no plans to grow the crop commercially in Australia or New Zealand.

Under Standard 1.4.2 - Maximum Residue Limits (MRLs), the use of glufosinate-ammonium herbicide in Australia pertains to a range of agricultural commodities including citrus fruits, olives, stone fruits and tree nuts, to list a few. There is no MRL for glufosinate-ammonium in corn and therefore, under the provisions of the standard, there must be no detectable residues of that herbicide in the food. This applies equally to food imported into Australia from overseas markets.

6. Regulatory Options

Option 1 – prohibit insect-protected and glufosinate-ammonium tolerant corn line 1507
Maintain the *status quo* by not amending the *Food Standards Code* to approve the sale of food derived from corn line 1507.

Option 2 – approve insect-protected and glufosinate-ammonium tolerant corn line 1507
Amend the *Food Standards Code* to permit the sale and use of food derived from corn line 1507, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

- food importers and distributors of wholesale ingredients;
- the manufacturing and retail sectors of the food industry;
- consumers, particularly those who have concerns about biotechnology; and
- Government, where a regulatory decision may impact on trade or WTO obligations.

The cultivation of corn line 1507 may have an impact (either positive or negative) on primary producers and the environment. However, it is unlikely that corn line 1507 would be planted commercially for food in Australia or New Zealand as it has been developed primarily for growers in the Northern Hemisphere. If planting in Australia or New Zealand ever became likely, a comprehensive environmental risk analysis would be required by various government agencies in Australia such as OGTR, the NRA and EA, or ERMA and MAF in New Zealand.

7.2 Impact of regulatory options

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

7.2.1 Option 1

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 corn line 1507 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: 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 corn line 1507.
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 is unlikely to impact on monitoring resources.

Industry: Benefit to growers in lower production costs and reduced exposure to agricultural chemicals used to manage insect pests and weeds.
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.

After consideration of the regulatory impact for food derived from insect-protected and glufosinate-ammonium corn line 1507, it is concluded that the benefits of option 2, in permitting this food, outweigh the potential benefits identified in option 1.

8. Consultation

8.1 Public consultation

The Initial Assessment (formerly referred to as the Preliminary Assessment) of this Application was advertised for public comment between 19 September 2001 and 31 October 2001. A total of 39 submissions were received and a summary of these is included in this report at Attachment 3. The majority of the public submissions expressed opposition to the approval of corn line 1507 on the grounds that all foods produced from GM crops are potentially unsafe or that the crops themselves are potentially harmful to the environment, irrespective of the case-by-case assessment. Several submitters also asserted that the process of assessment is flawed. There were additional concerns that not all foods derived from corn line 1507 will be labelled.

Two of the submissions supported approval of the Application on the grounds that a scientifically based risk assessment was sufficient to demonstrate safety of the food. The Australian Food and Grocery Council also expressed support for appropriate labelling of the foods to allow consumer choice.

FSANZ carried out an assessment of the Application, including a safety evaluation of the food, taking into account the comments received in the first round of consultation. In assessing the food for safety, specific issues relating to corn line 1507 have been addressed in this report. The general issues that are not specific to this case have been addressed in Attachment 4 of this report.

No additional submissions were received in response to the section 13A notice required under the ANZFA to FSANZ transitional provisions.

8.2 WTO notification

During the FSANZ assessment process, comments are also sought internationally from other Members of the World Trade Organization (WTO). As Members of the WTO, Australia and New Zealand are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreements). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

This Application has been recommended to the agencies responsible, that the WTO be notified under the TBT and/or SPS agreements, in order to enable other member countries to comment on proposed changes to standards that may have a significant impact on them.

The WTO notification is considered necessary in this case as there is significant international interest in the safety of GM foods, and the proposed amendments are likely to have a liberalising effect on international trade.

8.3 Invitation for public submissions

Pending the Board's approval of the Draft Assessment Report including the safety assessment of corn line 1507, this application will be advertised for a second round of public comment (at least 6 weeks) prior to preparation of a Final Assessment Report.

9. Conclusion and Recommendation

- based on the available information, there are no public health and safety concerns associated with the genetic modification used to produce insect-protected and glufosinate-ammonium tolerant corn line 1507;

- food derived from corn line 1507 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from corn line 1507 will be required if novel DNA and/or protein is present in the final food;

- the benefits of permitting food derived from corn line 1507 in Australia and New Zealand primarily accrue to the food industry and to consumers, and are considered to outweigh the costs to government, consumers and industry, provided the safety assessment does not identify any public health and safety concerns; and

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

Based on the data supplied with the Application and other available information, FSANZ concludes that food derived from corn line 1507 is as safe for human consumption as food from other commercial corn varieties, and therefore recommends that the *Australia New Zealand Food Standards Code* be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard 1.5.2 is provided in Attachment 1.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety Assessment Report
3. Summary of first round public submissions
4. General issues raised in public submissions

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

To Commence: On gazettal

[1] ***Standard 1.5.2*** *of Volume 2 of the Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2*, immediately after the last occurring entry –*

Food derived from insect-protected and glufosinate-ammonium tolerant corn line 1507

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT

APPLICATION A446

FOOD DERIVED FROM INSECT-PROTECTED AND GLUFOSINATE - AMMONIUM TOLERANT CORN LINE 1507

Summary and Conclusions

Insect-protected and herbicide-tolerant corn line 1507 has been assessed for safety as a food. This line has been developed primarily for agricultural purposes to provide growers with a variety of corn that is both resistant to attack from major Lepidopteran insect pests, including the European corn borer, and tolerant to glufosinate-ammonium herbicide.

1. History of use

Corn (*Zea mays* L.) has undergone substantial genetic breeding by conventional methods over many centuries of cultivation and has been safely consumed as food and feed for thousands of years. Products derived from corn include highly processed corn grain fractions such as flour, high fructose corn syrup, corn oil, breakfast cereals and other products.

The two introduced genes are bacterial in origin. One is derived from *Bacillus thuringiensis,* which has an established history of safe use as a biopesticide on agricultural crops, including in the organic farming industry. The second gene is also derived from a common soil bacterium, *Streptomyces viridochromogenes* that has no known pathogenicity.

2. Description of the genetic modification

The two genes introduced into corn line 1507 are *cry1F* (insect-protection) and *pat* (herbicide tolerance). The *cry1F* gene is a synthetic version of a gene from *B. thuringiensis* var. *aizawai,* and encodes a truncated version of an insecticidal protein, Cry1F. This protein specifically targets the larval stage of insect pests of major economic importance in corn.

The *pat* gene is derived from *S. viridochromogenes* and encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide phosphinothricin and its synthetic form glufosinate-ammonium. The action of the herbicide normally results in plant death due to interference with the plant mechanisms for detoxifying ammonia. However, in corn line 1507, the presence of the PAT enzyme specifically inactivates the herbicide allowing the plants to function normally when sprayed. The herbicide tolerance trait was used also as a selectable marker to facilitate selection of plants with both introduced genes.

Line 1507 contains one complete copy of the transformation cassette incorporating the two linked genes, *cry1F* and *pat*. Expression of the introduced genes is through constitutive promoters, one derived from plants and the other from the cauliflower mosaic virus. Because a purified segment of DNA was used in the transformation, only the genes of interest were

transferred. No antibiotic resistance marker genes were transferred to the plants. Molecular and genetic analyses of corn line 1507 indicate that the transferred genes are stably integrated into the plant genome.

3. Characterisation of novel protein

Expression of the two new genes results in very low levels of the proteins Cry1F and PAT in various tissues of the plant. The mean levels of Cry1F in the edible grain were approximately 100 pg/ug total protein and were similar across geographical regions.

Expression of the PAT protein in the transformed line was found only at measurable levels in leaf tissue and was below the limit of detection in grain samples, irrespective of the field locations where the corn was grown and tested. Human exposure to the two introduced proteins through the diet is therefore expected to be at very low levels.

The potential toxicity and allergenicity of the two novel proteins, Cry1F and PAT, were addressed in the assessment. Both introduced proteins were examined for their potential to be toxic to humans, including in acute animal toxicity tests. For Cry1F, no adverse effects were observed in mice at doses up to 576 mg/kg body weight. In a similar study using PAT, no adverse effects were observed in mice at doses up to 5000 mg/kg body weight. In addition, there is no amino acid sequence similarity between the two novel proteins and known toxins recorded in large public domain sequence databases.

The potential allergenicity of the novel proteins was investigated by evaluating whether either of the proteins exhibited any of the physical or biochemical characteristics of known allergens. Neither protein exhibited any significant amino acid sequence similarity with known allergens and both proteins are rapidly digested in simulated mammalian digestive systems. The weight of evidence therefore indicates that neither the Cry1F nor the PAT protein is toxic to humans and neither protein has properties in common with known food allergens.

4. Comparative analyses

Compositional analyses were completed to establish the nutritional adequacy of corn line 1507 compared to the conventional counterpart. The results of the compositional analyses on herbicide treated corn plants grown at multiple locations demonstrate that the levels of the important constituents in corn grain (protein, total fat, carbohydrate, ash, fibre, fatty acids, amino acids, minerals and moisture) were similar in corn line 1507 and the non-transformed control corns. In addition, there were no observed differences in results from the analyses of four vitamins (Vitamins B1, B2, E and folate) measured in the transformed and non-transformed corns.

The levels of naturally occurring toxins and anti-nutrients were also assessed. Corn contains no naturally occurring toxins but does contain a number of secondary plant metabolites and trypsin inhibitor as well as a known anti-nutrient. Grain from corn line 1507 and control corn was also analysed for five secondary metabolites: inositol, raffinose, p-coumaric acid, furfural and ferulic acid, as well as for trypsin inhibitor activity. The levels of these compounds were either well below the limit of detection or, where detectable, were very similar in both the modified corn line 1507 and the non-transformed controls.

5. Nutritional impact

Grain from corn line 1507 was shown to be nutritionally equivalent to the non-transformed counterpart in the ability to support typical growth and well being in rapidly developing broiler chickens, an animal species that is acutely sensitive to nutritional factors in the early stages of growth.

Conclusion

The assessment of the safety of food derived from insect-protected and glufosinate-ammonium tolerant corn line 1507 is based on:

(i) a thorough understanding of the genetic modification and identification of the new gene products;
(ii) characteristics of the two novel proteins, Cry1F and PAT, in relation to potential toxicity or allergenicity; and
(iii) compositional analysis of the modified corn line compared to traditional corn lines.

On the basis of the available evidence, food derived from corn line 1507 is as safe and wholesome as foods derived from other corn varieties.

1. Introduction

Dow AgroSciences Australia Pty Ltd has submitted an application to FSANZ to vary Standard 1.5.2 – Food Produced Using Gene Technology in the *Food Standards Code* to include foods derived from insect-protected and glufosinate-ammonium tolerant corn line 1507.

The new genetic traits in the corn resulted from the introduction of two new genes encoding the bacterial proteins Cry 1F, conferring resistance to certain insect pests, and phosphinothricin acetyltransferase (PAT), an enzyme conferring tolerance to the synthetic herbicide, glufosinate-ammonium.

Bacillus thuringiensis, a common soil bacterium, produces a number of Cry proteins, known also as Bt proteins, with very selective insecticidal activity. One of the family of Cry proteins, known as Cry1F, has been shown in field research to be effective in controlling certain lepidopteran insect larvae such as those from the European Corn Borer (*Ostrinia nubilalis*), Southwestern corn borer (*Diatraea grandiosella*), black cutworm (*Agrotis ipsilon*) and armyworms (*Spodoptera* sp.). These insects are common pests of corn in the United States where it is intended for this variety to be grown commercially. The Cry1F protein is encoded by the *cry1F* gene derived from *Bacillus thuringiensis* subsp. *aizawai*. The applicant claims that the presence of this genetic modification also results in a reduction in moulds and associated mycotoxins in the corn, in addition to the significant control of insect pests.

The PAT enzyme metabolises the herbicide glufosinate-ammonium (or L-phosphinothricin) into an inactive form (OECD, 1999). The enzyme is encoded by the *pat* gene, which is derived from *Streptomyces viridochromogenes*, a common soil bacterium.

Corn is used predominantly as an ingredient in the manufacture of breakfast cereals, baking products, extruded confectionery and corn chips. Maize starch is used extensively by the food industry for the manufacture of many processed foods including dessert mixes and canned foods.

Despite the diverse uses of corn products in many foods, corn is a relatively minor crop in both Australia and New Zealand, with a declining area planted over the last decade. When required, products such as high-fructose corn syrup and maize starch are imported from major corn growing regions in the Northern Hemisphere, to meet manufacturing demand.

The *cry1F* gene is registered for full commercial use in the United States in field corn originating from maize line 1507 (US EPA, 2001). Corn line 1507 has food, feed and environmental approval in Japan (2002) and food, feed and cultivation approval in Canada (2002). It is also undergoing assessment in Korea and is awaiting assessment in the European Union. Foods derived from corn line 1507 may enter the Australian and New Zealand markets in the future via imported products.

2. History of Use

2.1 Host organism

Maize (*Zea mays* L.), also known as corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of over 590 million tonnes in 2000 (FAOSTAT Database 2001). Almost half of the annual production is grown in the United States.

The majority of grain and forage derived from maize is used as animal feed, however corn also has a long history of safe use as food for human consumption. Corn grain is also 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 more minor 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 (Canadian Food Inspection Agency 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 applicant claims that, in the case of corn line 1507 hybrids, the presence of the insect-protected and herbicide-tolerance traits will provide producers with additional improvements to the available genetic stock.

2.2 Donor organisms

Bacillus thuringiensis

The source of the *cry1F* gene is the common bacterium *Bacillus thuringiensis* subsp. *aizawai*. *B. thuringiensis* are a diverse group of Gram-positive, spore-forming bacteria that were first isolated in 1901, and have proven to be a rich source of insecticidal proteins. Intensive research has identified a growing family of Bt proteins with different insecticidal specificities, including to coleopteran, dipteran and lepidopteran insect orders. While some discoveries are recent, the characterisation of individual Bt proteins and description of their insect specificity and mode of action is well described in the published literature.

The Bt organism has been used safely in spray form as a crop protective agent for at least 40 years (Schnepf *et al*. 1998; U.S. EPA 1996) as a useful alternative or supplement to synthetic chemical pesticide application in commercial agriculture, particularly in the organic farming industry, and in forest management. Several varieties of *B. thuringiensis* have been used as microbial insecticides since 1938 (Merritt 1998). The subspecies *aizawai* is commercially used to control wax moth larvae and various caterpillars, especially the diamondback moth caterpillar (Cornell University 1996).

Streptomyces viridochromogenes

The *pat* gene is derived from the common soil bacterium *Streptomyces viridochromogenes*. The bacterium produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide by Hoechst Ag. Over the past decade, the *pat* gene has been introduced into several other genetically engineered food crops to confer tolerance to PPT and the synthetic form glufosinate-ammonium. There have been no adverse effects on human health associated with its use in crops such as canola and several other corn varieties (OECD, 1999 & 2002).

Cauliflower mosaic virus

The 35S promoter and transcription termination sequences used in the genetic construct are derived from the commonly occurring cauliflower mosaic virus (CaMV), a DNA plant virus with a host range restricted primarily to cruciferous plants (ICTV Database 1998) that are common in the food supply. The DNA sequences originating from this virus have no pathological characteristics, other than in association with their target plant species (USDA 1995).

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.

