ANZFA
Australia New Zealand Food Authority
TE MANA WHAKARITE KAI MŌ ĀHITEREIRIA ME AOTEAROA

10/02
26 June 2002

FINAL ASSESSMENT REPORT
(INQUIRY - SECTION 17)

APPLICATION A380

FOOD FROM INSECT-PROTECTED AND GLUFOSINATE AMMONIUM-TOLERANT DBT418 CORN

THE AUSTRALIA NEW ZEALAND FOOD AUTHORITY

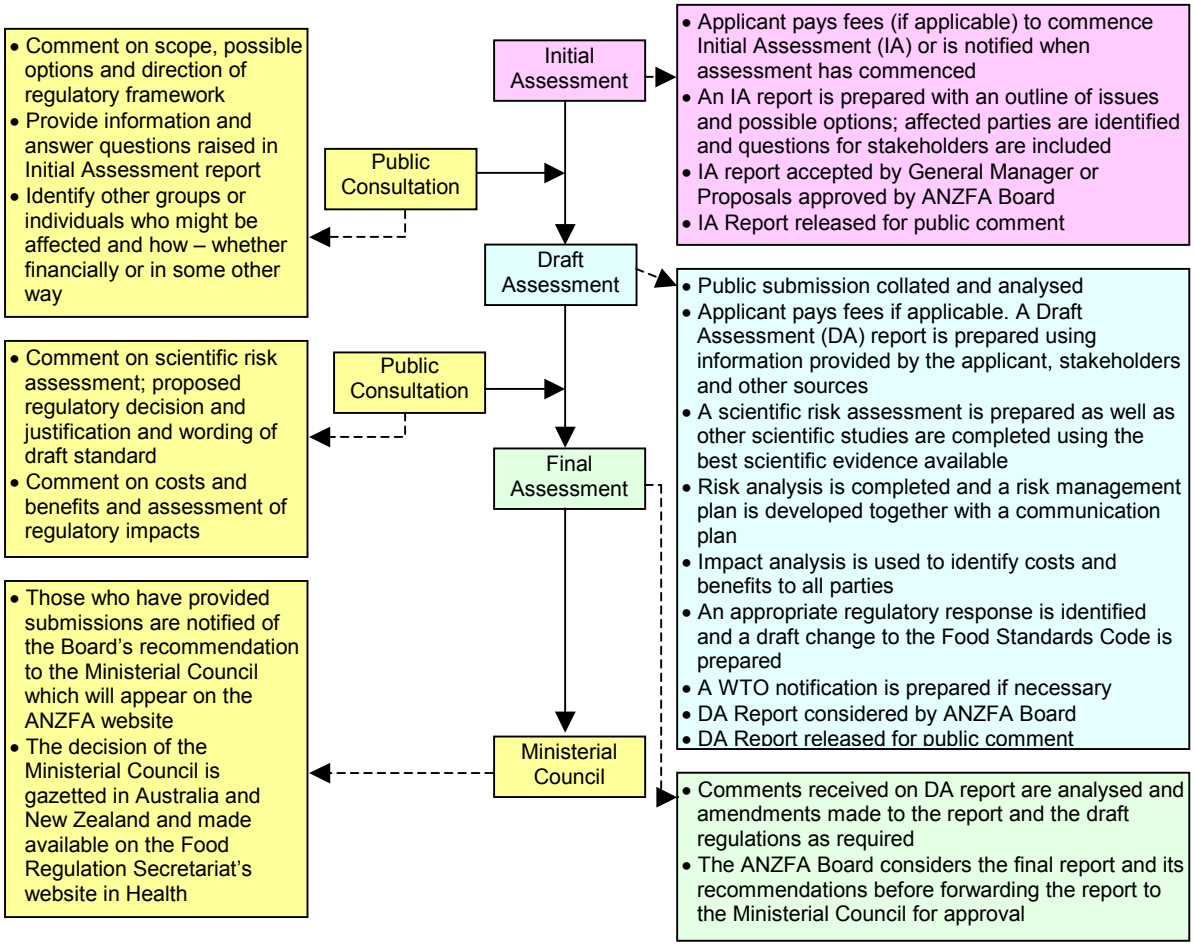
The Australia New Zealand Food Authority's (ANZFA) is a partnership between the Commonwealth Government, Australian State and Territory governments and the New Zealand Government. ANZFA is a bi-national, statutory body whose role, in association with others, is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply.

ANZFA seeks to achieve this goal by developing, varying and reviewing standards for food available for sale in Australia and New Zealand and through a range of other functions including national food surveillance and recall systems, conducting research, assessing policies about imported food and developing codes of practice with industry.

In developing and reviewing food standards for both Australia and New Zealand, ANZFA makes recommendations to change the food standards to the Australia New Zealand Food Standards Council, a Ministerial Council made up of Commonwealth, State and Territory and New Zealand Health Ministers. If the Council approves the recommendations made by ANZFA, the food standards are automatically adopted as regulations into the food laws of the Australian States and Territories and New Zealand.

STEPS IN DEVELOPING AND REVIEWING FOOD STANDARDS

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Australia New Zealand Food Authority Act 1991* (ANZFA 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.



CONTENTS

EXECUTIVE SUMMARY AND STATEMENT OF REASONS **4**
1. INTRODUCTION **5**
2. REGULATORY PROBLEM **5**
3. OBJECTIVE **6**
4. BACKGROUND **6**
5. RELEVANT ISSUES **7**
5.1 SAFETY ASSESSMENT 7
5.2 LABELLING OF FOOD DERIVED FROM DBT418 CORN 7
5.3 ISSUES ARISING FROM PUBLIC SUBMISSIONS 7
5.4 RISK ANALYSIS 11
6. REGULATORY OPTIONS **12**
7. IMPACT ANALYSIS **12**
8. CONSULTATION **13**
9. CONCLUSION AND RECOMMENDATION **14**
ATTACHMENT 1: DRAFT VARIATION TO THE *FOOD STANDARDS CODE* **16**
ATTACHMENT 2: SAFETY ASSESSMENT REPORT **17**
ATTACHMENT 3: SUMMARY OF PUBLIC SUBMISSIONS **51**
ATTACHMENT 4: GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS **65**

EXECUTIVE SUMMARY AND STATEMENT OF REASONS

Regulatory problem

An Application was received from Monsanto Australia Ltd on 30 April 1999 for the approval of food from insect-protected and glufosinate ammonium-tolerant DBT418 corn under the transitional arrangements of Standard A18 (clause 2A)/Standard 1.5.2 (clause 3). Food containing ingredients derived from DBT418 corn is thus already permitted in the food supply. The growing of DBT418 corn was discontinued in 1999 however as significant quantities were planted in the final year of production small amounts may still be present in certain imported food products.

Objective

In addressing the issue of approving the sale and use of food from DBT418 corn, the key objectives were the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, ANZFA also had regard for the need for standards to be based on risk analysis using the best available scientific evidence and the desirability of an efficient and internationally competitive food industry.

Options

Two regulatory options were considered. Option 1 involved the withholding of approval and Option 2 involved the granting of approval for the food. Option 1 would result in the removal from sale of food containing ingredients derived from DBT418 corn whereas Option 2 would maintain the *status quo* and allow food containing DBT418 corn to remain on the market.

Impact

The impact on all sectors of approving food from DBT418 corn (Option 2) was found to be minimal because firstly, DBT418 corn is no longer grown therefore its presence in the food supply has been steadily declining since 2000, and secondly, as food from DBT418 corn is already permitted in the food supply, the giving of approval merely serves to maintain the *status quo*.

Consultation

ANZFA undertook two rounds of public consultation in relation to this application. In response, 45 submissions were received during the first round, and 22 submissions were received in the second round. The majority of the submissions received were not supportive. Those opposing the application did so primarily on the basis of perceived health and environmental concerns. The food safety concerns raised in submissions have been addressed by the safety assessment report.

Conclusions and recommendation

Adoption of the draft variations, giving approval to the continued sale of food derived from DBT418 corn in Australia and New Zealand, is recommended for the following reasons:

- the genetic changes introduced into DBT418 corn are not considered to raise any additional public health and safety concerns;

- food from DBT418 corn is as safe and wholesome as food from other commercially available corn varieties;

- food products derived from DBT418 corn will require labelling if it can be shown that novel DNA and/or protein is present in the final food;

- the proposed amendments to the *Food Standards Code* are consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991; and

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

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

1. Introduction

An application was received from Monsanto Australia Ltd seeking approval of food from insect-protected and glufosinate ammonium-tolerant DBT418 corn.

The Application was received on 30 April 1999 and was accepted under the transitional arrangements of Standard A18 (clause 2A)/Standard 1.5.2 (clause 3), which allow food, containing ingredients derived from DBT418 corn, to be on the market prior to finalisation of the assessment process. This arrangement is subject to certain conditions being met (see below). The assessment is at the final assessment stage.

2. Regulatory Problem

The provisions of Standard A18 / 1.5.2 require that genetically modified (GM) foods undergo a pre-market risk assessment before being offered for sale in Australia and New Zealand. For those foods that were already on the market prior to the Standard coming into effect, an exemption from pre-market approval applies under transitional arrangements provided that an application was accepted by ANZFA on or before 30 April 1999, that the food is lawfully permitted in a country other than Australia or New Zealand, and that the Ministerial Council has not become aware of evidence that the food poses a significant risk to public health and safety. Foods assessed under the transitional arrangements, confirmed as safe, and subsequently approved by the Ministerial Council are listed in the Table to the Standard.

A new genetically modified variety of insect protected and glufosinate ammonium-tolerant corn – DBT418 corn – was developed for cultivation in the United States and was entering into Australia and New Zealand in imported food products. Monsanto Australia Ltd therefore made an application, under the transitional arrangements, to have the Standard amended to include food from DBT418 corn. Since making the Application, DBT418 corn has subsequently been discontinued, its last planting being in 1999. However as significant quantities of DBT418 corn were planted in its final year of production, there is still potential for DBT418 corn to be present in small amounts in certain imported food products.

3. Objective

To determine whether food regulations can be changed to permit the sale of a GM food. Such an assessment needs to consistent with the section 10 objectives of the ANZFA Act.