3. Nature of the Genetic Modification

3.1 Method used in the genetic modification

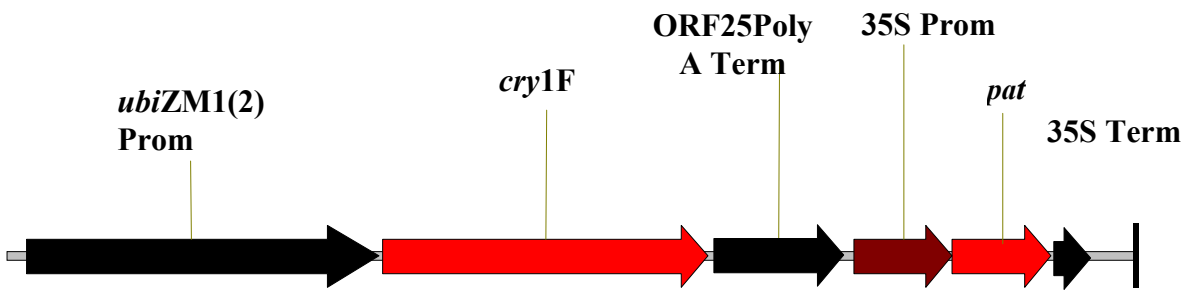
Corn line 1507 was generated by transformation of embryogenic Hi-II corn (*Zea mays*) cells, using a particle acceleration method. A purified linear DNA segment containing the *cry1F* and *pat* coding sequences, together with essential regulatory elements, was used in the transformation process. The DNA segment of 6235 bp was derived from plasmid PHP8999, and contained only the genes of interest. No additional plasmid DNA was used in the transformation event.

Following transformation, the plant embryos were transferred to cultivation medium containing the herbicide glufosinate-ammonium as the selection agent, allowing growth of cells expressing the PAT protein. As expected, the majority of explants were eliminated on this selective medium. Those that survived and produced healthy, glufosinate-ammonium tolerant callus tissue were subsequently regenerated into plants in the greenhouse. Following further testing and selection using European corn borer insects, corn line 1507 was eventually developed, based on the phenotypic characteristics, herbicide tolerance and resistance to lepidopteran insect pests.

3.2 Function and regulation of the novel genes

The purified linear segment PH18999A, used in the transformation, is illustrated in Figure 1. The 6235 bp DNA segment comprised two adjacent gene cassettes for expression of the two novel proteins, Cry1F and PAT. The *cry1F* gene is under the regulation of the ubiquitin promoter (ubiZM1(2)) from corn, and a 3' regulatory element derived from *Agrobacterium tumefaciens* (ORF25PolyA). The *pat* gene is regulated by the 35S promoter and the 35S transcription terminator, both from the Cauliflower Mosaic Virus (CaMV). The inserted DNA does not contain an antibiotic resistance gene or bacterial origin of replication sequences.

Figure 1. Arrangement of genetic elements comprising linear transformation segment



3.2.1 Gene cassettes

The DNA components present in the expression cassettes are described in Table 1. Each expression cassette consists of the genes of interest, flanked by regulatory elements derived from either plant or bacterial sources. The regulatory elements are described in the published literature, and their function in plants has been demonstrated (refer to Table 1). The gene sequences for *cry1F* and *pat* have been modified *in vitro* to optimise the production of the corresponding protein in plants (see below for further details on the expression of the genes).

Table 1: Genetic elements in insert PH18999A used to transform corn line 1507

Genetic element	Source	Size (bp)	Function
*ubi*ZM1(2)	Corn (*Zea mays*)	1986	The ubiquitin promoter (plus 5' untranslated region) from corn (Christensen *et al*., 1992) to enable protein expression in plants.
cry1F	*Bacillus thuringiensis* subsp. *aizawai*	1818	A truncated version of the coding region of the *cry1F* gene isolated from *B. thuringiensis*, in which codon usage has been optimised for expression in plants.
ORF25PolyA	*Agrobacterium tumefaciens*	714	DNA sequence corresponding to the transcription termination region from the pTi15955 of *Agrobacterium tumefaciens* (Fraley *et al*., 1983).
CaMV 35S promoter	Cauliflower Mosaic Virus (CaMV)	554	Promoter from a common plant virus, directing constitutive protein expression in the plant (Odell *et al*. 1985)
pat	*Streptomyces viridochromogenes*	552	The synthetic glufosinate-ammonium tolerance gene, optimised for expression in plants, based on the wildtype phosphinothricin acetyltransferase gene sequence from *S. viridochromogenes* (Wohlleben *et al*., 1988; Eckes *et al*. 1989; OECD 1999).
CaMV 35S transcription termination element	Cauliflower Mosaic Virus (CaMV)	204	A 3' untranslated region derived from the plant virus, terminating transcription and directing polyadenylation (Pietrzak *et al*. 1983)

3.2.2 cry1F gene

The bacterial *cry1F* gene sequence has been shown to provide high levels of protection against certain insect pests when it is expressed in plants. The gene encodes one of the families of Bt insecticidal proteins, Cry1F that specifically inhibits European and southwestern corn borer insects, black cutworm and armyworms.

Higher levels of field resistance in transgenic plants have been previously reported when the coding sequence of the introduced gene is modified to optimise plant codon usage (Vandemark, 1999). As naturally occurring Bt genes tend to be A:T rich, while plant genes have higher G:C content, the introduced *cry1F* gene in corn line 1507 has been resynthesised in the laboratory prior to transformation to optimise expression levels in the plant.

The corresponding amino acid sequence of the Cry1F protein is unchanged by the modified DNA sequence, except for one change at the carboxy terminus of the protein. A leucine residue occurs in place of a phenylalanine residue at position 604 of the 605 amino acids of the plant expressed protein. This single amino acid change results from an intended nucleotide change required to facilitate processing steps in the laboratory. The leucine substitution represents a conservative change in terms of the naturally occurring amino acid at the corresponding position in other Bt proteins.

Under the regulation of the constitutive Ubi-1 promoter element from corn, expression of the *cry1F* gene would be expected in all parts of the plant, conferring insect protection at the whole plant level.

3.2.3 pat gene

Tolerance to the herbicide phosphinothricin (glufosinate-ammonium) has been introduced to a variety of plant species using molecular techniques to insert a copy of the *pat* gene, which enables the plant to produce the PAT enzyme. Expression of PAT within the plant cell inactivates L-PPT thereby conferring tolerance to the herbicide (OECD 1999). The use of the *pat* gene in genetically modified corn and canola lines has undergone previous assessment by FSANZ.

As with the insect-tolerance gene, the codon usage pattern of the native *Streptomyces* gene has been modified in the laboratory prior to introduction into the plant. The amino acid sequence of the resulting PAT protein however is not changed (Eckes *et al.* 1989). In corn line 1507, the *pat* gene is under the regulation of the constitutive 35S promoter from CaMV and therefore the new protein is expected to be expressed in all parts of the plant, including the grain.

3.3 Characterisation of the genes in the plant

Genomic plant DNA from corn line 1507 was analysed using the standard methodology of Southern hybridisation blots and direct DNA sequencing to examine the presence of the insert, determine copy number, and provide information about the integrity of the inserted sequences. Northern hybridisation blots were also used to determine whether inserted sequences are functional.

Studies submitted:
Glatt, C.M. (2000) Genetic characterisation of maize event 1507: Southern blot analysis. DuPont de Nemours Company, Newark, Delaware, USA

Multiple Southern hybridisation blots were used to determine the nature and number of *cry*1F and *pat* gene insertions present in corn transformation event 1507.

The test material was root tissue taken from plants of two different generations, designated as T1S1 generation and BC4 generation, during the breeding of corn line 1507. The T1S1 generation seed consisted of the original transformed Hi-II corn line crossed to an elite inbred line to give an F1 hybrid, then self-crossed to give T1S1 seed. The BC4 generation seed consisted of the fourth backcross generation of the original transformed Hi-II line. Plants of both generations were grown in the glasshouse and root samples (four replicates) obtained for genomic DNA extraction and analysis.

Genomic DNA samples prepared from the non-transformed control Hi-II corn and line 1507 were used in the experiments, together with the plasmid DNA (PHP8999) from which the segment used in the transformation was derived. Reporter probes were generated by the use of specific primers to amplify five defined regions of plasmid PHP8999. The probes used to detect various regions of the transformation cassette were *ubiquitin*, *cry1F*, *CaMV 35S* and *pat*. A probe for the neomycin phosphotransferase (*nptII)* marker gene, which was present in the plasmid but not in the segment used to transform the corn, was also included in the analysis for comparison.

The results from these experiments indicate that one full-length copy of the transformation cassette is present in corn line 1507. The data also indicate that a partial-length insert is present at the same site of insertion (in both generations) and that this additional segment corresponds to a part of the *cry*1F and *pat* coding sequences. The results also confirm that, as expected, corn line 1507 does not contain the antibiotic resistance marker gene from plasmid PHP8999.

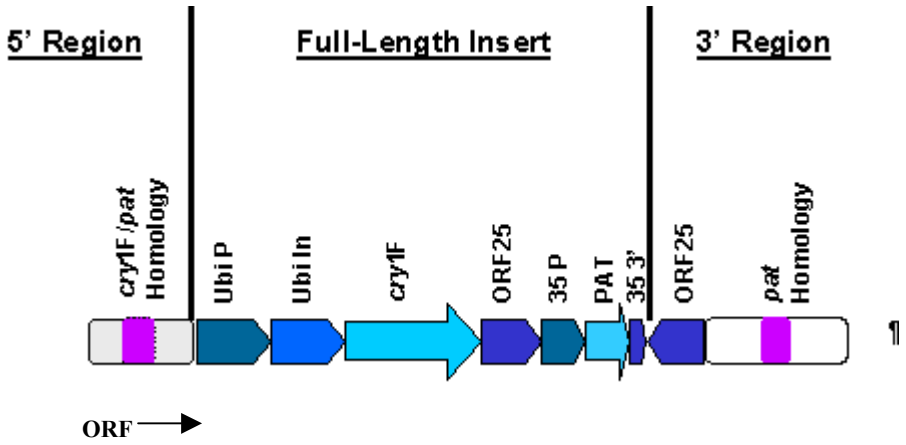
3.3.1 Verification of the nucleotide sequence

Nucleotide sequencing of the newly inserted segment and surrounding genomic regions was completed to confirm the characterisation of corn line 1507. The inserted DNA was amplified using polymerase chain reaction (PCR) methodology. Genomic DNA was extracted from two to three individual plants of corn line 1507 and the unmodified parental corn line (Hi II). PCR products unique to the transformed line were isolated by gel electrophoresis and sequenced directly, or sub-cloned into plasmid vectors and sequenced.

Analysis of the sequence data has identified that the transformation resulted in the insertion of one full-length copy of the gene cassette, together with partial fragments of the cassette at both the 5' and 3' ends, in the region adjacent to the site of insertion. A partial segment (335 bp) of the *cry*1F coding sequence was detected at the 5' end of the insert. In addition, a small fragment of the *pat* gene (comprising only the 5' portion of the gene) is also present at both the 5' (at least 19 bp) and 3' (188 bp) ends of the inserted DNA. Immediately adjacent to the 3'end of the full-length cassette, the nucleotide sequence corresponds to the majority (550 bp of the total 714 bp) of the ORF25 transcription termination element in the reverse orientation.

The sequence data enabled the construction of a complete map of the insert including the identification of restriction enzyme sites that give rise to DNA fragments that were correspondingly detected in the Southern Hybridisation analyses. The simplified map of the inserted DNA in corn line 1507 is depicted in Figure 2.

Figure 2. Diagram of inserted genes in corn line 1507



The nucleotide sequence and Southern blot data demonstrate that corn line 1507 contains one full-length copy of PHI8999A, one partial copy of *cry*1F in the 5' region, and two partial copies of *pat*; one in the 5' region and one in the 3' region relative to the insertion event.

3.3.2 Analysis of border regions

Overall, approximately 10,000 bp of sequence information has been provided covering the entire insert and extending some 2,500 nucleotides into plant genomic DNA at the 5' end and almost 2,000 nucleotides into plant genomic DNA at the 3' end of the insert. Homology searches were carried out to assist with identification of the border regions. In addition to homology searching using the BLAST program, the 5' and 3' border sequence was analysed for potential open reading frames (ORFs).

PCR analysis was used to compare the sequence in the 5' border region of corn line 1507 to the equivalent region in the unmodified parental corn line (Hi-II) used in the transformation. The sequence data revealed two ORFs in this region that are present in both the unmodified Hi-II line and corn line 1507, demonstrating that they are not novel to the transformed line. A third ORF (see Figure 2), spanning a total of 681 bp from the 5' *cry*1F fragment to the start of the *ubi*ZM1(2) promoter, is unique to corn line 1507 and is characterised by the presence of short fragments of the transformation cassette, including the very small fragment of the *pat* coding sequence, interspersed with corn genomic sequence.

There is no evidence to indicate that the third ORF, 5' to the full-length insert, could give rise to a protein product. The sequence is without many of the critical gene expression elements known to be associated with expression of stable proteins. Analysis of upstream sequences failed to detect any consensus promoter elements, and the G/C content of the ORF is low (46%) compared with the average for corn genes (56%). This latter property is known to adversely affect protein expression relative to native maize coding sequences. Northern blot analysis confirmed the prediction that there is no corresponding protein expression in the plant (see below).

As an added measure of assessment, the putative amino acid sequence arising from this ORF was analysed for homology with known allergenic proteins. No significant homology was found based on the criteria for a minimal domain size of identity across 8 contiguous amino acids.

Homology searching was conducted also on the 3' border sequence, again using the GenBank public databases and the BLAST program. These analyses confirmed the presence of 550 bp of the ORF25 termination element in the reverse orientation to the inserted DNA cassette, 520 bp of corn genomic sequences followed by a 188 bp fragment of the *pat* gene, then further corn DNA sequences. There are no significant ORFs (longer than 300 bp) occurring in the 3' border region in corn line 1507.

3.3.2 Northern blot analyses

In order to determine whether there is any expression resulting from the presence of partial *cry*1F and *pat* gene sequences, or the inverted ORF25 termination fragment, RNA was analysed by Northern blot for the presence of corresponding transcripts. Total RNA from leaf tissue of corn line 1507 (4 plants) and non-GM control corn (5 plants) was extracted for use in the experiments.

Based on the information gained from the nucleotide sequencing results, multiple genetic probes were prepared. No hybridising bands other than those corresponding to the expected full-length transcripts were observed with any of the probes. The northern blot results are therefore consistent with the conclusion that the partial copies of *cry*1F or *pat* occurring in the flanking regions to the full-length insert are not expressed as unique RNA transcripts in corn line 1507.

In addition, separate Northern blots were carried out to determine whether the potential ORF occurring in the 5' border region is producing a unique RNA transcript indicative of a level of expression in the plant. Sequence homology determined that this 681 bp ORF comprises 121 bp of the partial *cry*1F gene, 320 bp of a partial maize chloroplast *rpo*C2 gene, and the adjoining sequence up to and including the first 72 bp of the *ubi*ZM1 promoter in the full-length insert.

Total RNA from leaf tissue of corn line 1507 (9 plants) and non-GM corn (5 plants) was prepared in the same manner as for the previous Northern blot experiments. In this case, the probes corresponded to the 320 bp fragments of the maize chloroplast *rpo*C2 gene, and a positive control probe. No hybridisation signal was visible in either corn line 1507 or the non-GM control with the *rpo*C2 probe, but a strong signal was detected in all samples using the control probe. These results demonstrate that this potential ORF, novel to corn line 1507, is not producing a detectible RNA transcript and is therefore not expressed in the plant.

3.4 Stability of the genetic changes

Study submitted:
Song, P., Ernest, A.D., and Collins, A. (2002). Polymerase Chain Reaction (PCR) Analysis of *B.t.* Cry1F Maize Line 1507 and Its Hybrids. Laboratory Study GH-C 5371.

The presence of the transferred genes in corn line 1507 was investigated over multiple generations to ascertain genetic stability. The results from several rounds of backcrossing and self-crossing demonstrate that the *cry*1F and *pat* genes are stable in this line over at least six generations.

Observations of the phenotype indicated that the transgenes are inherited as dominant genes according to Mendelian segregation patterns. This method of analysis involved spraying each generation with glufosinate-ammonium to score and eliminate null segregants (those plants not containing a copy of the transgene). Further segregation data were obtained from plants derived from the F1 generation on the basis of herbicide tolerance, and later also challenged with neonate European corn borers. All of the plants determined to be tolerant to glufosinate-ammonium were also found to be resistant to European corn borer infestation.

Using PCR methodology, the presence of the same *cry*1F and *pat* gene sequences in corn line 1507 was confirmed in two independent hybrids derived from the T1S1 generation, confirming the stability of the introduced genes in the corn genome. Southern blot experiments, where similar hybridisation results were obtained for both the T1S1 and BC4F1 generations, also support the conclusion that the genetic modification is stable over multiple generations.

3.5 Antibiotic resistance genes

Antibiotic resistance genes can be present in some transgenic plants as a result of their use as marker genes in the laboratory or in the field. It is generally accepted that there are no safety concerns with regard to the presence in the food of the antibiotic resistance gene DNA *per se* (WHO 1993). There have been concerns expressed however that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of some antibiotics.

In this application, transformation of corn line 1507 was achieved using a specific segment of plasmid DNA corresponding to the genes of interest in conjunction with essential controlling elements. As transformed plant cells were selected using the introduced herbicide tolerance trait, no antibiotic resistance marker genes were necessary for this genetic modification. The molecular analyses have confirmed that no antibiotic resistance genes were transferred to corn line 1507.

3.6 Breeding pedigree

The applicant has provided details on the selective breeding program undertaken with the transformed line to demonstrate the production of a variety of elite corn lines with various commercial applications.

The *cry1F* and *pat* genes were transformed into the original parental line known as Hi-II, which was subsequently known as maize line 1507. The genetic makeup of this transformed line was 100% Hi-II. Maize line 1507 was crossed to an elite inbred line, so the resulting progeny contained 50% Hi-II germplasm and 50% elite inbred germplasm. Based on Mendelian genetics, only 50% of the progeny would contain the *cry*1F/*pat* genes (positive plants) and 50% of the progeny would not contain the new genes (null segregants).

The positive plants, with 50% Hi-II germplasm and 50% elite inbred germplasm, are then crossed again (or backcrossed) to the elite inbred. The resulting progeny contain 25% Hi-II germplasm and 75% elite germplasm. This process is repeated until the elite germplasm is very close to 100% and the *cry*1F and *pat* genes are also present.

High yielding hybrid corn seed sold to farmers is produced by crossing two distinct inbred corn lines. Each inbred corn line has a different genetic background that allows the hybrid seed to be optimised for a specific geographical region where corn is grown. Seed companies may sell over 100 different hybrid seed products requiring the development of hundreds of inbred corn lines. A new gene, such as *cry*1F in corn line 1507, is introduced into the many different inbred lines through conventional backcrossing.

3.7 Summary and conclusions from molecular characterisation

Corn line 1507 was produced using particle acceleration to insert a linear DNA segment of approximately 6.2 kb, comprising two bacterial genes and their defined controlling elements necessary for expression in plants. The encoded genes are *cry*1F (conferring insect protection) and *pat* (conferring tolerance to glufosinate-ammonium). Glufosinate-ammonium tolerance was used as a selectable marker for the transformation event and there was no transfer of any antibiotic resistance marker genes.

The insertion event was characterised using a range of molecular techniques including Southern and Northern hybridisation blots and DNA sequencing. The results of these analyses indicate that one complete, functional copy of the transformation cassette is present in corn line 1507. Using these tools, it was shown also that certain DNA rearrangements were present at both ends of the full-length insert, comprised of generally small fragments of both the *cry*1F and *pat* genes and the ORF25 transcription termination element. All of the studies on the 5' and 3' border regions indicate that the additional gene fragments do not result in detectable RNA expression products in the plant. Detailed studies, specifically targeting the putative open reading frame in the 5' border region, also failed to detect any RNA production.

Genetic rearrangements are known to occur with high frequency at the site of insertion of novel DNA during plant transformation, particularly using particle acceleration techniques. In this case, nucleotide sequencing was used to fully characterise the border region between corn genomic DNA and inserted DNA, providing a comprehensive picture of the molecular features that define corn line 1507. The conclusion from these analyses is that only the full-length *cry*1F and *pat* genes are expressed in the transformed plants.

Detailed phenotypic and molecular analyses demonstrate that the two new genes are physically stable and are inherited from one generation to the next according to predicted Mendelian patterns of inheritance.

4. Characterisation of Novel Protein

4.1 Biochemical characterisation

Studies submitted:
Evans, S.L. Equivalency of Microbial and Maize Expressed Cry1F Protein; Characterisation of Test Substances for Biochemical and Toxicological Studies. Project ID MYCO98-001. Completed October 1998.

Schafer, B.W. and Schwedler, D.A. Characterisation of the Recombinant Cry1F protein derived from *Pseudomonas fluorescens* and Transgenic Maize. Laboratory study GH-C 5294. Completed September 2001.

Gao, Y. Equivalency between transgenic corn-produced and microbially derived Cry1F protein. Dow AgroSciences, 2002.

Corn line 1507 contains two new bacterial proteins, Cry1F and PAT. Several techniques have been used to study the sites and level of expression of these two proteins in the modified plants.

Field studies representative of the conditions and growth stages corresponding to commercial corn production were undertaken during the 1998-1999 growing season in Chile, and during the 1999 growing season in France, Italy and the USA. The expression levels of Cry1F and PAT proteins in leaf, pollen, silk, stalk, whole plant, grain and senescent whole plant tissues from corn line 1507 and a non-GM control with comparable background genetics were measured using ELISA (Enzyme Linked Immunosorbent Assay), specifically developed for each protein. Western blot analysis was used to further characterise the specificity of the newly expressed proteins.

4.1.1 Cry1F

B. thuringiensis (Bt) occurs naturally in the soil and on plants including trees, vegetable crops and cotton. From intensive study of Bt species, four major classes of insecticidal protein genes (*cry1*, *cry2*, *cry3* and *cry4*) have been identified that are useful for the control of pest species among certain of the insect orders. This includes proteins that encode lepidoptera-specific (Cry1), lepidoptera- and diptera-specific (Cry2), coleoptera-specific (Cry3) and diptera-specific (Cry4) proteins respectively (Chambers *et al*., 1991). Cry1F was isolated from *B. thuringiensis* subsp. *aizawai* and is distinctly different in protein sequence and insecticidal specificity from the other Cry1 proteins (Chambers *et al*., 1991).

The mode of action of the insecticidal crystal proteins is to cause the death of susceptible insect larvae through the combined effects of tissue damage and a lack of feeding. Upon ingestion, the protein is solubilized and, in some cases, proteolytically processed by insect gut proteases to yield an active truncated toxin moiety. The activated protein toxin interacts specifically with gut receptors and leads to disruption of the osmotic balance of the cells in the insect midgut, ultimately leading to the death of the larvae.

In its natural form, Cry 1F is produced as a large protoxin of 1174 amino acids. Following solubilisation and proteolytic processing in the gut of susceptible insect larvae, the active toxin moiety corresponds to approximately 600 amino acids at the N-terminal end of the full-length protein. Although precise cleavage has not been shown, the activated toxin is estimated to correspond to amino acids 28-612, based on laboratory data and computer simulations. Therefore, to confer insect resistance, a truncated form of the *cry1F* gene, encoding only the active toxin moiety, was inserted into the corn plants.

The complete amino acid sequence of both the full-length Cry 1F protoxin from *B. thuringiensis*, and the truncated version used in corn line 1507 has been provided. The truncated Cry1F protein is identical to amino acids 1-605 of the N-terminal domain of the native Cry1F protoxin, with the exception of a single amino acid substitution, leucine in place of phenylalanine, at position 604 ($F_{604}L$).

The amino acid change was made to facilitate production in the laboratory of large quantities of a microbially produced Cry1F/Cry1A (b) chimeric protein that was used as a source of the Cry1F moiety required for toxicology studies. The chimeric protein is a fusion of the gene sequence coding for the Cry1A (b) C-terminal domain with the gene sequence coding for the Cry1F core toxin. The decision to use $F_{604}L$ substitution was based on the occurrence of leucine in the homologous position of other Cry1 proteins, and is therefore a conservative substitution.

The Cry1F/Cry1A (b) chimeric protein produced in *Pseudomonas fluorescens* strain MR872 enables high levels of expression of soluble protein. The MR872 fusion protein is subsequently enzymatically cleaved *in vitro* with trypsin to obtain the Cry1F core protein. The amino acid sequence of the microbially derived Cry1F protein (MR872) used in the toxicology studies was also submitted for comparison with the native and plant produced versions. Protein sequencing data showed that the N-terminal amino acid in both the plant derived Cry1F and the trypsin-processed microbial Cry1F corresponds to residue 28. On the basis of further experimental evidence, the biochemical characteristics and biological activity of the trypsin-released Cry1F core protein are equivalent to the corn-expressed core Cry1F protein (Evans, 1998 and Gao, 2002).

Although the DNA sequence of the *cry1F* transgene was altered from the native gene sequence to enable higher levels of expression of the core Cry1F protein in plants, there is no change in amino acid sequence compared to the corresponding wildtype protein sequence, except for the specific single amino acid change outlined above.

4.1.2 PAT

L-PPT, the active ingredient in glufosinate-ammonium herbicide, binds to, and inactivates, the enzyme glutamine synthetase in plants preventing the detoxification of excess ammonia, which ultimately results in plant death.

The activity of the PAT protein has been described in detail (OECD, 1999). The PAT enzyme is specific in catalysing the conversion of L-PPT to an inactive form, N-acetyl-L-PPT, which does not bind to the enzyme glutamine synthetase (De Block *et al*. 1987). The expression of PAT in corn line 1507 therefore results in the conversion of herbicide to the inactive form, allowing the detoxification of ammonia to continue in the plant in the presence of the herbicide. Plants expressing the PAT enzyme are therefore tolerant to the herbicide, enabling treatment of surrounding weeds without harm to the crop.

The *pat* gene from *S. viridochromogenes* encodes a polypeptide of 183 amino acids, and the mature PAT protein is known to be a homodimer of approximately 43 kDa in the native form (Wehrmann *et al*., 1996).

The *pat* gene has been resynthesised in the laboratory with a codon usage optimised for expression in plants. The synthetic *pat* gene encodes the same amino acid sequence as the native gene, and when expressed in plants, confers tolerance to glufosinate-ammonium (Eckes *et al*., 1989). Effective expression has been reported in numerous plant species including *N. tabacum*, *L. esculentum*, *M. sativa* as well as important food crops such as *B. napus* (canola) and *Z. mays* (corn).

4.1.3 Characterisation of the novel proteins expressed in corn line 1507

Study submitted:
Alarcon, C and Marshall, L., 2000. Characterisation of proteins as expressed in Bt Cry1F maize tissues. Performing Laboratory: Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number PH199-023

Gao, Y. and Collins, R.A., 2002. Gel-Electrophoresis, Western Blot, and ELISA of Truncated Cry1F Delta-endotoxin Following Heat Treatment. Dow AgroSciences Regulatory Laboratories, Indianapolis, USA. Study Number GH-C 5366.

Western blot techniques were used to examine biochemical properties including molecular weight and immunoreactivity of the CRY1F and PAT proteins expressed *in planta* comparatively against the respective microbially-derived protein produced in the laboratory. For this purpose, polyclonal antibodies that recognise multiple antigenic epitopes were used. Protein was extracted from a range of samples including leaf, pollen, grain and whole plant tissues from field grown corn line 1507 plants and a non-transformed control grown in Chile during the 1998/99 growing season.

Cry1F

The results of the analyses of Cry1F protein expression in plant tissues demonstrated that under denaturing conditions the Cry1F protein was detected as two bands with almost identical mobility (a doublet) of approximately 65 to 68 kDa in leaf, pollen, grain and whole plant tissue. No other bands indicative of a partial Cry1F protein or a fusion protein of greater molecular size were observed. Due to the presence of the known enzyme cleavage sites near the amino-terminus of the protein, the doublet is expected to have resulted from limited N-terminal processing by a plant protease with trypsin-like specificity.

PAT

In its native form, the PAT protein is known to be a homodimer of approximately 43 kDa, comprised of two identical components of approximately 22-23 kDa (Wehrmann *et al*., 1996, OECD, 1999). The immunoreactivity of the PAT protein extracted from leaf, grain, pollen and whole plant tissues derived from corn line 1507 was compared on a Western blot (under denaturating conditions) with that of a microbially-expressed PAT protein produced in the laboratory.

Plant-expressed PAT, of equivalent electrophoretic mobility to the microbially produced protein, was detected as a band of approximately 22 kDa only in leaf tissue from corn line 1507. There was no detectable PAT protein present in pollen, whole plant, or grain from the transformed line. These results are consistent with the levels and relative distribution of PAT protein detected by ELISA (see below). No other bands indicative of a partial PAT protein or a larger fusion protein with distinctive electrophoretic mobility were observed in these tissues from 1507 corn plants.

As expected, the Western blots did not detect immunoreactive bands corresponding to either of the novel proteins in the untransformed control corn tissue, using polyclonal antibodies.

4.2 Protein expression analyses

The introduced genes are each under the regulation of a constitutive promoter. However, at tissue level, the expression of either novel protein can vary and may be below the limit of detection. Three separate studies were undertaken to directly measure the levels of both novel proteins in a range of plant tissues derived from corn line 1507 and a non-GM control, when grown in different geographical locations representative of major commercial corn production regions.

Study submitted:
Stauffer, C. and Rivas, J., 1999. Quantitative ELISA Analysis of Cry1F and PAT Expression Levels in and Compositional Analysis of Maize Inbred and Hybrid Lines 1362 and 1507. Performing Laboratory: Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number 98-09-RA-NGLP-012.

The test system for this study consisted of four field sites located in the major corn growing regions of Chile, considered to be environmentally similar to corn growing regions in the United States where corn line 1507 would be a suitable agricultural product. At each site, multiple (usually 20) leaf, pollen, silk, stalk and grain samples were taken from five discrete plants. Whole plant and senescent whole plant samples consisted of three plants pooled together. CRY1F and PAT protein levels were measured in each of the samples using specific ELISAs developed for each protein.

Test seed from corn line 1507 (inbred and hybrid lines) were used in the planting of the field sites. The non-GM control seed was derived from Hybrid A_M and Inbred A_M that were representative of the transformed line in terms of their genetic background. Agricultural practices for growing the test and control plants were typical for producing corn in the regions chosen for this study. Chemical and fertilizer applications were appropriate for each location, and all test lines were sprayed with glufosinate-ammonium (Liberty®) using a hand spray at approximately the V5-V6 stage of development. The concentration of the active ingredient was 150 g/L, which is approximately four times the recommended label rate.