ANZFA's objectives in developing and varying food standards are (in descending priority order):

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

In developing and varying food standards, ANZFA must also have regard to the following:

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

In addressing the issue of approving the sale and use of food from DBT418 corn, the key objectives were the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, ANZFA also had regard for the need for standards to be based on risk analysis using the best available scientific evidence and the desirability of an efficient and internationally competitive food industry.

4. Background

DBT418 corn has been genetically modified to be protected against the insect pest European corn borer (*Ostrinia nubilalis*) and tolerant to the herbicide glufosinate ammonium. Protection against European corn borer is achieved through expression in the plant of a protein, called CryIAc that is produced naturally by the *kurstaki* subspecies of the spore-forming soil bacterium *Bacillus thuringiensis.* The majority of described *B. thuringiensis* strains produce proteins that have insecticidal activity against lepidopteran insects (larvae of moths and butterflies) although a few have activity against dipteran (mosquitoes and flies) and coleopteran (beetles) insects. Microbial pesticide products based on *B. thuringiensis* producing CryIAc (e.g. DIPEL®) have been approved for use on a variety of crops and for home garden use and have been available in both Australia and New Zealand since 1989.

Tolerance to glufosinate ammonium is achieved through expression in the plant of the enzyme phosphinothricin acetyl transferase (PAT). PAT inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium. Glufosinate-ammonium is currently registered in Australia under the commercial name of Basta® for non-selective uses, or Finale® for turf and home garden uses, and as Buster® in New Zealand. The herbicide tolerant trait of DBT418 corn is not exploited commercially and was incorporated into the corn for selection purposes only.

DBT418 corn was developed for cultivation in the United States and was never grown in Australia or New Zealand, therefore DBT418 corn would have entered the Australian and New Zealand food supply as imported food product only. The major imported corn product

is high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Corn products are processed into breakfast cereals, baking products, extruded confectionary and corn chips. Other corn products, including maize starch used by the food industry for the manufacture of dessert mixes and canned food, are also imported.

5. Relevant Issues

5.1 Safety assessment

Food from DBT418 corn has been evaluated according to the safety assessment guidelines prepared by ANZFA[1]. The assessment considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues.

On the basis of the available information, ANZFA concluded that food from DBT418 corn is as safe and wholesome as food from other commercial corn varieties. The full safety assessment report can be found at **Attachment 2** to this document.

5.2 Labelling of food derived from DBT418 corn

On 28 July 2000 the Ministerial Council agreed to a revised standard which requires labelling of food where novel DNA and/or protein is present in the final food and also where the food has altered characteristics. The revised standard (A18 in the Australian *Food Standards Code*, 1.5.2 in the Australia New Zealand Food Standards Code) was gazetted on 7 December 2000 and came into effect 12 months from the date of gazettal.

Under these new provisions, certain food products derived from DBT418 corn are likely to require labelling.

5.3 Issues arising from public submissions

General issues

Of the 67 submissions received following two rounds of public consultation, only a small number addressed issues specific to this Application. Rather, the majority of submissions raised issues of a general nature relating to gene technology or issues that have been addressed in the safety assessment report. The most commonly raised general issues were:

- environmental concerns about the growing of GM crops;
- concerns about the use of antibiotic resistance genes;
- concerns about the use of agricultural chemicals;
- labelling of GM foods;
- reliance by ANZFA on industry-generated data for the safety assessment; and
- viral recombination.

[1] ANZFA (2001) Guidelines for the safety assessment of genetically modified foods. In: *Information for Applicants – Amending Standard A18/Standard 1.5.2 Food Produced using Gene Technology*. (www.anzfa.gov.au/_srcfiles/GM Guidelines Nov 01.pdf)

A discussion of these and other general issues raised in public submissions for this and other applications can be found in **Attachment 4**. This includes matters such as the publishing of the report of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on "New Biotechnology Food and Crops: Science, Safety and Society", the United Kingdom Royal Society's report on "Genetically modified plants for food use and human health – an update" and the deliberations of various international committees and taskforces including those of the Codex Alimentarius Commission, the OECD and FAO/WHO Expert Consultations.

Specific issues

This section of the report will only address those issues raised in public submissions that are specific to an assessment of this application.

(i) Allergenic effects of novel genes

Diane Davie suggested that the use of herbicide-resistance genes could increase allergies.

Response

The safety assessment undertaken by ANZFA has addressed the issue of the potential allergenicity of PAT in some depth (see **Attachment 2**). Data were evaluated on a comparison of the amino acid sequence of PAT to that of known allergens, its resistance to acid and protease digestion, and its presence in the food as consumed. PAT does not come from a source that is known to be allergenic and has none of the characteristics that are common to food allergens, nor does it have any significant amino acid sequence similarity to known allergens. For these reasons it is considered to have very limited potential to become a food allergen.

(ii) Use of glufosinate ammonium

Several submitters including the Consumers' Association of South Australia Inc. and the National Council of Women of Australia (NCWA) raised the issue of herbicide toxicity and expressed concern that that the use of glufosinate-ammonium tolerant corn may lead to increased use of the herbicide on the crop, which in turn may necessitate an increase in the Maximum Residue Limit (MRL) for glufosinate-ammonium. The South Australia Public and Environmental Health Service consider that the ANZFA safety assessment should address the issue of whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.

Response

There is currently no MRL for either glufosinate ammonium or its metabolites in corn in Australia. Similarly, in New Zealand no MRL exists, although a level of 0.1 ppm is allowed under sub-regulations 257 (4) and (9) of the *Food Regulations 1984*. A Codex MRL of 0.1 ppm also exists. However, the glufosinate ammonium-tolerant trait that was introduced into DBT418 corn was used as a marker to facilitate the selection of transformed cells from non-transformed cells during the plant transformation procedure and was not exploited commercially in DBT418 corn. In other words, the herbicide was not used during the commercial cultivation of the plants and the Product Use Guide that accompanied batches of

DBT418 corn supplied to growers specifically recommended against its use. DBT418 corn was specifically marketed for its insect-protection trait only and no residues of glufosinate ammonium should be present on the kernels.

(iii) Use of Bt toxins

Mr Arnold Ward, the NCWA and the Western Australia Department of Health expressed concerns about the effect of *Bt* toxin on humans. The Australian GeneEthics Network stated that the *Bt* insecticidal proteins have no history of safe use in the animal and human food supplies and that their long-term impacts are unknown. The New Zealand Ministry of Health (NZMoH) noted the epidemiological evidence regarding the safety of *Bt* proteins used as the active ingredient of insecticidal sprays, but considered that ANZFA's assessment should address the biochemistry of the *Bt* protein, and why it is unlikely to cause any harmful effects when consumed by humans.

Response

The toxicity and allergenicity of the *Bt* toxin have been reviewed in the safety assessment report (**Attachment 2**). *Bt* toxins have a long history of safe use as insecticidal sprays applied directly to crops for over 30 years with no reports of human, or mammalian, toxicity or allergenicity.

While it is correct that the CryIAc protein is not used directly as a food or in a feed source, *Bacillus thuringiensis* is nevertheless ubiquitous in nature and commonly present as a contaminant on food. The donor organism *B. thuringiensis* subsp. *kurstaki (B.t.k.)*, which produces the insecticidal protein, is the basis of microbial formulations used commercially for Lepidopteran insect control for over 30 years. These microbial formulations have been used on a wide variety of crops, including fresh produce such as lettuce and tomato, with no reports of human, or mammalian, toxic or allergenic responses.

The mode of action of the *Bt* toxins has been thoroughly studied. The *Bt* toxin (Cry) proteins only bind to specific receptors on the surface of gut cells of specific insects. Binding of the Cry protein results in lysis of insect midgut epithelial cells, leading to gut paralysis, cessation of feeding and the eventual death of the insect. These receptors do not exist in humans or mammals and therefore the Cry protein cannot exert the same toxic effect in mammals, including humans. The CryIAc protein does not share the biochemical properties common to known allergens.

The Applicant provided direct experimental evidence of the absence of acute toxicity, with doses of up to 5000 mg/kg bodyweight in mice, far higher than those estimated to be ingested by humans through normal dietary intake. The level of the CryIAc protein in corn kernels, the only part of the plant used for human food, is very low – ranging from 36.0 – 42.8 ng/g dry weight (equivalent to about 0.0001% of the total protein). Furthermore, the processing steps for corn would be expected to remove and/or destroy the CryIAc protein. Thus the level of CryIAc protein present in processed products derived from DBT418 corn would be extremely low.

It is therefore concluded food products derived from corn containing CryIAc are safe for human consumption.

(iv) Molecular characterisation of the insert

The NZMoH stated in its submission that DBT418 corn contains an unusually long insert compared with other transformation events and although the sequence around the truncated *pinII* gene was assessed for open reading frames there is no comment at the 5' end of the construct, or the segments before the non-functional *bar* gene. The Ministry added that it is highly unlikely that there is any potential for expression from these small segments but an examination of sequence data would provide additional assurance.

Response

The sequence and open reading frame analysis which was done at the 3' end of the insert was necessary because of the significant number of rearrangements that had occurred in that region. As the rearrangements were quite complex the only way to analyse them with any certainty was by sequence analysis.

The 5' end of the insert was without significant rearrangement, with all the inserted gene cassettes being intact. The segments referred to by the NZMoH are actually located towards the middle of the insert and consist of a small *bar* fragment followed by a small Tr7 fragment (contains signals for transcription termination). Both fragments were minor and given their small size and lack of proximity to a functional promoter were considered unlikely to have any potential for generating an open reading frame capable of expression therefore additional analyses of this region were considered unnecessary.

(v) Compositional analysis of sprayed plants

The NZMoH stated that it would have strengthened the nutritional analysis to have also examined material from plants sprayed with glufosinate ammonium, as it is possible that this could occur in farming situations. However, it considered this would be unlikely to affect the outcome of the assessment.

Response

Ordinarily, when the composition of herbicide tolerant plants is assessed for the comparative analysis the constituents being measured (e.g. protein, vitamins, fatty acids, etc) are analysed in both sprayed and unsprayed plants. The purpose of this is to firstly establish if the herbicide has any unexpected effect on the composition of the plant (i.e. the unsprayed plants act as the control), and secondly, to reproduced as closely as possible the actual commercial growing conditions so that the composition data is representative of the food being produced for human consumption.

In the case of DBT418 corn, the glufosinate ammonium-tolerant trait was used during laboratory stages only and was never exploited commercially. Moreover, the growers of DBT418 corn were specifically instructed not to use the herbicide on the crop. Therefore, in this instance, it was the unsprayed plants that more closely resembled the actual growth conditions of plants used for commercial food production.