Total soluble protein in the corn tissue preparations was measured using the Bradford method (1976), using bovine serum albumin (BSA) as the protein standard. Reference standards were prepared in the laboratory from microbially expressed Cry1F (truncated toxin) and PAT proteins. The Cry1F protein was purified from *Pseudomonas fluorescens* (strain MR872) that contained a gene encoding the truncated Cry1F toxin. Characterisation of the standard was accomplished by electrophoretic mobility and amino acid analysis. The PAT protein was purified from recombinant *E. coli* (strain BL21) containing the *pat* gene. Characterisation of the PAT reference standard was accomplished by electrophoretic mobility (silver stain), sequencing and amino acid analysis. Both assay systems used polyclonal rabbit antibodies specific to the respective test protein.

The results of the ELISAs show that Cry1F was expressed in the transformed line at detectable levels in all collected tissues. The highest levels of expression of Cry1F were measured in the stalks (approximately 600 pg/µg total protein) while the lowest levels were detected in the corn silks (approximately 54 pg/µg total protein). The edible grain contained approximately 100 pg/µg total protein.

In contrast, expression of the PAT protein in the transformed line was found in only one of the leaf samples (21.4 pg/µg total protein); expression in all other plant tissues was below the limit of detection (<20 pg/µg total protein). As expected, expression of the Cry1F and PAT proteins was not detected in the non-transformed control plants.

Study submitted:
Stauffer, C., 2000. Quantitative ELISA Analysis of poCry1F and PAT Protein Expression Levels, Composition and Efficacy of Hybrid Lines 1360 and 1507 – EU Field Sites. Performing Laboratory: Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number PH199-005.

A second study was conducted using samples collected from three locations in France and three locations in Italy, all in major corn growing regions of the European Union. At each location in Italy, the trial consisted of transformed corn plants sprayed with glufosinate-ammonium, corn line 1507 unsprayed, and a non-transformed control hybrid with a genetic background representative of the transformed line. At the locations in France only, the trial involved corn line 1507, not sprayed with the herbicide, and the appropriate non-transformed control hybrid.

Tissue samples were collected from both the transformed and non-transformed lines as follows: leaf at V9 stage, whole plant at V9 stage, pollen, silk, stalk, whole plant at R1 stage, whole plant at R4 stage, grain and senescent whole plant. In addition, whole plant forage (R4 stage) and grain were collected from the glufosinate-ammonium sprayed plots. Grain was collected when plants were physiologically mature, corresponding to the time of typical commercial grain harvest. All tissue samples were analysed for Cry1F and PAT protein levels using the specific ELISA techniques. As before, each protein standard was characterised by electrophoretic mobility, sequencing and amino acid analysis.

The field studies show that expression of the Cry1F protein in transformed corn line 1507 occurs at measurable levels in all plant material sampled and tested. As found in the Chilean study, the stalks registered the highest expression level. However, in the European study, the lowest levels of Cry1F protein were measured in the grain (approximately 90 pg/µg total extracted protein). Overall, the pattern of expression in various plant tissues and at various stages of plant development in the studies conducted in Europe was similar to the results from the Chile studies. There was no significant difference in the expression level of Cry1F measured in the grain from sprayed (90.3 pg/µg) compared to unsprayed (96.4 pg/µg) plants.

The expression levels of the PAT protein in corn line 1507 were also similar in both studies; the amounts of PAT present were below the limit of detection of the assay (20 pg/µg total extracted protein) for all tissues and whole plant samples except for the leaf samples which again contained low levels (approximately 40 pg/µg total extractable protein). As expected, expression of the Cry1F and PAT proteins was not detected in any samples from the non-transformed control plants.

Study submitted:
Zeph, L., 2001. Quantitative ELISA Analysis of poCry1F and PAT Protein Expression Levels in Hybrid and Inbred Lines of TC1507 and an Inbred Line of TC1360. Performing Laboratory: Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number PH 199-001.

A third study was conducted on samples obtained from four locations in the midwestern corn growing regions of the United States. As before, plant tissues including leaf, pollen, silk, stalk and grain were collected from both corn line 1507 and a non-transformed control at each location. In addition, whole plant samples (entire plant except roots) were harvested approximately four weeks after pollination. Senescent whole plant samples, including ears, were harvested when the plant tissue had turned brown and dried.

The transgenic hybrid and inbred lines (test lines) used in this study were obtained from seed produced during the process of backcrossing the original transformant with elite inbred lines. The non-transgenic hybrid and inbred lines (control lines) were similar to the test lines in terms of background genetics but did not express Cry1F or PAT. As the population of plants in the test plots was segregating for the *pat* and *cry1F* genes, it was necessary to identify positive plants expressing both novel proteins. The test lines were therefore treated with glufosinate-ammonium herbicide by leaf painting plants at approximately the V4 to V5 stage of development. Since the two inserted genes are linked, plants exhibiting tolerance to glufosinate-ammonium herbicide are considered to also express the Cry1F protein.

In both the hybrid and inbred transgenic lines, expression of the Cry1F protein was found at measurable levels in all test tissues sampled. The results of this study are similar to those found in the other studies where it was observed that levels of Cry1F protein expression were highest in the stalks and lowest in either leaf, pollen or grain tissues. Although in this study the Cry1F protein expression was lowest in silk tissue, the edible grain contained only slightly higher levels than the silks, with 116 pg/µg total extractable protein (TEP) and 231 pg/µg TEP for the hybrid and inbred plants respectively. In addition, the pattern of expression of the Cry1F protein throughout the plant in both hybrid and inbred lines was identical.

Expression of the PAT protein was only found in leaf tissue samples (hybrid test lines) at detectable levels up to approximately 54 pg/µg TEP. These levels are nevertheless sufficient to confer tolerance to glufosinate-ammonium herbicide at the level of the whole plant. In all other tissues, the levels of PAT protein were below the limit of detection (20 pg/µg TEP). These results are comparable to those obtained in the other studies. As expected, expression of the Cry1F and PAT proteins was not detected in any samples from the control plants.

4.3 Potential toxicity of novel proteins

4.3.1 Cry1F

Studies submitted:

Evans, S., 1998. Equivalency of Microbial and Maize Expressed Cry1F Protein; Characterisation of Test Substances for Biochemical and Toxicological Studies. Mycogen Corporation Study ID MYC098-001.

Kuhn, J.O. 1998. Cry1F *Bacillus thuringiensis* subsp. *aizawai* Delta-endotoxin – Acute Oral Toxicity Study in Mice, conducted by STILLMEADOW, Inc. 12852 Park One Drive, Sugar Land, Texas 77478. Laboratory Study Number 4281-98.

Brooks, B.S. 2000. PAT Microbial Protein (FL): Acute Oral Toxicity Study in CD-1 Mice, conducted by Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Michigan, USA. Laboratory Study ID 991249.

As described previously under 4.1, to obtain quantities of the Cry1F protein sufficient for biochemical and toxicological tests, the protein was produced microbially in the laboratory from large-scale cultures of *Pseudomonas fluorescens* strain MR872.

A range of analyses was subsequently undertaken to establish that the microbially produced protein is equivalent to the protein produced by the transformed corn plants. The data collected demonstrate that the plant-derived protein exhibits characteristics expected of the *cry1F* gene product with respect to molecular weight, immunoreactivity, and apparent lack of post-translational modification, N-terminal amino acid sequence and bioactivity on susceptible insect larvae.

Acute oral toxicity

An acute oral toxicity study using laboratory mice was conducted to examine the potential toxicity of a single dose of the core Cry1F protein. The test substance, Cry1F delta endotoxin from *B. thuringiensis* subsp. *aizawai*, was administered by gavage in a 2% aqueous solution of carboxymethyl cellulose to each of 10 albino mice, 5 males and 5 females. The dose of test protein was equivalent to 576 mg/kg body weight. An individual dose was calculated for each animal based on its fasted body weight and administered in a total volume of 33.7 ml/kg, given as two half doses approximately one hour apart. Observations for mortality and clinical or behavioural signs of toxicity were carried out at least three times on the day of dosing (Day 0) and twice daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on days 7 and 14. At study termination, the animals were killed and examined for gross necropsy and any abnormalities recorded.

No animal deaths occurred during the course of the study and, with the exception of one female, all animals recorded normal relative weight gains at the mid-point and end of the study. There were no adverse clinical or behavioural signs of pathology and gross necropsy conducted at termination of the study revealed no observable abnormalities. The study conclusion was that the oral LD_{50}, as indicated by the data, was determined to be greater than 576 mg/kg in male and female mice.

Comparison of amino acid sequence with other proteins

The comparison of amino acid sequence of an introduced protein with that of known protein toxins is another means of evaluating the potential toxicity of a novel protein. A protein identified as having significant sequence similarity to a known toxin can then be further assessed using traditional toxicological approaches. The applicant submitted computer alignment analyses of the amino acid sequence of the truncated Cry1F protein (present in corn line 1507) against database entries with over 900,000 protein sequences.

As expected, the results of the amino acid comparison showed that Cry1F has significant sequence similarity with other Bt insecticidal proteins such as Cry 1A(b), a protein already approved for use in transgenic corn. In general, the family of Bt proteins is considered to lack mammalian toxicity based on studies that examine environmental and occupational exposures, and a history of safe use. Three other proteins from various sources were identified as having some limited degree of similarity with Cry1F, however none of these are known protein toxins.

4.3.2 PAT

Studies submitted:
Stauffer, C. and Rivas, J., 1999. Quantitative ELISA Analysis of Cry1F and PAT Expression Levels in and Compositional Analysis of Maize Inbred and Hybrid Lines 1362 and 1507. Performing Laboratory: Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number 98-09-RA-NGLP-012.

Brooks, B.S. 2000. PAT Microbial Protein (FL): Acute Oral Toxicity Study in CD-1 Mice, conducted by Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Michigan, USA. Laboratory Study ID 991249.

Acute oral toxicity

An acute oral toxicity study in mice was conducted using microbially derived PAT protein (84% purity) prepared as a reference standard also for use in other analyses. The test substance was administered by gavage tube to each of 10 CD-1 mice (5 males and 5 females) at a dosage equivalent to 5000 mg PAT protein /kg body weight, suspended in aqueous methylcellulose. Due to the calculated delivery volume exceeding 2 ml/100g body weight, the required dose was delivered in two halves approximately one hour apart. During the course of the study, detailed clinical observations and individual animal weights were recorded.

All of the mice survived for the two-week study period. There were no clinical observations associated with toxicity, and all animals, with the exception of one female, gained normal body weight. At the conclusion of the study, there were no gross pathologic lesions found in any of the test animals. It was concluded that the acute oral LD_{50} of microbially derived PAT protein in mice is greater than 5000 mg/kg.

The results and conclusions from this study are consistent with those obtained from other toxicology studies using the PAT protein. FSANZ has previously assessed scientific data in relation to the potential for toxicity of PAT in other genetically modified food commodities such as corn[2] and canola. The conclusion from these previous assessments is that the safety of the PAT protein has been well established. The synthetic *pat* gene used in corn line 1507 is identical to the one used in the other commodities, providing additional supporting evidence for the lack of toxicity of the encoded PAT protein.

4.4 Potential allergenicity of novel proteins

The potential allergenicity of novel proteins is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid 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 (Taylor and Hefle, 2001). The two new proteins in corn line 1507 were evaluated according to these criteria.

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[2] A372: Food derived from glufosinate-ammonium tolerant and fertility controlled canola
A375: Food derived from glufosinate-ammonium tolerant corn line T-25
A380: Food derived from insect-protected and glufosinate-ammonium tolerant DBT418 corn

4.4.1 Source of novel protein

The Cry1F insecticidal protein is encoded by the *cry1F* gene, derived from the soil bacterium *B. thuringiensis* subsp. *aizawai*. Microbial formulations of Bt are perhaps the most well known and widely used biopesticides and have a long history of safe use.

The subspecies *aizawai* is commercially used to control wax moth larvae and various caterpillars, especially the diamondback moth caterpillar (Cornell University, 1996).

The PAT enzyme introduced into corn line 1507 is naturally present in the soil bacterium *S. viridochromogenes*, a species not considered as pathogens of plants, humans or other animals (OECD, 1999). As described earlier in 4.3.2, PAT is present as a novel protein in several approved GM commodities (canola, corn) and is not associated with allergenicity.

4.4.2 Sequence comparison to known allergens

Study submitted:
Meyer, T. 1999. Comparison of Amino Acid Sequence Similarity of Cry1F and PAT Proteins to Known Allergen Proteins. Pioneer Hi-Bred International Inc. Johnston, Iowa. Study Number PH199-013.

A comparison of the amino acid sequence of the introduced proteins to that of known allergens can provide information on the extent to which an introduced protein is structurally similar to a known allergen. This is based on the identification of contiguous identical sequence matches that may be immunologically significant. This information may therefore suggest whether the introduced protein has allergenic potential.

A database was compiled using the Wisconsin Genetics Computer Group (GCG) sequence analysis program to search standard DNA and protein sequence databases. The results of the sequence alignment demonstrate that neither the Cry1F nor PAT proteins share significant amino acid sequence homology with known allergen proteins. This result was expected for the PAT protein, as it has been the subject of previous safety assessments.

4.4.3 Digestibility of Cry1F and PAT proteins

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 (Kimber *et al*., 1999; Astwood *et al*., 1996b; Metcalfe *et al*., 1996). Various physicochemical properties of the Cry 1F and PAT proteins were investigated, including the susceptibility of these proteins to proteolytic degradation in conditions that mimic digestion.

<u>*Cry1F*</u>

The *in vitro* digestibility of bacterially derived Cry1F protein was determined in laboratory experiments where the protein was exposed to simulated mammalian gastric fluid either with varying amounts of pepsin for a set incubation period, or a set amount of pepsin incubated in a time course experiment ranging from 0 to 60 minutes. The samples were analysed by SDS-PAGE and visualised by Commassie blue staining.

The results of the experiments show that Cry1F was completely digested to amino acids and small peptides within 5 minutes at molar ratios approximating 1:100 (Cry1F:pepsin). In separate experiments, the same molar ratio of 1:100 effected nearly complete proteolysis of

the Cry 1F within one minute. In the mammalian gut, further digestion occurs in the duodenum where pancreatic enzymes (lipase, amylase and the serine protease, trypsin) continue the degradative process. The results of the simulated upper gastrointestinal digestion with pepsin therefore indicate that Cry1F is readily degraded in environments simulating mammalian digestion.

PAT

A separate study was conducted to evaluate the stability of the PAT protein in a simulated gastric system containing 0.3% (weight /volume) pepsin, over various time intervals ranging from 5 seconds to 10 minutes. Following electrophoresis of reaction mixtures, all protein and digested fragments were visualised by Coomassie blue staining.

The gels show that PAT degrades to below detectable levels within 5 seconds of exposure to simulated gastric fluid (SGF), thus demonstrating very low stability in a normal mammalian digestive environment.

4.4.4 Glycosylation

As protein allergens are often glycosylated, studies were carried out to determine whether Cry1F is a glycoprotein. The potential glycosylation of Cry1F protein *in planta* and from a microbial source was examined by a sensitive immuno-blot technique used for glycoprotein detection. The studies provided no evidence for post-translational modification involving carbohydrates of the Cry1F in corn-derived extracts.

4.4.5 Heat stability

The stability of the Cry1F protein to heat was tested under bioassay conditions. Aqueous formulations of the microbially produced truncated Cry1F protein were incubated at various temperatures (60° C, 75° C and 90° C) for 30 minutes. The positive control formulation was treated at 4°C for the same period. The formulations were then applied to the surface of artificial insect diet in bioassay trays. Each well on the tray was infested with a neonate larva of tobacco budworm (*Heliothis virescens*). Insect mortality and weight were measured after 6 days exposure to the treated diet.

There was 96% growth inhibition of the larvae feeding on the diet coated with Cry1F protein pre-treated at the control temperature (4°C). Using protein that had undergone pre-treatment at temperatures of 75°C and 90°C, the percentage mortality was reduced to zero, with 8% and 3% larval growth inhibition respectively. At the intermediate temperature of 60°C, the protein gave rise to 25% mortality of insect larvae with 93% growth inhibition.

These results demonstrate that Cry1F is labile to temperatures above 60°C, and that the resultant denaturation of the protein leads to a concomitant reduction in insecticidal activity.

4.5 Summary and conclusions

Two bacterial proteins are expressed in corn line 1507 – Cry1F and PAT. Analyses of the modified line indicate that both proteins are present at low levels in some plant tissues and occur below the limit of detection in other parts of the plant. Using antibodies specific for each protein, Western blot data show the presence of immunoreactive bands corresponding to

the proteins of the expected size and mobility in the transformed corn when compared to laboratory-purified protein standards.

The levels of the novel proteins in plant tissues from corn line 1507 grown at multiple sites in locations in Chile, Europe and the United States were measured using protein-specific ELISA techniques. PAT protein was detected at very low levels only in leaf tissue.

In all other plant tissues tested, including the edible grain, the amount of PAT protein was below the limit of detection of the assay system. The mean levels of Cry1F in the edible grain were uniformly low across the three geographical areas, measuring 90, 100 and 116 pg/µg total protein. When tested, there was no significant difference in the expression level of Cry1F measured in grain from either herbicide-sprayed (90.3 pg/µg total protein) or non-sprayed plants (96.4 pg/µg total protein).

A range of biochemical and bioinformatic studies were conducted to determine whether Cry1F and PAT exhibit the potential to be either toxic or allergenic when present in foods. Both of these proteins are derived from bacterial sources that are not known to be either toxic or allergenic. The Bt organism has been used safely as a naturally occurring biopesticide over a long period, and the PAT protein has been present in a range of agricultural crops produced over the last decade with no apparent adverse effects. The protein expression studies indicate that dietary exposure to either of the introduced proteins would be very low. Neither protein exhibits significant amino acid sequence similarity with known toxins or allergens. Both proteins are readily broken down in conditions that mimic human digestion. Furthermore, no adverse effects were observed when the proteins were administered by gavage to mice at doses greatly exceeding the likely human level of exposure through consumption of corn products.

The biochemical and physicochemical investigations on the novel proteins therefore strongly support the conclusion that Cry1F and PAT are unlikely to be either toxic or allergenic to humans.

5. Comparative Analyses

Studies submitted:

Stauffer, C. and Rivas, J., 1999. Quantitative ELISA Analysis of Cry1F and PAT Expression Levels in and Compositional Analysis of Maize Inbred and Hybrid Lines 1362 and 1507. Pioneer Hi-Bred International Inc., Johnston, Iowa. Study Number 98-09-RA-NGLP-012.

Stauffer, C. and Zeph, L., 2000. Compositional Analysis of Maize MPS Hybrid Line 1507. Pioneer Hi-Bred International Inc, Johnston, Iowa and Woodson-Tenet Laboratories Inc, Delaware, Iowa. Study Number 98-09-RA-NGLP-012.

Stauffer, C. 2000. Quantitative ELISA Analysis of poCry1F and PAT Protein Expression Levels, Composition and Efficacy of Hybrid Lines 1360 and 1507 – EU Field Sites. Pioneer Hi-Bred International Inc, Johnston, Iowa. Study Number PH199-005.

The key constituents in corn have been evaluated in order to compare corresponding data from corn line 1507 expressing Cry1F and PAT proteins, the non-transformed counterpart and published literature values obtained for conventional varieties of corn. This evaluation includes a study of the major constituents that are characteristic of whole corn grain, taking account of the natural variation in composition that is known to occur due to genetic variability and geographical or environmental factors. As a reference tool, the OECD has

produced a consensus document on compositional considerations for new varieties of maize, which looks at key food (and animal feed) nutrients, anti-nutrients and secondary plant metabolites (OECD, 2002).

5.1 Key nutrients

Two major studies were conducted at different geographical areas to determine the compositional profile of key corn tissues collected from corn line 1507 and appropriate non-transformed control lines grown under field conditions.

The initial study was conducted at four trial sites located in the major corn growing regions of Chile. Plant tissue samples were collected from a hybrid line derived from 1507, an inbred line derived from 1507 and control lines designated as Hybrid A_M and Inbred A_M. The test lines were segregating for the two transgenes, *cry1F* and *pat*, and were sprayed with glufosinate-ammonium herbicide at approximately the V5-V6 stage of development. Plants that were damaged by the herbicide were considered to lack both transgenes and were removed from the plots. The control lines are not genetically modified and have background genetics representative of the test lines.

At physiological maturity, whole plant forage, consisting of the pooled material from three self-pollinated whole plants, and grain samples from the hybrid test and control lines were harvested for compositional analyses. The nutrient analyses included fat, protein and fibre content and moisture and ash analyses. In addition, grain was measured for fatty acid and amino acid profile, mineral content (calcium, phosphorus, copper, iron, magnesium, manganese, potassium and zinc), vitamin content (vitamins B1, B2, E and folic acid), tocopherols and the anti-nutrient substances phytic acid and trypsin inhibitor. The analyses were conducted at Woodson-Tenent Laboratories according to the methods of the Association of Official Analytical Chemists (AOAC).

The results obtained for the proximate analysis of the grain samples are presented in Table 2. Levels of these components in corn line 1507 and its non-GM counterpart were comparable, and also within previously reported ranges for corn grain. A small difference in the percentage of fat between the GM line and its comparator was statistically significant ($p<0.05$) but values were within the literature reported range for that variable.

Table 2: The means and p-values (across all sites) for the proximate analysis of grain from corn line 1507 and a control corn hybrid from samples collected in the 1998/1999 field trials in Chile.

Variable (% dry weight)	Corn Line 1507 (mean)	Control Line (mean)	P-value	Literature range*
Fat	**3.83**	**3.94**	**0.046**	**3.1-5.7**
Protein	11.20	11.32	0.611	6.0-12
ADF	3.55	3.68	0.250	3.0-4.3
NDF	10.47	10.08	0.315	8.3-11.9
Ash	1.51	1.50	0.335	1.1-3.9
Carbohydrates**	83.45	83.23	0.352	63.3-89.7

* Watson, 1982 and 1987.

** Carbohydrates calculated as the % dry weight less % protein, fat and ash.

The results for the proximate analyses on the forage samples collected during the same trial also show no statistically significant differences between corn line 1507 and the non-GM control samples (data not presented).

Amino acid analysis

The levels of eighteen amino acids in the grain from corn line 1507 and the non-GM control were compared, and the results are presented in Table 3 below. There were two values, cysteine and methionine, where the difference between the two lines was found to be statistically significant, but the magnitude of the difference was small and not considered to be biologically significant as the values fall within the ranges previously reported in the literature.

Table 3: Amino acid composition of corn grain. Values are means expressed as a percentage on a dry weight basis.

Amino acid	Corn Line 1507 (mean)	Non-GM control (mean)	p-value	Literature range a b
Glycine	0.39	0.40	0.150	0.26-0.47 0.24-0.41
Threonine	0.40	0.41	0.302	0.29-0.39 0.21-0.37
Valine	0.51	0.52	0.902	0.21-0.52 0.25-0.67
Isoleucine	0.40	0.40	0.952	0.26-0.40 0.19-0.39
Leucine	1.42	1.43	0.880	0.78-1.52 0.43-1.35
Phenylalanine	0.56	0.57	0.479	0.29-0.57 0.04-0.54
Histidine	0.29	0.30	0.822	0.20-0.28 0.21-0.32
Lysine	0.32	0.32	0.522	0.20-0.38 0.19-0.36
Arginine	0.44	0.45	0.672	0.29-0.59 0.28-0.55
Cysteine	**0.21**	**0.23**	**<0.0001**	**0.12-0.16** **0.13-0.27**
Methionine	**0.19**	**0.20**	**0.020**	**0.10-0.21** **0.12-0.26**
Tryptophan	0.08	0.08	0.065	0.05-0.12 0.05-0.10
Serine	0.54	0.55	0.390	0.42-0.55 0.25-0.46
Alanine	0.84	0.85	0.727	0.64-0.99 0.37-0.81
Glutamic Acid	2.14	2.18	0.472	1.24-1.96 0.89-2.02
Proline	1.01	1.03	0.679	0.66-1.03 0.43-1.01
Aspartic Acid	0.77	0.81	0.102	0.58-0.72 0.37-0.80
Tyrosine	0.20	0.20	0.954	0.29-0.47 0.17-0.31

a: Watson, 1982

b: Data from analyses of 22 commercial Pioneer® Hybrids

Fatty Acid Analysis

Corn oil is an excellent source of polyunsaturated fatty acids, with a high level of the essential fatty acid linoleic acid (18:2). In addition, it has naturally low levels of the saturated fatty acids, palmitic acid (16:0, 11%) and stearic acid (18:0, 2%). It is known also that corn oil from cooler regions has a higher proportion of unsaturated fatty acids than corn oil from warmer areas, which appears to be an adaptation to climatic conditions. However, genotype has a greater influence on fatty acid composition than any environmental factor. The biochemical variability for fatty acid composition among corn genotypes is known to cover a broad range.

Analyses of five major fatty acids in corn grain were conducted: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). There was a small statistically significant difference between corn line 1507 and the non-GM control in all of these measurements except for levels of palmitic acid that were similar in both lines. However, the values for these fatty acids in corn line 1507 and the control were within the literature reported range for maize.

Mineral analysis

The levels of nine minerals (calcium, phosphorus, copper, iron, magnesium, manganese, potassium, sodium and zinc) were analysed in grain from corn line 1507 and the non-GM control line. The levels of sodium however, were below the limit of quantitation in all samples. For the remaining eight mineral components, no significant differences between the transformed and non-transformed lines were detected. There was also no difference between either of the lines and the range of values found in the literature (from Watson, 1982).

In addition to a published range, data were compiled on calcium levels in 22 current commercially grown hybrids (Pioneer®). The modifications made by the testing laboratory (Woodson-Tenent) to the method for determining calcium levels typically results in lower amounts of calcium than those reported in the literature. It was therefore considered more accurate to compare the tested lines to the compiled data from the Pioneer hybrids, which were analysed by the same method, rather than to previously published data produced using a different method of analysis.

Vitamin analyses

Grain from corn line 1507 and the control corn was analysed for content of four vitamins. Vitamin B1, B2, total tocopherols (Vitamin E) and folic acid levels in both transformed and non-transformed lines were determined for comparison to the published literature range. The results show small statistically significant variations between the transformed and non-transformed corn in terms of the total tocopherols (slightly higher in corn line 1507) and in vitamin B1 levels (slightly lower in corn line 1507). However, the values obtained for all vitamin analyses were within the respective range previously reported in the literature (Watson 1982 and 1987). It should be noted that there is no typical range available for folic acid in grain, although an average value of 0.3 ppm is reported (Watson, 1987). Levels of folic acid in corn line 1507 and the control corn were not significantly different.

European field trial

Corn line 1507 and a non-GM control corn with genetics representative of the test line, were grown also in field sites in locations in France and Italy. At each of the sites in Italy, plots comprising transformed plants sprayed with glufosinate-ammonium herbicide, unsprayed transformed plants, and unsprayed control plants were cultivated. Glufosinate-ammonium herbicide was not applied to any of the plants at the trial sites in France. Grain and whole plant tissue samples were collected for measurement of various compositional and nutritional parameters.

As in the Chilean field trials, the nutrient analyses included moisture, fat, protein, ash, fibre and carbohydrates for whole plant samples. Grain samples were analysed for moisture, crude fat, crude protein, ash, fibre, and carbohydrate content, as well as fatty acid and amino acid composition, levels of minerals, certain vitamins, and the anti-nutrient compounds phytic acid and trypsin inhibitor. In addition, tocopherol levels in the grain were measured.

The forage samples were dried to between 7% and 14% moisture before processing. The grain was dried to between 9% and 12% moisture before shelling. Laboratories at Woodson-Tenent determined the exact moisture content for each sample so that the results could be reported on a dry weight basis.

The results of the proximate analyses on grain are presented in Table 4 below. The values are estimated means (across all sites) and carbohydrate levels are arithmetically calculated using measured percentages of protein, fat and ash.

Table 4: Proximate analysis of grain across all sites.

Variable (% dry weight)	Corn line 1507 (unsprayed) (mean ± SE)	Corn line 1507 (sprayed) (mean ± SE)	Non-GM control (mean ± SE)	Literature Range*
Fat	4.21 ± 0.12	4.41 ± 0.14	4.41 ± 0.12	3.1 – 5.7
Protein	11.73 ± 0.24	12.04 ± 0.28	10.98 ± 0.24	6.0 – 12.0
ADF	2.37 ± 0.17	2.52 ± 0.18	2.29 ± 0.17	3.0 – 4.3
NDF	10.16 ± 0.30	10.54 ± 0.35	10.13 ± 0.30	8.3 – 11.9
Carbohydrates	82.46 ± 0.57	81.97 ± 0.25	83.00 ± 0.28	63.3 – 89.7
Ash	1.60 ± 0.04	1.67 ± 0.05	1.56 ± 0.04	1.1 – 3.9

* Watson, 1982 or Watson, 1987.

The results show that grain from transformed corn line 1507, either sprayed or unsprayed, has similar levels of the proximate components to the non-transformed control corn line. Furthermore, with the exception of ADF (Acid Detergent Fibre), the measurements were all within the respective literature range for that variable. The levels of ADF in both the control and transformed line were both slightly lower than the published range indicating a small genotypic variation that is not noteworthy.

The proximate analyses on the forage samples in the European study also show no statistically significant differences between corn line 1507 and the non-GM control samples (data not presented). Furthermore, there was close correlation in the proximate results obtained from both the Northern and Southern hemisphere studies for both the grain and the forage.

Mineral analysis

As was observed in the field trials in Chile, the levels of eight minerals (calcium, phosphorus, copper, iron, magnesium, manganese, potassium and zinc) present in the grain from corn line 1507, either from sprayed or unsprayed plants, and the non-GM control, reveal no differences of any biological significance between the comparators. Furthermore, all of the values were within the reported literature range, noting that the accepted range for calcium levels was derived from data obtained from an analysis of 22 commercial hybrid corn lines (not genetically modified). Sodium levels were again below the limit of quantitation for all the lines, including the non-GM control.

Amino acid analysis

The results of the amino acid analysis of the grain from the transformed and non-transformed lines grown in the European field trials are presented in Table 5.

There are small statistically significant ($p<0.05$) differences between corn line 1507 and its comparator in the amino acid levels indicated in Table 5 (bold print). In general, individual amino acid levels in corn line 1507 are marginally elevated when compared to those of the control line, although the measurements show no difference between the sprayed and unsprayed GM plants. In addition, the levels of threonine and glutamic acid in 1507 corn, unsprayed and sprayed, were marginally higher than the respective published literature range.