(vi) Analysis for anti-nutrients

The West Australian Department of Health suggested it would have been useful to have some

anti-nutritional tests carried out on the corn, especially for the trypsin and chymotrypsin protein inhibitors. This was suggested in consideration of the potential for the gene transformation to result in increases in these anti-nutritional components. The WA Department of Health added the argument – that these compounds are low in corn and therefore do not need to be tested – is inconsistent with rigorous scientific evaluation.

Response

In undertaking a comparative analysis of a GM food relative to the traditional or non-GM counterpart, the assessment focuses on the key constituents of the particular food in question. The purpose of such an assessment is not to establish that the GM food is identical or equivalent to the non-GM counterpart in terms of every single food constituent, but rather to concentrate on those substances most relevant to the safety and nutritional adequacy of the food. For this reason, the assessment focuses on those constituents that have a substantial impact in the overall diet and therefore these constituents will differ from crop to crop.

In the case of corn, anti-nutrients such as trypsin and chymotrypsin inhibitors are present at very low levels and are not considered nutritionally significant and are therefore not routinely analysed in either conventional or genetically modified corn varieties.

(vii) Approval of a discontinued line

The NCWA noted that DBT418 corn has been discontinued and that approval is being recommended for a line that is no longer planted. They stated that manufacturers should be able to ascertain whether food from this line is entering Australia or not and if it is entering Australia, they object to approving it simply to avoid looking for it upon entry or for purposes of trade facilitation. As the line is not being grown now, they see no valid reason why it should be approved in Australia or New Zealand.

Response

The Application for approval of food from DBT418 corn was received on 30 April 1999 and was accepted under the transitional arrangements; meaning food containing ingredients derived from DBT418 corn was already on the market prior to the GM food standard taking effect. During the course of the assessment of this application, the applicant advised ANZFA that the crop was being discontinued, the last plantings being in 1999 in the United States. At that time, and on subsequent occasions, ANZFA discussed with the applicant whether the application was still necessary. As significant quantities of the crop were planted in 1999 the applicant has argued, and ANZFA accepted, that there is still a distinct possibility that certain food products may contain ingredients derived from DBT418 corn. Given ANZFA's subsequent assessment confirming that food derived from DBT418 corn is as safe as food derived from conventional corn varieties, ANZFA has recommended approval of the food. Manufacturers using corn-derived ingredients will still be required to firstly ascertain whether they are using approved ingredients in their food products and secondly whether labelling is required.

5.4 Risk analysis

Under the *Food Standards Code*, a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines.

On the basis of the safety assessment, together with a consideration of the public submissions, it is concluded there are no public health and safety concerns associated with the consumption of food derived from DBT418 corn. Food from DBT418 corn will require labelling if it can be established that novel DNA or protein is present in the final food.

In relation to the concerns raised in the public submissions with regard to gene technology and GM food, ANZFA has prepared a public discussion paper on the safety assessment process for GM food[2]. This is widely available and may assist in addressing some of the concerns raised by the public. In addition, in collaboration with Biotechnology Australia, ANZFA has produced an information pamphlet entitled *Genetically Modified Foods* that has been distributed throughout Australian supermarkets.

Other government agencies such as the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand, and industry bodies are also addressing the broader concerns in relation to gene technology.

6. Regulatory Options

The following regulatory options were considered:

Option 1 – do not approve food derived from insect-protected and glufosinate ammonium-tolerant DBT418 corn. Standard A18/1.5.2 of the *Food Standards Code* would not be amended to include food from DBT418 corn. Food already in the food supply containing ingredients derived from DBT418 corn would need to be removed from sale.

Option 2 – approve food derived from insect-protected glufosinate ammonium-tolerant DBT418 corn. Amend Standard A18/1.5.2 of the *Food Standards Code,* as sought by the applicant, and include food from DBT418 corn in the Table to the Standard. Food containing ingredients derived from DBT418 corn already in the food supply could continue being sold. Food containing ingredients derived from DBT418 corn would have to be labelled if novel DNA and/or protein are present in the final food.

7. Impact Analysis

7.1 Affected parties

The affected parties are:

1. Governments – State, Territory and New Zealand Health Departments, AQIS;
2. Consumers; and
3. Manufacturers, producers and importers of food products.

[2] ANZFA (2000) GM foods and the consumer: ANZFA's safety assessment process for genetically modified foods. ANZFA Occasional Paper Series No. 1.

7.2 Impact of Option 1

Consumers:
- would mainly impact on those consumers who perceive GM food to be unsafe, and who may consider the prohibition of food derived from DBT418 corn, and its subsequent removal from the market, to provide a public health and safety benefit.
- costs on consumers would be minimal as very few foods derived from DBT418 corn probably remain on the market due to the crop being discontinued in 1999.

Industry:
- DBT418 corn was last grown in 1999 therefore there would be very few products remaining in commerce containing ingredients derived from DBT418 corn, however, small disruptions could occur from the removal of the remaining products from sale.

Government:
- enforcement agencies would need to identify those few remaining products that contain food derived from DBT418 corn in order to remove them from the food supply or prevent importation.
- as prohibition is unlikely to affect many products, a challenge under the World Trade Organization is doubtful.

7.3 Impact of Option 2

Consumers:
- no direct impact as *status quo* maintained.

Industry:
- no direct impact as *status quo* maintained

Government:
- no direct impact other than minor costs associated with amending the *Food Standards Code*

The regulatory impact on all sectors of approving food derived from DBT418 corn is minimal because firstly, DBT418 corn is no longer grown therefore its presence in the food supply has been steadily declining since 2000, and secondly, as food from DBT418 corn is already permitted in the food supply under transitional arrangements, the giving of approval merely serves to maintain the *status quo*. It is concluded that the amendment to the *Food Standards Code* is necessary, cost effective and of net benefit to both food producers and consumers.

8. Consultation

8.1 Public consultation

The Initial Assessment (Preliminary Assessment – section 13) of this Application was released for public comment on 3 November 1999. A total of 45 submissions were received in response to the initial assessment report. A Draft Assessment (Full Assessment – section 15) of the Application, including a comprehensive safety evaluation of the food and consideration of issues raised by public submissions, was subsequently released for public comment on 6 February 2002 for a period of six weeks. A total of 22 submissions were received.

This Final Assessment Report completes the assessment by ANZFA, again taking into account comments received from the public. ANZFA's recommendation will then be submitted to the Ministerial Council for consideration. **Attachment 3** contains a summary of all submissions received.

8.2 Notification to the World Trade Organization

During the ANZFA 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.

As there is significant international interest in the safety of GM foods, and the proposed amendments are likely to have a liberalizing effect on international trade, this application was notified to the WTO as a potential TBT or SPS matter.

9. Conclusion and Recommendation

Adoption of the draft variation (**Attachment 1**), giving approval to the continued sale of food derived from DBT418 corn in Australia and New Zealand, is recommended for the following reasons:

- the genetic changes introduced into DBT418 corn are not considered to raise any additional public health and safety concerns;
- food from DBT418 corn is as safe and wholesome as food from other commercially available corn varieties;
- food products derived from DBT418 corn will require labelling if it can be shown that novel DNA and/or protein is present in the final food;
- the proposed amendment to the *Food Standards Code* is consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991; and
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The process concluded that the amendment to the *Food Standards Code* is necessary, cost effective and of net benefit to both food producers and consumers.

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

Submissions

No submissions on this matter are sought as the Authority has completed its assessment and the matter is now with the Australia New Zealand Food Standards Council for consideration.

Further Information

Further information on this and other matters should be addressed to the Standards Liaison Officer at the Australia New Zealand Food Authority at one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2258
email: slo@anzfa.gov.au

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
email: nz.reception@anzfa.gov.au

Assessment reports are available for viewing and downloading from the ANZFA website www.anzfa.gov.au . People without access to internet facilities may request paper copies of reports from the Information Officer.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety assessment report
3. Summary of public comments
4. General issues raised in public comments

ATTACHMENT 1

DRAFT VARIATIONS TO THE *FOOD STANDARDS CODE*

APPLICATION A380 – FOOD DERIVED FROM INSECT-PROTECTED AND GLUFOSINATE AMMONIUM-TOLERANT DBT418 CORN

To commence: On gazettal

[1] ***Standard A18*** *of Volume 1 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 DBT418 corn

[2] ***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 DBT418 corn

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

APPLICATION A380 – FOOD DERIVED FROM INSECT-PROTECTED AND GLUFOSINATE AMMONIUM-TOLERANT DBT418 CORN

SUMMARY AND CONCLUSIONS

Insect-protected and glufosinate ammonium-tolerant DBT418 corn has been assessed by ANZFA to evaluate its safety as a food. A number of criteria have been addressed in this assessment including: a characterisation of the genes, their origin and function; the changes at the DNA, protein and whole food levels; stability of the introduced genes in the corn genome; compositional analyses; evaluation of intended and any unintended changes; and the potential of the newly expressed proteins to be allergenic or toxic.

Nature of the genetic modification

Insect-protected and glufosinate ammonium-tolerant DBT418 corn was generated through the transfer of the *cryIAc* and *bar* genes to the inbred corn line, AT824. The *cryIAc* gene is derived from *Bacillus thuringiensis* subspecies *kurstaki* and encodes the insecticidal crystal protein CryIAc, the toxic effect of which is specific to Lepidopteran insects, including the European corn borer (ECB). The *bar* gene is derived from *Streptomyces hygroscopicus* and encodes the enzyme phosphinothricin acetyltransferase (PAT) which inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides. The herbicide tolerant trait was used as a marker to facilitate the selection of transformed cells from non-transformed cells during the plant transformation procedure and is not exploited commercially in DBT418 corn.

Other genes transferred along with the *cryIAc* and *bar* genes were *bla* and *pinII*. The *bla* gene is derived from *Escherichia coli* and is used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It codes for the enzyme β-lactamase and confers resistance to a number of β-lactam antibiotics such as ampicillin. The *pinII* gene is derived from potato (*Solanum tuberosum* L.) and encodes a serine protease inhibitor that is reported to enhance the insecticidal activity of CryIAc against various lepidopteran pests. The *pinII* gene in DBT418 corn is non-functional and does not give rise to any protein products.