In general, the data indicate that protein levels in corn line 1507 are marginally higher than in the non-GM control corn, although the difference is small and not statistically significant. This difference is reflected in slightly elevated measurements of several of the amino acids in the transformed line that are considered typical for the genetic background of this particular corn.

Fatty acid analysis

To allow a comparison between studies, the same five major fatty acids in corn grain were analysed in the European study: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). There was no statistically significant difference between the transformed line (sprayed or unsprayed) and the non-transformed counterpart in the observed levels of these five fatty acids. All samples tested were also within the previously established literature range for the respective fatty acid component.

Vitamin analysis

As in the Chilean study, grain from corn line 1507 and the control was analysed for its content of four vitamins: thiamine hydrochloride (B1), riboflavin (B2), folic acid and total tocopherols. The levels of these vitamins in the GM line were not different from the control, whether the plants were spayed or unsprayed.

Table 5: Amino acid composition of corn grain. The values are estimated mean values across all sites ± standard errors. Data presented as percentage of dry weight.

Amino acid	Corn Line 1507 (unsprayed)	Corn Line 1507 (sprayed)	Non-GM Control	Literature range a b
Glycine	**0.41 ± 0.0090**	**0.42 ± 0.0102**	**0.38 ± 0.0090**	**0.26-0.47** **0.24-0.41**
Threonine	**0.41 ± 0.0080**	**0.41 ± 0.0094**	**0.37 ± 0.0080**	**0.29-0.39** **0.21-0.37**
Valine	**0.51 ± 0.0106**	**0.52 ± 0.0125**	**0.47 ± 0.0106**	**0.21-0.52** **0.25-0.67**
Isoleucine	0.41 ± 0.0098	0.41 ± 0.0116	0.36 ± 0.0098	0.26-0.40 0.19-0.39
Leucine	**1.38 ± 0.03**	**1.41 ± 0.04**	**1.23 ± 0.04**	**0.78-1.52** **0.43-1.35**
Phenylalanine	0.55 ± 0.018	0.56 ± 0.014	0.49 ± 0.012	0.29-0.57 0.04-0.54
Histidine	0.31 ± 0.0065	0.32 ± 0.0076	0.29 ± 0.0065	0.20-0.28 0.21-0.32
Lysine	0.32 ± 0.008	0.33 ± 0.009	0.31 ± 0.008	0.20-0.38 0.19-0.36
Arginine	0.47 ± 0.012	0.48 ± 0.014	0.44 ± 0.012	0.29-0.59 0.28-0.55
Cysteine	0.22 ± 0.004	0.23 ± 0.005	0.22 ± 0.004	0.12-0.16 0.13-0.27
Methionine	0.20 ± 0.0035	0.21 ± 0.0041	0.20 ± 0.0035	0.10-0.21 0.12-0.26
Tryptophan	0.10 ± 0.0035	0.10 ± 0.0037	0.09 ± 0.0035	0.05-0.12 0.05-0.10
Serine	**0.55 ± 0.012**	**0.56 ± 0.014**	**0.50 ± 0.012**	**0.42-0.55** **0.25-0.46**
Alanine	**0.83 ± 0.018**	**0.85 ± 0.022**	**0.74 ± 0.018**	**0.64-0.99** **0.37-0.81**
Glutamic Acid	**2.12 ± 0.050**	**2.18 ± 0.060**	**1.90 ± 0.050**	**1.24-1.96** **0.89-2.02**
Proline	**1.00 ± 0.0212**	**1.04 ± 0.0258**	**0.92 ± 0.0217**	**0.66-1.03** **0.43-1.01**
Aspartic Acid	**0.79 ± 0.0157**	**0.81 ± 0.0186**	**0.71 ± 0.0157**	**0.58-0.72** **0.37-0.80**
Tyrosine	0.21 ± 0.0048	0.21 ± 0.0057	0.19 ± 0.0048	0.29-0.47 0.17-0.31

a Watson, 1982.
b Data from analysis of 22 Pioneer® brand hybrids.

5.2 Key toxicants

In general, more than 70% of edible corn grain is composed of starch, with smaller amounts of protein, oil and other nutritionally valuable substances. There are no known naturally occurring toxins in corn. While mycotoxins can be detected in corn, these are metabolites produced by fungal contamination of corn kernels as a result of production and storage under adverse conditions. They are not a natural component of sound corn.

5.3 Key anti-nutrients and secondary metabolites

Corn contains insignificant levels of anti-nutrient compounds. The levels of trypsin inhibitor in particular are known to be very low (Del Valle *et al*., 1983; Watson, 1987). Lectins, carbohydrate-binding proteins with haemagglutination activity, have been found at low levels in the endosperm and germ (Newberg and Concon, 1985). Phytic acid is also present in low amounts in corn, and levels in maize grain vary from 0.45 to 1.0% of dry matter (Watson, 1987).

Grain from corn line 1507 and the control corn, collected from sites in both field trials, was analysed for five secondary metabolites. The secondary metabolites measured were inositol, raffinose, p-coumaric acid, furfural and ferulic acid. The trypsin inhibitor activity of the transformed and non-transformed corn grain was also compared using an enzyme activity assay (limit of detection was 2000 TIU/g dry weight of sample). In addition, data on the levels of phytic acid were provided. As the sites in Italy were sprayed with glufosinate-ammonium, the data available are for both sprayed and unsprayed plants. A literature range was available only for raffinose and phytic acid, as other compounds occur at such low levels that they are not generally measured.

The levels of trypsin inhibitor and furfural were below the limit of quantitation. The levels of the other metabolites and phytic acid in corn line 1507 are similar to the levels found in the untransformed control grain. There were no observed differences between the sprayed and unsprayed plants and all values were within the literature reported range where that was available.

5.4 Summary and conclusions from compositional analyses

In general, examination of data from the comparative analyses of both the Chilean and European studies, generated over a period of two years, reveal no compositional differences of biological significance in the grain from the transformed line and the non-transformed control. Several observed differences in some nutrient components such as amino acids are not indicative of an overall pattern of change arising from the genetic modification. Whereas measurements are similar across sites for the transformed line, the compositional analyses for the control line are marginally lower overall in the European study. The data are explained by the known natural variation in composition that arises due to a broad range of factors that influence plant growth and biochemistry. In addition, the spraying of corn line 1507 with glufosinate-ammonium herbicide does not have a measurable effect on the composition of the grain.

6. Nutritional Impact

6.1 Animal feeding studies

In assessing the safety of a genetically modified food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well being. In most cases, this can be achieved through an understanding of the genetic modification and its consequences together with an extensive compositional analysis of the food. Where, on the basis of available data, there is still concern or doubt in this regard, carefully designed feeding studies in animals may provide further reassurance that the food is nutritionally adequate.

Such studies may be considered necessary where the compositional analysis indicates significant differences in a number of important components or nutrients, or where there is concern that the bioavailability of key nutrients may be compromised by the nature of the genetic changes to the food.

In this case, the applicant commissioned a feeding study using chickens to investigate the nutritional performance of corn line 1507. Rapidly growing broiler chickens are sensitive to changes in nutrient quality in diets, and therefore serve as a useful model species to evaluate the wholesomeness of protein/amino acid sources.

Feeding study in Broiler Chickens

Study submitted:
Zeph, L., 2000. Nutritional Equivalency of B.t. Cry1F Maize – Poultry Feeding Study. Performing Laboratories PARC Institute Inc., 30 North Harrison Street, Easton, MD 21601. PARC Trial Number 98-PIO-25a-BB, Study Number PHI99-010.

This study compares the performance and processing parameters of rapidly growing broiler chickens (*Gallus domesticus*) raised on a diet containing either corn line 1507, a non-transformed control corn line (Hybrid 7250) or four commercially available reference corn lines, over approximately 42 days. The non-transformed control hybrid line has a genetic background representative of the test line, but is not genetically modified and does not express either the Cry1F or PAT proteins.

A total number of 245 healthy male chickens (Cobb x Cobb) were randomly assigned to the various treatment groups. All diets were formulated to meet nutritional recommendations (National Research Council, 1994), and consisted of a commercial corn/soy ration. From days 0-20, chickens were fed a starter diet containing 54.21% w/w corn while from days 21-42, chickens were fed a grower/finisher diet containing 57.03% w/w corn. These rations applied to all treatments. Both the solid diets and water were provided to the chickens *ad libitum* and no medication was administered during the course of the study.

To ensure the integrity of the grain used in the study, sub-samples of the test corns (hybrid corn 1507 and the control) were analysed for expression of Cry1F using a specific ELISA. This assay system confirmed the presence of the novel protein in grain from corn line 1507 and its absence in the control grain. In addition, samples of whole corn grain, starter and grower diets for each treatment were analysed for nutrient composition (proximates, amino acids, calcium and phosphorous). The results demonstrate a close similarity in composition of all diets and dietary components in the study.

During the course of the study, the birds were examined daily for general health, and any abnormal observations were recorded. Individual body weight was recorded on days 0 and 42, and body weight gain over the period (trial days 0 to 42) was calculated from these data. Standard statistical methods were applied in the calculation for feed conversion over the starter and grower/finisher periods. The results are presented in Table 6 below.

Table 6: Summary of performance measures; values are means for all animals per treatment.

Parameter	Diet Treatment					
	Ref #1	Ref #2	Ref #3	Ref #4	**Control hybrid 7250**	**Hybrid corn line 1507**
Mortality %	5.71	5.71	2.86	5.71	2.86	5.71
Body weight (kg) day 0	0.044	0.043	0.043	0.043	0.044	0.043
Body weight (kg) day 42	1.730	1.739	1.738	1.728	1.739	1.757
Daily gain (g per bird per day)	0.040	0.040	0.040	0.040	0.040	0.041
Feed Conversion (body wt. corrected)	1.797	1.806	1.808	1.804	1.802	1.775

The results of the broiler feeding study show there were no differences in parameters tested between birds fed a diet containing corn line 1507 and four non-transformed reference lines or a control line. These results support the results of the compositional analyses and indicate that corn line 1507 is equivalent to non-transformed corn in the ability to provide adequate nutrition to rapidly growing broiler chickens.

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ATTACHMENT 3

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

1. **Janet Ablitt (Aus)**
   - Opposes application.
   - Contends that environmental containment of GM crops and transgenes cannot be assured.
   - Maintains that there will be an increased use of herbicides from release of corn line 1507 with consequent effect on the environment.
   - Submits quotation and references from Prof Joe Cummins that cauliflower mosaic virus promoter could recombine to produce virulent new viruses.
   - Believes that there is a lack of long-term safety studies for GM foods.
   - Contends that expression of Bt toxin could increase pest resistance and reduce efficacy of Bt sprays used in organic agriculture.

2. **Tony & Diane Achtzehner (NZ)**
   - Oppose application.
   - Maintains that there would be detrimental health and environmental effects of Bt toxin and increased use of herbicide.

3. **Neil Archer, Debbie Howey (NZ)**
   - Opposed to application.
   - Concerned about the use of herbicides on crops.

4. **Australian Food and Grocery Council**
   - Supports application.
   - Supports a regulatory system that applies appropriate safeguards to matters concerning public (and environmental) health and safety that in turn provide a framework for companies and individuals to make independent decisions.
   - Supports the OGTR/GTTAC regulatory control of release of GMOs to the environment and supports commercialisation of corn 1507 in Australia under this system.
   - Considers there is no intrinsic safety issue with gene technology and that developers of technology must prove safety. States that if assessed to be safe, corn 1507 should be approved so that manufacturers can make their own choice as to its use.
   - Supports a labelling framework for GM foods that provides consumers with factual information to enable an informed choice.
   - Suggests that FSANZ should seek to reduce regulatory burden for assessing safety of GM foods by entering "equivalence" agreements with other international food agencies.

5. **Solange Bely NZ)**
   - Opposed to application.
   - Considers that labelling regulations for GM foods are inadequate.

6. **Pam Bourne (Aus)**
   - Opposes application.
   - States that herbicide tolerant GM crops will increase use of herbicides with consequent effect on the environment.
   - Maintains that there is inadequate proof regarding the safety of GM foods.
   - States that studies show that transgenes from GM crops may transfer to native crops with unknown consequences to the environment and humans.
   - Maintains that expression of Bt toxin in GM crops could increase pest resistance and reduce efficacy of Bt sprays used in organic agriculture.
   - Contends that GM crops are a threat to conventional and organic seed and food industries.

7. **Melanie Closs (NZ)**
   - Opposed to application.
   - Maintains that there is inadequate proof regarding the safety of GM foods.

8. **Katherine Dewar (NZ)**
   - Opposed to application.
   - Considers that release of corn line 1507 may have detrimental long-term impact on the environment.

9. **Bryan Divers (NZ)**
   - Opposed to application.
   - Maintains that there is inadequate proof of the safety of GM foods.
   - Considers that labelling regulations for GM foods are inadequate.

10. **Carole Fisher (NZ)**
    - Opposed to application.
    - Considers that there is insufficient long-term human health and safety data.
    - Considers that application be rejected until labelling regulations for GM food are in place.

11. **Food Technology Association of Victoria Inc. (David Gill)**
    Supports application as long as health and safety concerns addressed.

12. **GE Free New Zealand In Food And Environment (Rage) Inc. (Susie Lees)**
    - Submitted on behalf of 3,500 members opposed to GM foods.
    - Provides a list of health and medical concerns that are claimed to be attributable to gene technology.
    - Lists a number of references relating to purported health and environmental problems associated with GM foods. These include:
      - Instability of transgenic DNA as reflected by unknown sequences in RoundUp ready soy.
      - Increased herbicide residues leading consequent to increased use of herbicide-tolerant GM crops would result in increased toxicity to human health and environment.
      - Inability to control differential use of GM crops for food and agricultural purposes, as exemplified by dietary corn contamination with Starlink.
      - Ingested foreign DNA may transfer to cells of hosts or host flora inducing potential mutagenicity or antibiotic-resistance respectively.

- Insufficient characterisation of gene insert and potential for internal genetic rearrangements in instable GM crops.

- Application specific concerns include:
  - Bt toxins have environmental impacts
  - Horizontal gene transfer of Bt gene may occur into soil organisms
  - Bt proteins have been found to disrupt gut cells of mice.

13. Susan Gulotta (NZ)

- Opposed to application. No reasons supplied.

14. Irmgard Habl (NZ)

- Opposed to application.
- Maintains that Bt expressed in maize 1507 may have health and safety implications to soil microbes, people or animals.
- Considers that environmental release of GM crops in NZ could impact on environment, marketing and trade.

15. Susan Hards (NZ)

Opposed to application. No reasons supplied.

16. Stephen Hards (NZ)

Opposed to application. No reasons supplied.

17. David Head (NZ)

- Opposed to application.
- Contends that Bt expressed in maize 1507 may have health and safety implications to soil microbes, people or animals.
- States that insect pest resistance to Bt may arise due to increased levels of Bt in the environment and that increase in herbicide use will similarly impact on health.
- Contends that environmental release of GM crops in NZ will impact on marketing and trade.

18. Colin Hewens (NZ)

Opposed to application. No reasons supplied.

19. Oliver Hoffman (NZ)

- Opposed to application.
- Considers that GM corn would impact on the NZ environment and increase level of herbicide in food.

20. Craig Hutchinson (NZ)

- Opposed to application.
- Considers that corn line 1507 may have health and safety implications including increased allergenic potential.
- States that US EPA declared in October 2001 that it had not conducted any allergenicity studies.

21. Kerikeri Organics (Martin Robinson, NZ)

- Opposed to application.
- Bt expressed in corn line 1507 may have health and safety implications to soil microbes, people or animals.
- Glufosinate-ammonium could be toxic and increased use may have human and animal health and safety implications.
- Environmental release of GM crops in NZ could impact on marketing and trade.

22. Salvatore Lauria (NZ)

- Opposed to application and GM foods in general.
- Raises environmental concerns.

23. Baerbal Leeker (NZ)

- Opposed to application.
- Contends that Bt expressed in maize 1507 may have health and safety implications to soil microbes, people or animals.
- Believes that environmental release of GM crops in NZ will impact on marketing and trade.

24. G. E. Mabbs (NZ)

- Opposed to application.
- Opposed to general use of herbicides and GM foods.

25. Dale Mallet (Aus)

- Opposed to application.
- Contends that environmental containment of GM crops and transgenes cannot be assured.
- Believes there will be an increased use of herbicides with consequent effect on the environment with release of herbicide resistant GM crops.
- Claims that inbuilt pest resistance genes in GM crops will lead to evolution of tolerant pests with consequences to agriculture.
- Claims that RoundUp Ready soy carried an allergen from the donor organism.
- Contends that expression of Bt toxin could increase pest resistance and reduce efficacy of Bt sprays used in organic agriculture impacting on trade.
- States that environmental containment of GM crops and transgenes – especially 'terminator' genes - cannot be assured and will have deleterious effects on agriculture and the environment.

26. Kate Moss (NZ)

- Opposed to application.
- Believes that environmental release of GM crops in NZ will impact on marketing and trade.

27. National Council of Women of Australia (Judith Parker, Elaine Attwood) endorsed by Consumers' Association of South Australia (Jill Bailey)

- Oppose the approval of corn line 1507.
- Believe that Bt toxin from GM crops will impact on environment. Cite monarch butterfly study as evidence that Bt may have unintended environmental effects. Also allude to Bt being an allergen.

- Highlight concerns raised by Public Health Association of Australia regarding PAT gene in Application A375. These were:
  - the enzyme specificity of the PAT protein and the potential for it to acetylate or deacetylate other proteins in human and farm animals; and
  - that measurements other than gross pathology had not been done on animals subjected to acute oral toxicity testing of the PAT protein.
- State that glufosinate-ammonium is not approved for use on corn in Australia and that OGTR should deal with this prior to FSANZ.
- Submit that additional uncharacterised genetic inserts may be present within corn 1502.
- Consider that lack of long term feeding trials is a shortfall of the FSANZ assessment process.
- Consider that use of 'substantial equivalence' as outmoded and unscientific. Claim that EU has discarded this approach and so should FSANZ.
- State that GM food labelling provisions are not comprehensive and do not provide adequate information for consumers regarding use of gene technology in food production. Claim that EU labelling approach lead in this respect and that lack of adequate labelling compromises FSANZ's objective – 'the provision of information to consumers to provide an informed choice'.
- In relation to Costs and Benefits in the Initial Assessment:
  - the argument that costs to government may accrue from enforcing an import prohibition for corn line 1507 implies that all GM foods will ultimately be approved.
  - the argument that consumers will bear increased costs and be adversely affected by decreased availability of corn products if corn line 1507 is prohibited is unrealistic. Contend that conventional corn lines will still be on the market, this argument is thus misleading.
  - the argument that 'consumers wishing to avoid consuming products from corn line 1507 may be negatively affected because GM foods are not currently identified by labels' could be avoided if a comprehensive labelling scheme was in place. Similarly that the argument that such consumers may need to pay more for non-GM corn food products if GM corn line 1507 is approved are unsubstantiated and illogical.
  - state that FSANZ ignored the majority of submissions who were opposed to the 'stock-in-trade' provisions regarding the commencement of GM food labelling.
  - consider that the statement 'consumers may be positively affected should approval not be granted' is hard to comprehend and is unsubstantiated.
  - consider that the statement that 'manufacturers would be able to source raw commodities from their usual suppliers if corn line 1507 is approved' is ambiguous and irrelevant.
  - contend that approval of corn line 1507 ended in the USA in September 2001. Current application implies it will be available on international markets but not in the USA.
  - contend that rejection of the application would result in a minimal impact on trade similar to the statement in the Initial Assessment regarding acceptance of the application.
- Indicate that more detailed comment on the safety evaluation is deferred until the next step in the process when the Draft (Full) Assessment report is released.

28. Lynden Over (NZ)

- Opposed to application.
- Believes that there is inadequate proof that GM crops are safe for human health and to the environment.
- Contends that labelling of GM food insufficiently comprehensive for consumer choice.

29. Kirsty Patton (NZ)

- Opposed to application.
- Considers there is insufficient evidence that GM foods are safe for the environment and to health.

30. Christine Robb (NZ)

- Opposed to application.
- Believes that there is inadequate proof that GM crops are safe for human health and to the environment.
- States that current labelling requirements for GM food is inadequate to allow for consumer choice.

31. Alistair Robinson (NZ)

- Opposed to application.
- Contends that gene technology is inherently risky.
- Considers that toxicological tests for GM foods are inadequate.
- Considers that a workable traceability system for GM foods is required to withdraw product if found unsafe.

32. Cliff Royal (NZ)

- Opposed to application.
- Considers that there is insufficient evidence that GM foods are safe for the environment and to health.
- Considers that genetic modification is against Maori principles regarding protection of the environment.

33. Leif Thomaes (NZ)

- Opposed to application.
- Contends that there is insufficient evidence that GM foods are safe for the environment and to health.

34. Andrew Thomson (NZ)

- Opposed to application.
- Contends that there are health and safety uncertainties with GE foods.
- Submits that labelling regulations do not transfer adequate information for informed choice.

35. Sharyn van Heerden (NZ)

- Opposed to application
- Has no desire to eat any GM food products.

36. Chrissy Walmsley (NZ)

- Opposed to application.
- Maintains that there is inadequate proof regarding the safety of GM foods.
- Considers that labelling regulations for GM foods are inadequate.

37. Kath Watzig (NZ)

- Opposed to application.
- Maintains that there is insufficient evidence that GM foods are safe for the environment or to human health.
- Contends that long-term toxicological and consumption tests required.
- Contends that use of antibiotic resistance marker genes and viral promoters may impact on human and environmental health.
- Considers that labelling regulations for GM foods are inadequate.

ATTACHMENT 4

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received in response to Gazette Notices in relation to GM foods express general views opposed to the use of gene technology and assert that food produced using this technology is unsafe for human consumption. The general issues, which are not necessarily specific to the application, are addressed below.

1. FSANZ's processes

FSANZ's general processes for the risk assessment of GM foods have been criticised by several submitters from Australia and New Zealand.

Response

The processes used by FSANZ for safety assessment and labelling of GM foods were subject to an independent assessment by the New Zealand Royal Commission on Genetic Modification which was conducted during the first quarter of 2001. In its deliberations, the Royal Commission considered that both the New Zealand Environmental Risk Management Authority (ERMA) and FSANZ provided a robust regulatory environment and stated that the authorities acted conscientiously and soundly in carrying out their duties. The Commission expressed confidence in the FSANZ safety assessment process, stating that it considered it unlikely that foods that have satisfied the food standard will have harmful effects. The Commission also considered that FSANZ carries out its functions with an appropriate degree of independence not only from political influence but also from the influence of commercial interests. In reaching this view, it should be noted that the Commission examined the criticisms levelled at FSANZ's processes and the detailed rebuttal of those criticisms supplied to the Commission by FSANZ, including issues such as adequacy of the toxicological studies, use of substantial equivalence, sources and independence of data, and the use of antibiotic resistance marker genes.

The Report can be accessed at http://www.gmcommission.govt.nz.

2. Sources of data

The use of company data from the applicant during the assessment is seen by some submitters to compromise the independence and validity of the safety evaluation.

Response

It is a requirement of the FSANZ assessment process that raw data from experiments supporting the safety of a GM food are submitted to FSANZ for assessment. These data are assessed in detail by FSANZ scientists and then the assessment report undergoes a robust process of internal review by FSANZ's own scientific experts and external review by FSANZ's expert panel and senior health officials from State and Territory and New Zealand Health Departments. The quality and sources of the data supplied to FSANZ in support of applications for approval of GM foods was the subject of particularly intense scrutiny during FSANZ's evidence at the New Zealand Royal Commission on Genetic Modification.

FSANZ submitted a full data package (15 volumes of raw data on Roundup Ready Soybeans) to the Commission for inspection. The Commission states that it looked closely at the quality of this data and came to the view that FSANZ did receive and assess raw data and that the processes were valid in this regard.

Furthermore, in relation to the issue of the independence, integrity and different sources of data submitted in support of applications for approval of GM foods, at the recent OECD Conference "New Biotechnology Food and Crops: Science, Safety and Society" held on 16-20 July 2001 in Bangkok, there was agreement by participants (as stated in the Conference Rapporteurs report) attending the Conference that "There is information for regulatory dossiers – where there is a high level of quality assurance and validation – and information in general scientific literature which is peer-reviewed but not necessarily subject to quality assurance procedures (e.g. Good Laboratory Practice). The frameworks and designs for work generating data are important determinants of quality."

3. Imported GM foods versus GM crops

Some submitters have argued that approvals for GM foods or commodities as imports to Australia and New Zealand is a tacit approval for the GM crop to be grown in either country.

Response

The regulatory framework for approval by FSANZ of safety of GM foods (imported foods and derived from GM crops grown in Australia) is separate from that of the Office of the Gene Technology Regulator (OGTR) and the Environmental Risk Management Authority (ERMA), which have responsibility for approving the environmental release of GM crops in Australia and New Zealand respectively. FSANZ's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard 1.5.2 in the joint *Food Standards Code* cannot be regarded as tacit approval for the environmental release of the crop in Australia since the environmental issues are completely separate and entirely different to food safety issues.

4. Compositional studies

The compositional analysis occasionally reveals that some of the components of the genetically modified plant line under assessment are statistically different to the control line. Some submitters therefore claim that the GM line is not comparable to the control line.

Response

Statistical differences observed in the compositional analyses are assessed by FSANZ in terms of their relevance in a biological system. In order to determine if any differences have biological significance, FSANZ compares these values to published ranges for each component. Many of the significant differences observed have been small differences, are usually within the range that would be expected for other commercially available varieties and do not indicate a trend, as they do not occur consistently. Additionally, many of the differences can be explained by differences between locations or seasons.

The use of published ranges and historical control data in safety assessment studies is standard procedure in the interpretation of biological and analytical components of variation. Although the most appropriate control group for interpretative purposes is always the concurrent control, there are instances in which the use of historical control information can aid an investigator in the overall evaluation of safety data. Studies (Carokostas and Banerjee (1990), *Interpreting Rodent Clinical Laboratory Data in Safety Assessment Studies: Biological and Analytical Components of Variation*, Fundamental and Applied Toxicology) suggest that statistically significant laboratory findings that are not biologically or toxicologically important will be present in many safety assessment studies with a standard design. An over-reliance on the result of standard prepackaged statistical analyses for determining the presence of toxicologically significant findings can lead to misinterpretation of laboratory data. It is well recognized that sound judgement must be applied to laboratory findings using appropriate statistical analyses as a tool for pattern recognition.

5. The safety of genetically modified foods for human consumption

Many submitters raise the issue of public health and safety in relation to food produced using gene technology. In particular, it is often stated that there has been inadequate testing of genetically modified foods, that there is limited knowledge concerning the risks associated with the technology and that there may be potential long–term risks associated with the consumption of such foods.

Response

It is a reasonable expectation of the community that foods offered for sale are safe and wholesome. In this context, *safe* means that there is a reasonable certainty of no harm. As with other aspects of human activity, the absolute safety of food consumption cannot be guaranteed. Conventionally produced foods, while having a long history of safe use, are associated with human disease and carry a level of risk, which must be balanced against the health benefits of a nutritious and varied diet.

Because the use of gene technology in food production is relatively new, and a long history of safe use of these foods has yet to be established, it is appropriate that a cautious approach is taken to the introduction of these foods onto the market. The purpose of the pre–market assessment of a food produced using gene technology under Standard A18/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

The safety assessment focuses on the new gene product(s), including intentional and unintentional effects of the genetic modification, its properties including potential allergenicity, toxicity, compositional differences in the food and it's history of use as a food or food product.

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD).

The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

6. The need for long-term feeding studies

Concerns are often expressed in relation to the lack of long-term toxicity studies on genetically modified foods.

Response

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to laboratory animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

By contrast, foods are complex mixtures of compounds characterised by wide variations in composition and nutritional value. Due to their bulk, they can usually be fed to animals only at low multiples of the amounts that might be present in the human diet. Therefore, in most cases, it is not possible to conduct dose-response experiments for foods in the same way that these experiments are conducted for chemicals. In addition, a key factor to be considered in conducting animal feeding studies is the need to maintain the nutritional value and balance of the diet. A diet that consists entirely of a single food is poorly balanced and will compromise the interpretation of the study, since the effects observed will confound and usually override any other small adverse effect, which may be related to a component or components of the food being tested. Identifying any potentially adverse effects and relating these to an individual component or characteristic of a food can, therefore, be extremely difficult. Another consideration in determining the need for animal studies is whether it is appropriate from an ethical standpoint to subject experimental animals to such a study if it is unlikely to produce meaningful information.

If there is a need to examine the safety of a newly expressed protein in a genetically modified food, it is more appropriate to examine the safety of this protein alone in an animal study rather than when it is part of a whole food. For newly expressed proteins in genetically modified foods, the acute toxicity is normally examined in experimental animals. In some cases, studies up to 14 days have also been performed. These can provide additional reassurance that the proteins will have no adverse effects in humans when consumed as part of a food.

While animal experiments using a single new protein can provide more meaningful information than experiments on the whole food, additional reassurance regarding the safety of newly expressed protein can be obtained by examining the digestibility of the new protein in laboratory conducted *in vitro* assays using conditions which simulate the human gastric system.

7. Substantial equivalence

Some submitters express concern regarding the use of the concept of substantial equivalence as part of the assessment process and reject the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

Response

Substantial equivalence embodies the concept that, as part of the safety assessment of a genetically modified food, a comparison can be made in relation to the characteristics and properties between the new food and traditionally produced food. This can include physical characteristics and compositional factors, as well as an examination of the levels of naturally occurring allergens, toxins and anti-nutrients.

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

The concept of substantial equivalence allows for an evaluation of the important constituents of a new food in a systematic manner while recognizing that there is general acceptance that normally consumed food produced by conventional methods is regarded by the community as safe. It is important to note that, although a genetically modified food may be found to be different in composition to the traditional food, this in itself does not necessarily mean that the food is unsafe or nutritionally inadequate. Each food needs to be evaluated on an individual basis with regard to the significance of any changes in relation to its composition or to its properties.

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for over ten years and has been an integral part of the safety assessment of some 50 products.