Molecular and genetic analyses of the DBT418 corn indicate that the transferred genes are stably integrated into the plant genome and are stably inherited from one generation to the next.

General safety issues

Corn (*Zea mays* L.) is used as a staple food by a significant proportion of the world's population. Corn-based products are routinely used in a large number and diverse range of foods, and have a long history of safe use. Products derived from DBT418 corn may include highly processed corn products such as flour, breakfast cereals, high fructose corn syrup and other starch products.

DBT418 corn was shown to produce two new proteins at very low levels – CryIAc and PAT. PAT is expressed at significantly higher levels than CryIAc in DBT418 corn. In kernels, mean CryIAc levels ranged from 36.0 – 42.8 ng/g dry weight (equivalent to about 0.0001% of the total protein) and mean PAT levels ranged from 3.1 – 6.0 µg/g dry weight (equivalent to about 0.0175% of the total protein). Higher levels of CryIAc and PAT were detected in other parts of the plant, particularly the leaves, however these are not used for human consumption.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells in the human digestive tract. Much of the concern in this regard relates to the presence of antibiotic resistance genes. In the case of DBT418 corn, it was concluded that the *bla* gene would be extremely unlikely to transfer to bacteria in the human digestive tract because of the number and complexity of the steps that would need to take place consecutively. More importantly however, in the highly unlikely event that transfer did occur, the human health impacts would be negligible because ampicillin resistant bacteria are already commonly found in the human gut and in the environment. Transfer of other novel genetic material from DBT418 corn to human cells via the digestive tract was also considered to be equally unlikely.

Toxicological issues

The presence of naturally occurring toxins and allergens in DBT418 corn was investigated, as well as the potential toxicity and allergenicity of the two novel proteins — CryIAc and PAT. Corn contains no naturally occurring toxins or allergens and, as noted above, has a long history of safe use. In addition, the Cry proteins from *B. thuringiensis* have a long history of safe use as insecticides.

The newly expressed CryIAc and PAT proteins in DBT418 corn were evaluated for their potential to be toxic to humans using acute toxicity testing in animals. For CryIAc, no deaths or other adverse signs were recorded in mice at doses up to 3825 mg/kg bodyweight. In a similar study using PAT, no deaths or other adverse signs were recorded at doses up to 2500 mg/kg bodyweight. No deaths or other adverse signs were also observed in an acute toxicity study with birds using 200 000 ppm of lyophilised DBT418 leaf tissue. As the CryIAc and PAT expression levels in corn kernels are low, exposure to both proteins through the consumption of DBT418 corn products would be very low, and certainly well below the levels found to be safe in acute toxicity tests using animals.

The potential allergenicity of the novel proteins was investigated by evaluating whether either of the proteins exhibited any of the characteristics of known allergens. Both proteins are rapidly digested in simulated mammalian digestive systems and a comparison of their amino acid sequence with that of known allergens did not reveal any biologically or immunologically significant similarities. Furthermore, both proteins are expressed in corn kernels at low levels indicating there would be little potential for allergic sensitisation.

Therefore, the evidence does not indicate that there is any potential for either CryIAc or PAT to be toxic to humans and also indicates that both proteins have limited potential as food allergens.

Nutritional issues

Compositional analyses were done to establish the nutritional adequacy of DBT418 corn, and to compare it to non-transformed control lines. The components measured were protein, oil, moisture, starch, fibre, ash, fatty acids, amino acids, as well as the minerals phosphorous and calcium. No significant differences in the levels of these major constituents or nutrients between transgenic and control lines were observed. Therefore, on the basis of the data submitted in the present application, DBT418 corn can be considered compositionally no different to other commercial corn varieties.

Conclusion

No potential public health and safety concerns have been identified in the assessment of DBT418 corn. Therefore, on the basis of the data provided in the present application, and other available information, foods derived from DBT418 corn can be considered as safe and wholesome as foods derived from other corn varieties.

1. BACKGROUND

Monsanto Australia Ltd has made an application to ANZFA to amend Standard A18/Standard 1.5.2 of the *Food Standards Code*, to include food derived from corn, genetically modified to be protected from lepidopteran insects, particularly the European corn borer, and tolerant to the herbicide glufosinate ammonium. The corn is commonly known as 'DBT418 corn' and when DBT418 hybrids are sold commercially the suffix 'BtX' is incorporated into the name of the hybrid corn (e.g. DK493BtX).

Protection against European corn borer (*Ostrinia nubilalis*) is achieved through expression in the plant of a protein – called CryIAc – that is produced naturally by the *kurstaki* subspecies of the spore-forming soil bacterium *Bacillus thuringiensis*. The majority of described *B. thuringiensis* strains produce proteins that have insecticidal activity against lepidopteran insects (larvae of moths and butterflies) although a few have activity against dipteran (mosquitoes and flies) and coleopteran (beetles) insects. Microbial pesticide products based on *B. thuringiensis* producing CryIAc (e.g. DIPEL®) have been approved for use on a variety of crops and for home garden use and have been available in both Australia and New Zealand since 1989.

Tolerance to glufosinate ammonium is achieved through expression in the plant of the enzyme phosphinothricin acetyl transferase (PAT). PAT inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium. Glufosinate-ammonium is currently registered in Australia under the commercial name of Basta® for non-selective uses, or Finale® for turf and home garden uses, and as Buster® in New Zealand. The herbicide tolerant trait of DBT418 corn is not exploited commercially and was incorporated into the corn for selection purposes only.

Corn varieties containing the DBT418 transformation event were developed for cultivation in the United States. This variety has since been discontinued, its last planting being in 1999, however as significant quantities were planted in its final year of production, there is still potential for DBT418 corn to be present in corn products imported into Australia and New Zealand from the United States. The major imported corn product is high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Corn products are processed into breakfast cereals, baking products, extruded confectionary and corn chips. Other corn products, including maize starch used by the food industry for the manufacture of dessert mixes and canned food, are also imported.

2. DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modification

DBT418 corn was produced by the simultaneous introduction of DNA from three different plasmids (pDPG699, pDPG165 and pDPG320) into embryogenic cells of the inbred corn line AT824 using the technique of microprojectile bombardment (Gordon-Kamm *et al* 1990).

2.2 Function and regulation of the novel genes

Transformation of corn with plasmids pDPG699, pDPG165 and pDPG320 resulted in the transfer of three gene expression cassettes — *cryIAc*, *bar* and *pinII*. Each of these expression cassettes is described in Table 1 below.

Table 1: Gene expression cassettes in pDPG699, pDPG165 and pDPG320

Cassette	Genetic element	Source	Function
pDPG699:			
CryIAc	OCS-35S promoter	OCS is a 20 bp enhancer sequence derived from the T-DNA of *Agrobacterium tumefaciens* (Benfey and Chua 1990, Bouchez *et al* 1989). Two copies of OCS were positioned upstream of the 90 bp A domain of the cauliflower mosaic virus (CaMV) 35S promoter (Odell *et al* 1985).	A chimeric promoter for high level gene expression in plant cells. The OCS enhancer is known to promote expression of genes in most vegetative plant tissues.
	adh1 intron VI	The intron VI from the maize alcohol dehydrogenase I (*adh1*) gene (Dennis *et al* 1984).	Used to improve transcription of the *cryIAc* gene.
	cryIAc	Synthetic gene encoding the first 613 amino acids of the HD73 CryIAc endotoxin from *B. thuringiensis* (Adang *et al* 1985).	Confers protection against lepidopteran insects, including the European corn borer.
	pinII 3'	The putative 3' untranslated region and transcription termination region of the protease inhibitor II (*pinII*) gene from potato (Thornburg *et al* 1987).	Contains signals for termination of transcription and directs polyadenylation.
pDPG165:			
bar	35S promoter	A promoter derived from the cauliflower mosaic virus (Odell *et al* 1985).	A promoter for high-level constitutive gene expression in plant tissues.
	bar	Gene from *Streptomyces hygroscopicus* encoding phosphinothricin acetyltransferase (De Block *et al* 1987, White *et al* 1990).	Confers tolerance to phosphinothricin, the active constituent of glufosinate ammonium herbicides.
	Tr7 3'	The 3' untranslated region from *A. tumefaciens* T-DNA transcript 7 (Dhaese *et al* 1983).	Contains signals for termination of transcription and directs polyadenylation.
pDPG320:			
pinII	35S promoter	As above.	As above.
	adhI intron I	The first intron from the maize *adhI* gene (Dennis *et al* 1984).	As above.
	pinII	Gene from potato encoding protease inhibitor II (Thornburg *et al* 1987).	Inhibits serine proteases and has been shown to inhibit both trypsin and chymotrypsin (Ryan 1990).
	Tr7 3'	As above.	As above.

The cryIAc gene

The *cryIAc* gene used is a synthetic version of the native c*ry1Ac* gene derived from the soil bacterium *B. thuringiensis* subsp. *kurstaki* strain HD73 (Adang *et al* 1985). The gene is one of several that have been isolated from *B. thuringiensis* species which encode a group of proteins known as the δ-endotoxins or the crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1μm in size. These proteins are selectively active against several Orders of insects such as the Lepidoptera, Coleoptera, and Diptera. The crystal proteins are produced by the bacterium during sporulation. The protein product of the *cryIAc* gene, CryIAc, is selectively active against Lepidopteran insects (MacIntosh *et al* 1990b).

When ingested by susceptible insect species, the highly alkaline pH of the insect midgut promotes solubilisation of the protoxin–containing crystals. The protoxin is then activated by trypsin–like proteases in the insect gut which cleave off domains from the carboxy and amino–termini leaving a protease–resistant core representing the active protein. The active protein binds to highly specific glycoprotein receptors on the surface of the midgut epithelial cells in the insect (Rajamohan 1998). This binding of the protein to specialised receptors has been shown to be essential for the onset of toxicity (Wolfersberger 1990, Ferré *et al* 1991). Aggregation of the protein molecules results in formation of a pore through the cell membrane. These cells eventually swell and burst, causing loss of gut integrity and resulting in larval death within 1 to 2 days (Hofte and Whitely 1989, Schnepf *et al* 1998).

The bacterial c*ry1Ac* gene has a high content of the nucleotides guanosine (G) and cytosine (C) that is not typical of plant genes, so it is not well expressed in plants. To optimise its expression in plant cells the native *cryIAc* gene was re-synthesised to lower the GC content. This was achieved without altering the amino acid sequence so the synthetic gene encodes a protein that is identical to the first 613 amino acids of the native bacterial CryIAc protein.