Although the concept of *substantial equivalence* has attracted criticism, it remains as the most appropriate mechanism for assessing the nutritional and food safety implications of foods produced using gene technology. It is generally agreed also that continual review of the concept, in response to the criticism, provides a useful stimulus to ensure that safety assessment procedures are kept at the forefront of scientific knowledge (Nick Tomlinson, Food Standards Agency, United Kingdom: Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, 2000), and reflect the support of international bodies such as Codex Alimentarius, OECD, FAO/WHO, other regulators such as the UK, the EU, Japan, Canada and the recent report of the Canadian Royal Society.

8. The nutritional value of food produced using gene technology

A small number of submitters express concern that the genetic alteration of food decreases its nutritional value.

Response

The assessment of food produced using gene technology by FSANZ entails an exhaustive evaluation of analytical data on any intentional or unintentional compositional changes to the food. This assessment encompasses the major constituents of the food (fat, protein, carbohydrate, fibre, ash and moisture) as well as the key nutrients (amino acids, vitamins, fatty acids). There is no evidence to suggest that genetic modification *per se* reduces the nutritional value of food.

In the future, genetic modification may be used intentionally to improve the nutritional value of food. In this regard, GM foods may be able to assist in addressing the general nutritional needs of the community and also specific dietary needs of sub-populations.

9. Potential toxins and allergens

Some submitters express concerns about the risks of the introduction of new toxins or allergens.

Response

This issue is considered in detail as part of the safety assessment conducted on each new genetic modification applied to a food or commodity crop. New toxins or allergens may be introduced into food by either gene technology or by traditional breeding techniques, or by altered production processes. It is also possible to use these techniques to develop foods specifically where such compounds are significantly reduced or eliminated. One advantage of gene technology, in comparison with these other methods, is that any transferred genes are well characterised and defined, thus the possibility of developing a food with a new toxic or allergenic compound is likely to be reduced.

10. Antibiotic resistance

Some submitters raise concerns about an increase in antibiotic resistance resulting from the use of gene technology. Some consider that it would be reassuring if independent biomedical advice were available to inform the public that the use of antibiotic resistance markers does not pose a risk to the future use of antibiotics in the management of human disease.

Response

The human health considerations in relation to the potential for the development of antibiotic resistance depend on the nature of the novel genes and must be assessed on a case-by case basis. This issue arises because of the use of antibiotic resistance marker genes in the generation of genetically modified plants. In some circumstances, antibiotic resistance genes are linked to the gene of interest, to enable the initial selection of the engineered cells in the laboratory.

Those cells that contain the antibiotic resistance marker gene, and hence the gene of interest, will be able to grow in the presence of the antibiotic. Those cells that failed the transformation process are eliminated during the selection procedure.

Concern has arisen that ingestion of food containing copies of antibiotic resistance genes could facilitate the transfer of the gene to bacteria inhabiting the gut of animals and humans. It is argued that these genes may then be transferred to disease causing bacteria and that this would compromise the therapeutic use of these antibiotics.

In 1993, the World Health Organisation Food Safety Unit considered this issue at a Workshop on the health aspects of marker genes in genetically modified plants. It was concluded at that Workshop that the potential for such gene transfers is effectively zero, given the complexity of the steps required. Since this time, several separate expert panels (Report to the Nordic Council, Copenhagen 1996; Advisory Committee on Novel Foods and Processes, UK 1994, 1996; The Royal Society, UK 1998) and numerous scientific papers published in peer reviewed journals have also considered the available evidence on this issue. It is generally agreed that the presence and subsequent transfer of an intact functional gene from transgenic food to microorganisms in the human intestine is an extremely unlikely event. Furthermore, if this were to occur, bacteria would not normally retain the resistance genes unless there was an environment for positive selection. The majority of these genes provide for resistance to antibiotics whose use is confined to the laboratory and are not considered to be of major therapeutic use in humans.

Antibiotic resistant bacteria are naturally occurring, ubiquitous and normally inhabit the gut of animals and humans. There is a general consensus that the transfer of antibiotic resistance genes is much more likely to arise from this source and from associated medical practices, rather than from ingested genetically modified food. Even so, at the OECD Conference (GM Food Safety: Facts, Uncertainties, and Assessment) held in Edinburgh on 28 February – 1 March 2000, there was general consensus that the continued use of antibiotic marker genes in GM food crops is potentially unnecessary given the existence of adequate alternatives, and therefore should be phased out.

The recent JETACAR (Joint Expert Technical Advisory Committee on Antibiotic Resistance) Report states (page 117, referring to a specific gene, *nptII*) that the use of antibiotic resistance genes in GM foods is unlikely to contribute in any significant way to the spread of antibiotic resistance in human pathogens. The issue of the use of antibiotic resistance marker genes in GM foods was discussed at the Ministerial Council meeting held in late July 2000. At that meeting, Professor John Turnidge, former Chair of JETACAR and now Chair of the NHMRC Expert Advisory Group on Antibiotic Resistance (EAGAR), appeared at the Council meeting as expert adviser on this matter in support of FSANZ's assessment on this issue.

11. Transfer of novel genes to humans

Some submitters have expressed the view that the transfer of any novel gene within the human digestive tract may be a health concern.

Response

It is extremely unlikely that novel genetic material will transfer from GM foods to bacteria in the human digestive tract because of the number of complex and unlikely steps that would need to take place consecutively. It is equally unlikely that novel genetic material will transfer from GM foods to human cells via the digestive tract. In considering the potential impact on human health, it is important to note that humans have always consumed large amounts of DNA as a normal component of food and there is no evidence that this consumption has had any adverse effect on human health. Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in GM foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

12. Viral recombination

Some submitters express concern about the long-term effects of transferring viral sequences to plants.

Response

This is an issue that is commonly raised because some of the genes that are transferred to plants use a plant virus promoter. Promoters are controlling DNA sequences which act like a switch and enable the transferred genes to be expressed (i.e. to give rise to a protein product) in a plant cell. The routine use of these viral promoters is often confused with research which has shown that plant virus genes, which have been transferred into plants to render them virus–resistant, may recombine with related plant viruses that subsequently infect the plant, creating new viral variants. This research demonstrates that there may be a greater risk to the environment if viral genes are transferred to plants because it may lead to the generation of new plant virus variants capable of infecting a broader range of plants. This is a matter that is considered by the scientific technical committee of the Office of the Gene Technology Regulator (OGTR) on a case–by–case basis when assessing such projects.

However, the presence of plant viruses, plant virus genes or plant virus segments in food is not considered to pose any greater risk to human health as plant viruses are ubiquitous in nature and are commonly found in food eaten by animals and humans. Plant viruses are also biologically incapable of naturally infecting human or animal cells.

13. Labelling of foods produced using gene technology

Submissions generally call for comprehensive labelling of foods produced using gene technology, based on perceptions that the foods are potentially not as safe as conventional foods, even where no novel genes are present. Based on consumer “right to know” arguments, it is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

Response

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods.

Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended standard (A18 in Volume 1 and 1.5.2 in Volume 2) of the *Food Standards Code* came into effect on 7 December 2001, allowing 12 months implementation period for compliance to the new provisions.

The revised Standard requires the labelling of food and food ingredients where novel DNA and/or protein are present in the final food or where the food has altered characteristics.

Exempt from these requirements are:

- highly refined food, other than with altered characteristics, where the effect of the refining process is to remove novel genetic material and/or protein;

- processing aids and food additives, except where novel genetic material and/or protein is present in the final food;

- flavours which are present in a concentration less than or equal to 0.1 per cent in the final food; and

- food intended for immediate consumption which is prepared and sold from food premises and vending vehicles, including restaurants, takeaway outlets, caterers or self-catering institutions.

In addition, the revised Standard allows for a maximum of 1 per cent of unintended presence of genetically modified product before labelling is required. The comprehensive provisions of the new Standard represent the culmination of extensive consultation between governments; consumers and the food industry to ensure practical and relevant information is available to all in relation to the sale of genetically modified foods.

A User Guide has been prepared by the Authority under direction of the Ministerial Council, to assist with compliance with the amended labelling provisions of the Standard. A copy of the guide is available on the FSANZ website (www.foodstandards.gov.au).

14. The need for post marketing surveillance of genetically modified foods

A number of submitters have commented on the need for post-market surveillance of genetically modified food consumption.

Response

Surveillance of potential adverse or beneficial effects of GM foods is seen by many as a logical follow-up to the initial scientific risk assessment. Nevertheless, it is recognised that there are limitations to the application of epidemiology studies, particularly in relation to food components. A key requirement for post-market surveillance systems is that a clear hypothesis be identified for testing. Establishing a system for the surveillance of potential health effects of exposure to novel foods requires monitoring of the consumption patterns of novel foods in the population, and health effects in both “exposed” and “non-exposed” individuals/populations, so that risk estimates can be derived.

For any such monitoring system to be useful, there needs to be a range of exposures, otherwise, any variation in health outcome would be unexplainable by that exposure. Variations in exposure could be apparent over time (temporal trends), space (geographical trends) or both.

Availability of robust data on consumption of the foods in question is vital in order to establish a surveillance system. The other side of the equation is the need for access to data on population health outcomes. Such a system could also be used to identify potential positive health outcomes, such as improved nutritional status or lower cholesterol levels. The availability of linked basic data (e.g. date of birth, sex, geographical location), and the ability to correlate with demographic data, could potentially offer the means of establishing links with food consumption.

The possibility of setting up a post-market health surveillance system for novel foods, including GM foods, has been examined by the UK's Advisory Committee on Novel Foods and Processes (ACNFP). Recognising the many difficulties involved in developing such a system, an initial feasibility study to look at the available data and its usefulness has been proposed. Work is currently being commissioned; when completed in 18 months, it will be subject to peer review. If such a feasibility study suggests that post-market surveillance is practical, methods and details concerning data collection will be determined in the UK, but common strategies might be able to be harmonised internationally in order to minimise the use of resources while maximising the reliability of the final results. This is an area that FSANZ will be monitoring closely, along with international regulatory bodies such as the OECD Taskforce for the Safety of Novel Foods and Feeds.

15. Public consultation and information about gene technology

A number of submitters were concerned that the public has not been properly consulted or informed by government or FSANZ on the introduction of foods produced using gene technology. Some submitters urged to undertake wider consultation with all affected parties including growers, the food industry and consumers before these food commodities are introduced, and to ensure that adequate consultation is undertaken as part of its assessment process.

Response

The issue of gene technology and its use in food has been under consideration in Australia since 1992. The Agreement between the Governments of Australia and New Zealand for a joint food standard setting system, however, did not occur until 1995, and the New Zealand community therefore had not been consulted on this matter by the Authority until after that time. Consequently, the proposed standard for GM foods underwent only one round of public comment in New Zealand at which time significant objections were raised by the New Zealand community to the use of gene technology in food production. Many New Zealand consumers, in previous submissions to the Authority, have expressed the view that there has been insufficient consultation and a consistent lack of information about gene technology.

Although Standard A18 came into force in May 1999, the public have a continuous and ongoing opportunity to provide comment in relation to applications under the standard. FSANZ's statutory process for all applications to amend the *Food Standards Code* normally involves two rounds of public comment.

Furthermore, all the documentation (except for commercial in confidence information) relating to these applications is available in the public domain, including the safety assessment reports. There is ample evidence that the provision of such information by FSANZ has already significantly stimulated public debate on this matter.

In addition, other government departments including the Environmental Risk Management Authority (ERMA) are potential sources of information about gene technology available to consumers in New Zealand. ERMA is a statutory authority set up by the New Zealand Government to administer the *Hazardous Substances and New Organisms* (HSNO) *Act 1996*, and has responsibility for assessing the risks to the environment from genetically modified organisms. This body has been assessing applications for the approval of genetically modified organisms since July 1998 and this has involved a number of public meetings.

In response to the concerns raised in public submissions with regard to gene technology and GM foods, FSANZ has prepared a public discussion paper on the safety assessment process for GM foods[3], available at no charge on request. Since completion, this document has been widely distributed and may assist in addressing some of the safety concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

16. Maori beliefs and values

Some New Zealand submitters stated that Maori people find genetic engineering in conflict with their beliefs and values and that, out of respect to Maori, no genetically modified foods should be allowed into New Zealand until a wider discussion, both within Maori and non–Maori, is held.

Response

This issue was also raised during consideration of the proposal for the establishment of Standard A18. At that time, it was stated that the likely implications for Maori regarding genetically modified organisms surround the issues of the rights of Maori to the genetic material from flora and fauna indigenous to New Zealand and the release into the environment of genetically modified organisms. The HSNO Act requires that these matters be considered by ERMA.

17. Environmental concerns and the broader regulatory framework

A number of submitters have raised concerns that genetically modified crops may pose a risk to the environment.

Response

These issues are considered as part of the comprehensive assessment processes of the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand. Since June 2001, OGTR regulates all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

[3] Gm foods and the consumer – FSANZ Occasional Paper Series No.1, Australia New Zealand Food Authority, June 2000.

FSANZ does not have the legal authority to assess matters relating to potential environmental risks resulting from the release of foods produced using gene technology into the environment. However, links exist between FSANZ and other government regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs.

In Australia, government agencies with a legal remit to regulate some aspects of GM products (such as imports, food, agricultural and veterinary chemicals) are:

- Food Standards Australia New Zealand (FSANZ)
- Therapeutic Goods Administration (TGA)
- National Registration Authority for Agricultural and Veterinary Chemicals (NRA)
- National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
- Australian Quarantine and Inspection Service (AQIS).

All GM foods are assessed by FSANZ, approved by the Board of FSANZ and then notified to the Ministerial Council (Australia New Zealand Food Regulation Ministerial Council) comprised of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister. However, an interface between FSANZ and OGTR has been established through amendments to the FSANZ Act arising from the Gene Technology Bill 2000. These amendments to the FSANZ Act require the Authority to advise OGTR of outcomes of the Board's consideration of applications at initial, draft and final assessment in relation to the standard for foods produced using gene technology (Standard 1.5.2).

Similarly, in New Zealand various other government departments and agencies play a specific role in the regulatory process. These are:

- the Ministry of Agriculture, Fisheries (MAF)
- the Ministry of Health (MoH)
- the Ministry of Research, Science and Technology (MoRST)

18. Maximum residue levels of agricultural/veterinary chemicals

A number of submitters have raised concerns that residues of agricultural and veterinary chemicals in genetically modified (e.g. herbicide tolerant) crops may pose a health risk.

Response

Residues of these chemicals can only legally be present if the chemical has been registered for use in Australia and/or New Zealand, and it has been demonstrated that the residue at specified levels does not lead to adverse health impacts. The concentration of a chemical residue that may be present in a food is regulated through maximum residue limits (MRLs). The MRL is the highest residue concentration that is legally permitted in the food. Food products have to meet the MRL, whether or not they are derived from genetically modified organisms. The MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

It is important to note that MRLs are not direct public health and safety limits but rather, are primarily indicators of appropriate chemical usage. MRLs are always set at levels lower than, and normally very much lower than, the health and safety limits. The MRL is determined following a comprehensive evaluation of scientific studies on chemistry, metabolism, analytical methods and residue levels. In Australia, the National Registration Authority (NRA) applies to FSANZ to amend the MRLs in the Food Standards Code and the application is considered by FSANZ through its legislated decision making processes. In New Zealand MRLs are set by the Ministry of Health, generally following a request from, and in collaboration with, the Ministry of Agriculture and Forestry. Only following demonstration that the use of agricultural and veterinary chemicals will not result in unsafe residues will the MRL enter into food law, through its inclusion in either the Food Standards Code in Australia, or the New Zealand Mandatory Food Standard 1999 (Maximum Residue Limits of Agricultural Compounds).