The bar gene

The *bar* gene, encoding phosphinothricin acetyl transferase (PAT), has been cloned from the soil bacterium *Streptomyces hygroscopicus* (ATCC 21705) (De Block *et al* 1987) and its full DNA sequence of 549 base pairs has been published (White *et al* 1990). The GTG translation initiation codon present in the native *bar* gene from *S. hygroscopicus* was mutated to ATG to conform to plant codon usage.

PAT is produced by *S. hygroscopicus* to protect itself from the toxicity of the antibiotic (phosphinothricin alanyl alanine or bialaphos) that it produces. The PAT enzyme catalyses two reactions in the bacterium: the acetylation of demethylphosphinothricin, which is an intermediate step in the biosynthesis of bialaphos; and the acetylation of phosphinothricin, which is the activity that serves to protect *S. hygroscopicus* from phosphinothricin toxicity.

Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, was initially characterised as bialaphos produced by another bacterium *Streptomyces viridochromogenes* (Comai and Stalker 1986) and was later shown to be effective as a broad-spectrum herbicide. PPT can also be chemically synthesised. PPT is a potent competitive inhibitor of glutamine synthase (GS; EC 6.3.1.2) in plants. GS plays a central role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants.

It is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration. Inhibition of GS in plants by PPT causes rapid accumulation of ammonia leading to cell death (De Block *et al* 1987).

In DBT418 corn, the *bar* gene acts only as a selection marker, allowing plants to be distinguished from non-transformed plants; DBT418 corn is not marketed as a herbicide-tolerant variety.

The pinII gene

The *pinII* gene, encoding an inhibitor of serine proteases, was originally cloned from potato (Thornburg *et al* 1987). The encoded protein, referred to as protease inhibitor II, contains two active sites, one of which inhibits trypsin and the other which inhibits chymotrypsin (Plunkett *et al* 1982). In potato, the protease inhibitor protein is naturally expressed in leaves in response to chewing insects or other severe mechanical damage, and is thought to help defend the plant against insect predators by reducing the digestibility and nutritional quality of the leaves (Ryan 1978).

The *pinII* gene was transferred into DBT418 corn because it had been reported that high level expression of protease inhibitor II in tobacco plants had conferred resistance to a lepidopteran pest, *Manduca sexta* (Johnson *et al* 1989) and that the presence of serine protease inhibitors has served to enhance the insecticidal activity of the crystal proteins from *B. thuringiensis* subsp. *kurstaki* (MacIntosh *et al* 1990a).

Other genetic elements

The plasmid vectors also each contained a number of additional genetic elements and these are described in Table 2 below. These genetic elements are present in most *Escherichia coli* cloning vectors and are well described (Sambrook *et al* 1981). They are used to assist in the manipulation of DNA sequences as well as direct gene expression in *E. coli*.

Table 2: Additional genetic elements in plasmids pDPG699, pDPG165 and pDPG320

Genetic element	Source	Function
lac	An incomplete copy of the *lac* operon which contains a partial *lac* repressor (*lac*I) coding sequence, the promoter P_{lac}, and a partial coding sequence for β-galactosidase (*lac*Z) from the phagemid pBluescripte SK(-) (Stratagene).	Sequences used to assist in the cloning of genes into plasmids.
fl (-) ori (not in pDPG165)	Bacteriophage f1 origin of replication from phagemid pBluescript SK(-) (Stratagene).	Used to produce single stranded DNA. The f1 origin is not recognised unless bacteriophage f1 is present.
bla	The β-lactamase gene from phagemid pBluescript SK(-) (Stratagene).	Confers resistance to ampicillin and other penicillins (Sutcliffe 1978).
ColE1 ori	Plasmid origin of replication from the *Escherichia coli* high copy phagemid pBluescript SK(-) (Stratagene).	Allows plasmids to replicate in *E. coli*.

The *bla* gene is derived from the bacterium *Escherichia coli* and encodes the enzyme β-lactamase that confers resistance to a number of β-lactam antibiotics, including the moderate-spectrum penicillin, and ampicillin. The *bla* gene is under the control of a bacterial promoter and was included as a marker to allow for selection of bacteria containing pDPG699, pDPG165 and pDPG320 prior to transformation of the plant cells. Bacterial cells are plated onto medium containing ampicillin, and only those that have been transformed with the plasmid conferring antibiotic resistance will grow. As the *bla* gene is under the control of a bacterial promoter it is therefore not expressed in transformed plant cells.

2.3 Characterisation of the genes in the plant

Study submitted by Monsanto:

Stephens, M. *et al* (1996). Molecular characterization of transgene content and stability in transgenic corn hybrid line DK.DL (DBT418). Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A07.

Albee, L.D. *et al* (2001). Amended report for: Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the insert in corn event DBT418. Performing laboratory: Monsanto Company. Study No. 00-01-39-52.

Selection and derivation of plant lines

A transformed callus line, designated DBT418, was selected and individual plants were regenerated. Regenerated DBT418 plants (referred to as the T_0 generation) were then crossed with non-transformed, inbred corn lines to produce T_1 seed inheriting the DBT418 transformation event. Repeated backcrossing to various inbred lines resulted in hybrid germplasm containing the DBT418 transformation event. During the backcross program, segregating populations of plants were sprayed with glufosinate ammonium to identify positive segregants. Typically, about one half of the plants resulting from a backcross were found to be tolerant to the herbicide. The herbicide tolerant plants were also protected from European corn borer infestations, indicating that both genes were linked, possibly resulting from the same insertion event.

Characterisation of DBT418 corn

The DBT418 transformation event was created by the introduction of the three plasmids — pDPG165, pDPG320 and pDPG699. These plasmids encode the *bar*, potato *pinII*, and *cryIAc* genes, respectively. In addition, each plasmid contains the *bla* gene as well as the ColE1 origin of replication. The DBT418 transformation event was characterised using Southern blot, polymerase chain reaction (PCR) and nucleotide sequence analyses.

The maize genotype used as the test substance for this study is designated DK.DL(DBT418) and is the result of a cross between a female inbred line, DK, that is homozygous for the DBT418 event, and a male inbred line, DL, that does not contain the DBT418 event. Consequently, the resultant test plant, DK.DL(DBT418) is hemizygous for the DBT418 event. The control used for this study was non-transformed hybrid seed of the genotype DK.DL. In both the Southern and PCR analyses, plasmids bearing the target sequences were used as positive control references. Leaf material for genomic DNA extraction was harvested from each germinated plant between 54 and 60 days post-planting.

The Southern blot analyses were primarily used to determine the copy number of each transferred gene. Copy number was determined by comparing the hybridisation signal for each genetic element with standards prepared from each of the plasmids diluted to represent 0.5, 1, 2, 4 and 8 maize genome copy number equivalents.

The results of the Southern blot analyses are summarised in Table 3 below.

Table 3: Gene copy number determination of DBT418 corn using Southern blot analysis

	Approximate gene copy number	
Region	**Intact**	**Rearranged**
cryIAc	2	0
bar	1	1
pinII	0	0.5
adhI intron I	0	0.5
bla	4	0.5
ColE1	4	0

PCR analysis was then used to further characterise the inserted DNA in corn event DBT418. Numerous overlapping, long PCR products (4.8 – 13.0 Kb) were generated which spanned the length of the DNA insert in event DBT418. The PCR fragments were analysed with restriction enzymes and this data was used to construct a map of the inserted DNA. To further define the 5' and 3' ends of the inserted DNA, the relevant PCR fragments were sub cloned and sequenced and this information was used to complete the map of the inserted DNA (see Figure 1).

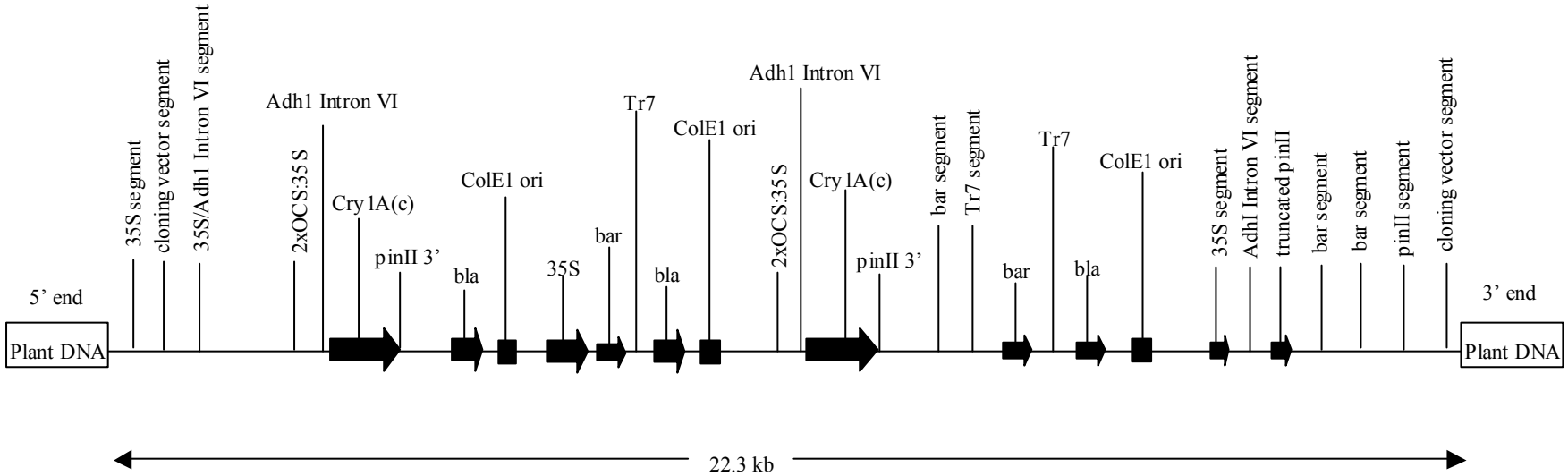
The PCR and sequencing analysis confirmed the estimations of the gene copy number by Southern blot analysis (Table 3) although the more detailed information indicated that there are three, rather than four copies of the *bla* gene and ColE1 origin of replication.

PCR analysis was also used to further characterise the *pinII* insertion in DBT418 corn, which according to the Southern blot analysis was not present as a fully intact copy. A 370 bp PCR product spanning the *pinII* region in the DBT418 insertion site was cloned and then sequenced. Analysis of the DNA sequence confirmed that the *pinII* gene in DBT418 corn is not intact. The sequence analysis of this region indicated there are two potential open reading frames (ORFs), of 94 and 104 amino acids, which overlap the region corresponding to the *pinII* sequence and which are present on the two complementary DNA strands. To confirm that these ORFs are not expressed, Northern blot analysis was done using total RNA isolated from DBT418 leaves and kernels. The RNA was probed with a sequence specific to the *pinII* gene. No hybridisation signals were observed indicating that the two ORFs are unlikely to be expressed.

Conclusion

The molecular analyses indicate that transformation event DBT418 contains two copies of the *cryIAc* expression cassette, one functional copy of the *bar* expression cassette, three copies of the *bla* gene and the ColE1 origin of replication, plus several non-functional partial fragments of the *bar* and *pinII* genes all at the one insertion site. The evidence strongly indicates that the partial copy of the *pinII* gene is not expressed.

Figure 1: Map of inserted DNA in event DBT418



2.4 STABILITY OF GENETIC CHANGES

Studies submitted by Monsanto:

Stephens, M. *et al* (1996). Molecular characterization of transgene content and stability in transgenic corn hybrid line DK.DL (DBT418). Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A07.

Walters, D. (1996). Demonstration of stable Mendelian inheritance of *cryIAc* and *bar* genes in DBT418. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A14.

Southern blot analysis

As a demonstration of genetic stability, a large number of DBT418 plants were analysed using Southern blot analysis to determine the frequency at which variations in *cryIAc* content occurred. Non-segregating hybrid seed, produced by crossing a female elite inbred (DK) homozygous for the DBT418 event with a non-transformed male inbred (DL), were planted in the field and leaf tissue was collected from 190 of these plants.

Out of the samples analysed, the vast majority exhibited the expected Southern hybridisation pattern indicating that both copies of the *cryIAc* gene had been stably inherited. Only four out of the 190 plants analysed had a hybridisation pattern that was atypical. Three of these four plants contained a single copy of the *cryIAc* gene, i.e. had lost one copy of the *cryIAc* gene through normal genetic processes. The other plant had neither copy of the *cryIAc* gene. These plants were not further characterised. A low level of genetic variation is considered normal.

PCR analysis

DBT418 plants have been repeatedly backcrossed to non-transformed inbred plants to introgress the DBT418 event into elite inbred germplasm for the production of hybrids. Progeny of DBT418 plants that were backcrossed to non-transformed inbreds were tested to determine if the DBT418 event is inherited in a predictable manner consistent with that expected of a single nuclear genetic locus, that is, in a Mendelian manner. Progeny derived from a cross between a parent hemizygous for the DBT418 event and a non-transformed parent would be expected to contain the DBT418 event at a frequency of about 50% (that is, the ratio of transgenic to non-transgenic should be 1:1).

One early generation backcross population and three late generation backcross populations were analysed for segregation of the *cryIAc* and *bar* genes using PCR. The genotypes tested are listed in Table 4 below.

Table 4: DBT418 and control genotypes tested by PCR

Genotype code	Genotype designation	No. of crosses
AW/BC5/DBT418	Late generation	6
BS/BC5/(AW.DBT418)	Late generation	7
DK/BC6/(AW.DBT418)	Late generation	8
DBT418(AW)08(aBK)	Early generation	2
AW	Non-transformed control	-

Approximately 100 seeds per DBT418 genotype and 50 seeds from the non-transformed control were planted and grown using standard methods for propagation of corn in a greenhouse. Samples of leaf tissue for PCR analysis were taken from young seedlings at 12 to 13 days post planting. Samples were taken from about 48 seedlings of each DBT418 genotype and 24 control seedlings. PCR analysis was subsequently performed on 43 samples from each set of samples. A non-transformed control was analysed with each set of 43 samples of a given genotype.

Chi-square analysis was done to assess the hypothesis that segregation is Mendelian (that is, occurs at a ratio of 1:1), as would be expected from a single genetic locus in a cross involving a transformed hemizygote and a non-transformed individual. The hypothesis that segregation occurred in a 1:1 ratio was accepted if chi-square values gave a probability of 5% (P value of 0.05) or greater.

The extracted DNA was analysed for *cryIAc*, *bar* and an endogenous maize gene, *adh* (coding for alcohol dehydrogenase), which serves as an internal control. Included in each set of PCR reactions for each genotype were two positive control reactions containing the relevant plasmid DNA. Two negative control reactions, containing no DNA, were included with each set of PCR reactions.

The results of the PCR analyses are summarised in Table 5 below.

Table 5: Results of segregation analysis using PCR

Genotype	No. of plants tested	Gene tested	No. of PCR positive plants	No. of PCR negative plants	χ^2	P
AW/BC5/DBT418	43	*cryIAc*	22	21	0	0.95
		bar	22	21	0	0.95
		adh	43	0		
BS/BC5/(AW.DBT418)	43	*cryIAc*	24	19	0.37	0.50
		bar	24	19	0.37	0.50
		adh	43	0		
DK/BC6/(AW.DBT418)	43	*cryIAc*	24	19	0.37	0.50
		bar	24	19	0.37	0.50
		adh	43	0		
DBT418(AW)08(aBK)	43	*cryIAc*	20	23	0.10	0.70
		bar	19	24	0.37	0.50
		adh	43	0		
AW	5	*cryIAc*	0	5		
		bar	0	5		
		adh	5	0		

In all the genotypes tested, *bar* and *cryIAc* appear to segregate together in plants in approximately a 1:1 ratio to plants that lack the two genes.

Conclusion

The *bar* and *cryIAc* genes in DBT418 corn are tightly linked and segregate together in a Mendelian fashion suggesting that both genes are inserted at the same genomic location. This further supports the results of the molecular characterisation. In the vast majority of cases, both genes are stably maintained in the corn genome through several generations of backcrosses.

3. GENERAL SAFETY ISSUES

3.1 History of use

Corn (*Zea mays* L., also called maize) has been cultivated for centuries and is used as a basic food item by people throughout the world. A large part of corn production is used for human food products, and a wide variety of food products are derived from corn kernels. Grain and by-products from processing of corn are also used as animal feedstuffs.

In developed countries, corn is consumed mainly as popcorn, sweet corn, corn snack foods and occasionally as corn bread. However, most consumers are not aware that corn is an important source of the sweeteners, starches, oil and alcohol used in many foods, beverages and numerous other products.

Two milling procedures are used for the processing of corn: dry milling and wet milling. Dry milling is a mechanical process in which the endosperm is separated from the other components of the kernels and fractionated into coarse particles (grits). The process is used to produce meal and flour for use in cereals, snack foods and bakery products, or for use in brewing (Alexander 1987). Human food products derived from dry milling include corn flakes, corn flour and grits.

The wet milling process for corn is designed to physically separate the major component parts of the kernel: starch, protein, oil and fibre. Wet milling produces primarily starch (typically 99.5% pure). In this process grain is steeped in slightly acidic water for 24–48 hours at 52°C before being milled. Starch is separated from other solids through a number of grinding, washing and sieving steps. Washed starch may contain 0.3-0.35% total protein and 0.01% soluble protein (May 1987). Starch is largely converted to a variety of products for human consumption, such as sweetener and fermentation products including high fructose corn syrup and ethanol. Oil is produced from wet-milled corn by solvent extraction and heat (120°C, May 1987) and corn oil is considered to be free of protein.

In Australia and New Zealand crop planting regimes are variable. Due to the diverse uses of corn products, there is a requirement to import corn products, mainly in the form of high-fructose corn syrup, to meet manufacturing demand.

3.2 Nature of novel proteins

On the basis of the molecular and phenotypic characterisation, corn containing the DBT418 event would be expected to express two new proteins – a truncated form of the insecticidal protein CryIAc and PAT.

No β-lactamase expression would be expected in DBT418 corn, as the *bla* gene does not have the appropriate regulatory sequences for plant expression. Expression of the potato protease inhibitor II would also not be expected because the *pinII* expression cassette is not intact.

CryIAc

The synthetic *cryIAc* gene encodes the CryIAc protein of 613 amino acids with a predicted molecular weight of 66 kDa

Like other insecticidal crystal proteins produced by *Bacillus thuringiensis*, CryIAc is naturally produced in the bacterium as a 130 kDa protoxin that is cleaved by trypsin in the insect gut to a tryptic core protein of approximately 66 kDa. It is this 66 kDa trypsin resistant core that is toxic to susceptible lepidopteran larvae (Bietlot *et al* 1989, Hofte and Whitely 1989). The trypsin resistant core is essentially composed of the amino-terminal half of the protein excluding the first 28 amino acids of the protoxin which are also cleaved off by trypsin. The CryIAc coding sequence introduced into DBT418 corn only differs from the native trypsin resistant core fragment in that the 28 amino acids at the amino-terminus have been retained.

Characterisation of CryIAc

Studies submitted by Monsanto:

Millham, R.D. *et al* (1996). Characterization of the Cry1Ac protein from transgenic plants and demonstration of equivalence to microbially produced Cry1Ac. Performing laboratory: DEKALB Genetics Corporation. Study No.DGC-95-A19.

In this study CryIAc protein isolated from DBT418 plants was analysed to assess its equivalence to CryIAc protein purified from *Bacillus thuringiensis* — characteristics analysed were molecular weight, immunogenicity, amino-terminal sequence, insecticidal activity and glycosylation.

The DBT418 plant used for this study had the genotype AW/BC2/DBT418.BS/BC1/DBT418 (2Bt). The non-transformed control plant was of the DK.DL genotype. Leaf tissue was collected from more than 50 plants (field and glasshouse grown) and then pooled into a single sample so that adequate protein could be extracted for the analyses.

The apparent molecular weights and immunogenicity of the DBT418 corn and *B. thuringiensis* produced CryIAc proteins were compared using Western blot analysis. A single immunogenic protein band of approximately 66 kDa was detected in extracts from both DBT418 corn and *B. thuringiensis*. As expected, no such band was seen in the control samples.

The CryIAc protein encoded by the DBT418 event contains six potential glycosylation sites however as the encoded protein has not been specifically targeted to the endoplasmic reticulum where glycosylation occurs, no glycosylation would be expected. A glycoprotein detection assay confirmed that CryIAc expressed in DBT418 corn had not been glycosylated.

Amino-terminal sequencing of the native CryIAc protein yielded a match with the predicted sequence of the trypsinised CryIAc core fragment from which the first 28 amino acids have been cleaved, leaving Ile_{29} of the protoxin sequence as the terminal residue. Amino-terminal sequencing of the CryIAc from DBT418 corn also yielded a sequence that is an exact match with amino acid residues 26 through 33 of the CryIAc coding sequence of the native *Bacillus cryIAc* gene.

This indicates that the amino-terminus of CryIAc extracted from DBT418 begins at amino acid Gly_{26}, which is three residues longer at the amino-terminus than the native CryIAc. It is not known if the removal of the first 25 amino acids of the DBT418 CryIAc occurs *in vivo* or whether it occurred during the purification of the protein for analysis.

The functional activity of the DBT418 plant CryIAc protein was evaluated using tobacco hornworm bioassays on lyophilised leaf material. Results from these assays demonstrated nearly 100% mortality of insects in a 5-day assay.

Conclusion

The native and DBT418 CryIAc are identical in their electrophoretic mobility and immunogenicity and are virtually identical in their amino-terminal sequence. There was no evidence of glycosylation of either the DBT418-derived or native CryIAc. The DBT418 corn derived CryIAc is also insecticidally active.

Phosphinothricin acetyl transferase

PAT is a protein consisting of 183 amino acids with a molecular weight of about 22 kDa. The PAT enzyme catalyses the transfer of an acetyl group from acetyl CoA to the amino group of phosphinothricin. The enzyme is highly substrate specific (Thompson *et al* 1987). The substrate affinity for phosphinothricin is more than 30 times higher than affinity for demethylphosphinothricin (the biosynthetic pathway intermediate) and over 300 times higher than affinity for the amino acid glutamate. Therefore as its affinity for related PPT compounds is very low and no additional substrates have ever been reported it is highly unlikely that any naturally occurring compounds in corn would react with PAT.

Characterisation of PAT

Study submitted by Monsanto:

Laccetti LB, Adams WR, Nutkis JE, Millham RD and Walters DS (1996). Characterization of the phosphinothricin acetyltransferase protein from transgenic plants and demonstration of equivalence to microbially produced phosphinothricin acetyltransferase. Performing laboratory: DEKALB Genetics Corporation. Study No.DGC-95-A20.

In this study PAT protein isolated from DBT418 plants was analysed to assess its equivalence to PAT protein purified from *Escherichia coli* — characteristics analysed were molecular weight, immunogenicity, amino-terminal sequence, enzyme activity and glycosylation.

Leaf tissue collected from field and glasshouse grown AW/BC2/DBT418.BS/BC1/DBT418 (2Bt) and DK.DL lines was used as the source of test and control proteins for analysis. The reference protein was a microbial produced PAT containing an amino-terminal extension of 20 amino acids, including six histidine residues (a His-tag) and a thrombin protease cleavage site. The His-tag facilitates the one-step purification of the protein using affinity chromatography with nickel resin. The majority of this extension can subsequently be removed from the affinity-purified protein through treatment with thrombin protease, leaving a protein with only four additional amino acids at its amino-terminus.

The apparent molecular weights and immunogenicity of the plant and microbial produced PAT proteins were compared using Western blot analysis with antibodies specific to PAT. An immunogenic PAT band of approximately 23 kDa was clearly present in tissue from DBT418 plants. No such band was present in the extract from control plants. In the lane in which the microbial produced PAT was run a band of approximately 25 kDa was detected. This molecular weight is consistent with what would be expected given that the microbial produced PAT has an amino-terminal extension that adds an additional 1.9 kDa to the molecular weight of the protein. Elimination of the His-tag by thrombin cleavage produced a band having a similar electrophoretic mobility as the DBT418 PAT.

Results of glycoprotein detection assays showed, as expected, no evidence of glycosylation of either DBT418 PAT or microbial PAT.

Amino-terminal sequence data was obtained for DBT418 PAT, microbial PAT with the His-Tag intact and thrombin-treated microbial PAT. These experimentally determined sequences were compared to the deduced amino acid sequence from the *bar* gene inserted into DBT418 corn. The experimentally determined amino-terminal sequence of the DBT418 PAT protein matched with the deduced amino acid sequence of the *bar* gene used to transform the corn. The amino terminal sequence of the His-Tag PAT is identical to that of the DBT418 plant PAT except for the presence of the His-Tag. The thrombin-treated His-Tag PAT is identical DBT418 PAT except for the four additional amino-terminal residues – glycine, serine, histidine, and methionine.

The enzymatic activity of DBT418 PAT was compared to microbial His-Tag PAT. The PAT activity of the His-Tag PAT was similar to, although slightly lower than, that observed for DBT418 PAT. This result demonstrates that the addition of the His-Tag to PAT has not altered the essential characteristics of the enzyme, as expressed in DBT418 corn.

Conclusion

The DBT418 plants produce a single PAT protein of the expected molecular weight that is recognised by PAT antibodies. The DBT418 PAT and microbial-produced PAT exhibited similar electrophoretic mobility, immunogenicity and enzymatic activity, and their amino terminal amino acid sequence matched the deduced amino acid sequence derived from the *bar* gene used to transform corn and *E. coli*. Neither the DBT418 nor microbial PAT showed any evidence of glycosylation.

3.3 Expression of novel protein in the plant

Study submitted by Monsanto:

Kruger, D.E. *et al* (1996). Magnitude of transgenic protein accumulation in transformed DBT418 corn lines. Performing laboratory: DEKALB Genetics Corporation, Mystic. Study No. DGC-95-A01.

The genotypic backgrounds containing the DBT418 insertion event and control genotypes that were analysed in this study are detailed in Table 6 below.

Table 6: Genotypic backgrounds used for protein level determinations

Genotype	Abbreviation	DBT418 allele	Description
AW/BC2/DBT418 S4	S4 inbred	Segregating	Unfinished inbred
AW/BC2/DBT418.BS/BC1/DBT418 (2Bt)	2Bt hybrid	Homozygous	Unfinished hybrid
DK.DL(DBT418)	DK.DL(DBT418)	Hemizygous	Finished hybrid
DK.DL	DK.DL	None	Control hybrid
AW	AW	None	Control hybrid

Tissue samples were collected from three field locations during the 1995 growing season. Each field study site consisted of one plot with thirteen rows, each row with a separate corn line (either a DBT418 line or a control line). Tissue samples were collected at five distinct time points over the course of the growing season and the samples analysed to determine the concentration of the novel proteins. The tissue sampled at the various time points were:

(a) leaf and root tissue from the V6-V7 growth stage (collar of the 6 or 7th true leaf is visible);
(b) leaf, stalk, root, ball, pollen, silk, whole plant including root of the pollen shedding stage;
(c) whole plant not including roots from the dough stage (typical for silage corn);
(d) leaf, stalk, root ball, and ear (husk removed) of the harvest stage; and
(e) whole plant including roots of the senescence stage.

The data relating to the kernel are the most important as the kernel is the only part of the plant used for human consumption.

CryIAc

An enzyme-linked immunosorbent assay (ELISA) was used to quantify the CryIAc protein in the various tissue samples. For each DBT418 tissue type analysed for CryIAc expression, control non-transformed plants were also analysed. No CryIAc expression was detected in any of the control genotypes analysed.

The protein expression data indicates that the pattern of expression is similar in all three genotypes analysed. The 2Bt hybrid (homozygote) generally exhibited higher levels of CryIAc expression than the hemizygote hybrid (DK.DL(DBT418). An exception to this pattern was observed in kernel tissue where both lines produced similar levels of CryIAc.

The highest tissue expression levels were found in the leaves with the highest levels occurring at the harvest stage. The levels of CryIAc in kernels was generally low but detectable at the time of harvest. Mean levels ranged from 36.0 to 42.8 ng/g dry weight for the three genotypes. This is equivalent to about 0.0001% of the total kernel protein.

A summary of the CryIAc expression data is presented in Table 7 below. The data for the kernel expression is highlighted.

Table 7: CryIAc protein levels during DBT418 corn development

Tissue	Genotype	Mean protein levels (ng/g dry weight [n; SE])		
		V6-V7	Pollen shed	Harvest
Leaf:	S4 inbred	217.9 (7; 46.23)	335.0 (8; 74.93)	459.6 (8; 99.84)
	DK.DL(DBT418)	177.8 (8; 42.22)	93.7 (8; 7.39)	620.6 (8; 84.39)
	2Bt hybrid	289.6 (4; 36.74)	174.2 (4; 36.69)	1198.4 (4; 270.78)
Stalk:	S4 inbred	N/A	28.5[c] (3;4.19)	123.6[a] (7; 46.72)
	DK.DL(DBT418)	N/A	BLD[*] (8)	40.9 (8; 7.34)
	2Bt hybrid	N/A	BLD (4)	115.1 (4; 25.19)
Root ball:	S4 inbred	69.8 (7; 17.34)	78.2 (8; 12.56)	58.7 (8; 25.19)
	DK.DL(DBT418)	50.9 (8; 9.58)	57.7[a] (7; 16.24)	58.0[b] (5; 8.22)
	2Bt hybrid	117.9 (4; 17.08)	72.0 (4; 20.56)	125.4 (4; 16.93)
Kernel:	S4 inbred	N/A	N/A	42.8 (6; 16.60)
	DK.DL(DBT418)	N/A	N/A	37.1 (8; 3.97)
	2Bt hybrid	N/A	N/A	36.0 (4; 8.14)
Silk:	S4 inbred	N/A	BLD (8)	N/A
	DK.DL(DBT418)	N/A	110.5[d] (2; 10.70)	N/A
	2Bt hybrid	N/A	BLD (4)	N/A
Pollen:	1Bt hybrid[#]	N/A	BLD (8)	N/A
	DK.DL(DBT418)	N/A	BLD (4)	N/A
	2Bt hybrid	N/A	BLD (8)	N/A
Whole plant:	S4 inbred	N/A	147.1 (8; 47.87)	N/A
	DK.DL(DBT418)	N/A	35.9 (8; 5.44)	N/A
	2Bt hybrid	N/A	75.0 (4; 15.03)	N/A

[*] BLD below the limit of detection of the assay (6.7 ng/g dry weight)
[a] 1 of 8 samples were BLD, [b] 3 of 8 samples were BLD, [c] 5 of 8 samples were BLD, [d] 6 of 8 samples were BLD
[#] AW/BC2/DBT418.BS/BC1/DBT418 (1Bt) genotype was substituted for the S4 hybrid because insufficient pollen was available from the S4 hybrid.

PAT protein

A quantitative immunoblot was used to determine the quantity of PAT protein, using an enhanced chemiluminescence system in conjunction with scanning densitometry.

The tissue distribution for PAT expression was similar to that found for CryIAc except that levels of PAT were significantly higher. Leaf tissue is the site of highest PAT expression in DBT418 plants with means for the three genotypes ranging from 501.8 to 1099.4 µg/g dry weight at the pollen shed stage. Relatively low levels of PAT were found in the kernel, mean levels for the three genotypes ranging from 3.1 to 6.0 µg/g dry weight. This is equivalent to about 0.0175% of the total kernel protein.

In many of the tissues analysed the PAT levels found in the homozygous line were approximately double the levels found in the hemizygous line. This would be expected if expression levels were additive based on the number of DBT418 events present. In general, the genetic background was not found to exert a great deal of influence on PAT expression levels, with all three genotypes examined expressing similar protein levels characteristic for the particular tissue type

A summary of the PAT expression data is presented in Table 8 below. The data for kernel expression is highlighted.

Table 8: PAT protein levels during DBT418 corn development

Tissue	Genotype	Mean protein levels (µg/g dry weight [n; SE])		
		V6-V7	Pollen shed	Harvest
Leaf:	S4 inbred	351.1 (7; 52.91)	522.0 (6; 59.04)	60.8[a] (6; 12.46)
	DK.DL(DBT418)	276.3 (8; 25.51)	501.8 (8; 34.75)	180.5 (8; 24.68)
	2Bt hybrid	554.9 (2; 136.03)	1099.4 (3; 76.29)	213.6 (4; 61.92)
Stalk:	S4 inbred	N/A	75.8 (8; 12.24)	95.2 (6; 16.86)
	DK.DL(DBT418)	N/A	60.0 (8; 11.98)	64.4 (8; 8.23)
	2Bt hybrid	N/A	77.0 (4; 11.66)	136.3 (2; 12.74)
Root ball:	S4 inbred	95.1 (7; 16.91)	54.1 (8; 9.15)	24.5 (7; 3.71)
	DK.DL(DBT418)	59.4 (8; 3.53)	27.5 (8; 6.25)	21.3 (8; 2.23)
	2Bt hybrid	88.1 (4; 21.45)	69.5 (4; 23.58)	28.8 (3; 7.37)
Kernel:	S4 inbred	N/A	N/A	6.0 (6; 1.88)
	DK.DL(DBT418)	N/A	N/A	3.1 (8; 0.35)
	2Bt hybrid	N/A	N/A	4.9 (4; 0.63)
Silk:	S4 inbred	N/A	128.2 (8; 17.21)	N/A
	DK.DL(DBT418)	N/A	29.1 (8; 2.97)	N/A
	2Bt hybrid	N/A	133.3 (2; 60.01)	N/A
Pollen:	1Bt hybrid[#]	N/A	BLD[*] (8)	N/A
	DK.DL(DBT418)	N/A	BLD (8)	N/A
	2Bt hybrid	N/A	BLD (4)	N/A
Whole plant:	S4 inbred	N/A	111.1 (8; 16.50)	N/A
	DK.DL(DBT418)	N/A	72.8 (8; 5.88)	N/A
	2Bt hybrid	N/A	119.5 (4; 25.63)	N/A

[*] BLD below the limit of detection of the assay (12.10 µg/g dry weight)
[a] 2 of 8 samples were BLD and not used to calculate the mean or standard error
[#] AW/BC2/DBT418.BS/BC1/DBT418 (1Bt) genotype was substituted for the S4 hybrid because insufficient pollen was available from the S4 hybrid.

PIN II protein

DBT418 corn does not contain an intact copy of the *pinII* gene and is therefore not expected to produce the serine protease inhibitor. Consequently, the PIN II protein analysis was done as a qualitative assay only to determine the presence or absence of the protein in a variety of DBT418 plant tissues.

The three plant genotypes analysed were the same as those analysed for CryIAc and PAT expression. The limit of detection of the PIN II immunoblot assay for most tissues (leaf, stalk and root) is 400 ng/g dry weight. The results of the PIN II protein expression analysis are summarised in Table 9 below.

Table 9: Summary of PIN II analysis in various lyophilised DBT418 tissues

Tissue	Growth stage	No. of genotypes evaluated	Total No. of DBT418 plants analysed	Assay limit of detection (per g dry weight)	PIN II detection
Leaf	V6-V7	3	10	400 ng	ND
Leaf	Pollen shed	3	10	400 ng	ND
Stalk	Pollen shed	3	6	400 ng	ND
Root	Pollen shed	3	6	400 ng	ND
Pollen	Pollen shed	3	6	Indeterminate	ND
Kernel	Harvest	3	10	1800 ng	ND

No evidence was found for the presence of PIN II in any of the tissues analysed. Comparison of control lanes with DBT418 lanes on the immunoblot revealed no additional immunogenic bands that were not present in the control extracts.

β-lactamase

Several copies of the *bla* gene are present in DBT418 corn. As the *bla* gene is under the control of a bacterial promoter it should not be expressed in DBT418 corn. To determine whether DBT418 corn produced any ß-lactamase, plant tissue samples were assayed using an immunoblot for the presence of the enzyme in a variety of DBT418 tissues. The DBT418 genotypes analysed were the same as for the previous protein expression analyses discussed above. The limit of detection of the ß-lactamase immunoblot was less than 9 μg/g dry weight.

No evidence was found of expression of ß-lactamase protein in DBT418 corn. Comparison of control lanes with DBT418 lanes also revealed no additional immunogenic bands in the DBT418 lane that were not present in the control extracts.

Conclusion

CryIAc and PAT protein expression was detected in several tissue types and throughout plant development in three different genetic backgrounds containing the DBT418 event. PAT was expressed at significantly higher levels than CryIAc in the corresponding tissues in which it was detected. The highest protein expression levels were in leaf, with significantly less protein being expressed in kernels. In kernels, mean CryIAc levels ranged from 36.0 – 42.8 ng/g dry weight (equivalent to about 0.0001% of the total protein) and mean PAT levels ranged from 3.1 – 6.0 μg/g dry weight (equivalent to about 0.0175% of the total protein). In general, the different genetic backgrounds did not appear to influence protein expression levels, which appeared to be more greatly influenced by tissue type. No evidence for either PIN II or ß-lactamase expression was found in any of the DBT418 plants tested.

3.4 Impact on human health from the potential transfer of novel genetic material to cells of the human digestive tract

The human health considerations in regard to the potential transfer of novel genetic material to cells of the human digestive tract depend on the nature of the novel genes and must be assessed on a case-by-case basis.

In 1991, the World Health Organization (WHO) issued a report of a joint consultation between WHO and the Food and Agriculture Organization, which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). The consultation concluded that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

The major concern in relation to the transfer of novel genetic material to gut microorganisms is with antibiotic resistance genes. Antibiotic resistance genes can be present in some transgenic plants as a result of their use as marker genes to select transformed cells. It is generally accepted that there are no safety concerns with regard to the presence in the food of antibiotic resistance gene DNA *per se* (WHO 1993).

However, concerns have been expressed 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 antibiotics. This section of the report will therefore concentrate on evaluating the human health impact of the potential transfer of antibiotic resistance genes from insect-tolerant and glufosinate ammonium tolerant corn to microorganisms present in the human digestive tract.

In the DBT418 corn lines, PCR analysis demonstrated that DBT418 corn contains three copies of the *bla* gene under the control of a bacterial promoter. The *bla* gene encodes the enzyme β-lactamase and confers resistance to a number of β-lactam antibiotics such as penicillin and ampicillin. The *bla* gene is not expressed in DBT418 corn.

The first issue that must be considered in relation to the presence of an intact *bla* gene in DBT418 corn is the probability that this gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract. The following steps are necessary for this to occur:

- excision of DNA fragments containing the *bla* gene and its bacterial promoter;
- survival of DNA fragments containing the *bla* gene in the digestive tract;
- natural transformation of bacteria inhabiting the digestive tract. This requires the recipient cells to be physiologically competent (competence depends on growth conditions, the age of the cells and environmental conditions) (Stewart and Carlson 1986);
- survival of the bacterial restriction system by the DNA fragment containing the *bla* gene;
- stable integration of the DNA fragment containing the *bla* gene into the bacterial chromosome or plasmid; and
- maintenance and expression of the *bla* gene by the bacteria.

The transfer of a functional *bla* gene to microorganisms in the human digestive tract is therefore considered highly unlikely because of the number and complexity of the steps that would need to take place consecutively. It should also be noted that the processing steps for corn typically include heat, solvent or acid treatments that would be expected to remove and destroy DNA. Intact fragments of the *bla* gene are unlikely to survive the processing steps making the chance of horizontal gene transfer even more unlikely. The processing steps can also lead to the release of cellular enzymes (nucleases) that are responsible for degrading DNA into smaller fragments.

The second and most important issue that must be considered is the potential impact on human health in the unlikely event that successful transfer of a functional *bla* gene to microorganisms in the human digestive tract occurred.

In the case of transfer of the *bla* gene from DBT418 corn to microorganisms of the digestive tract, the human health impacts are considered to be negligible. This is because ampicillin-resistant bacteria are commonly found in the digestive tract of healthy individuals (Calva *et al* 1996) as well as diseased patients (Neu 1992).

Therefore, the additive effect of a *bla* gene from DBT418 corn being taken up and expressed by microorganisms of the human digestive tract would be insignificant compared to the population of ampicillin resistant bacteria already naturally present.

In relation to considering the potential impact on human health from the transfer of other novel genetic material to human cells via the digestive tract, 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 genetically modified 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.

Conclusion

It is extremely unlikely that the ampicillin resistance gene or other novel genetic material will transfer from foods derived from DBT418 corn to bacteria or other cells in the human digestive tract because of the number and complexity of steps that would need to take place consecutively. In the highly unlikely event that the resistance gene was transferred the human health impacts would be negligible because ampicillin-resistant bacteria are already commonly found in the human gut and in the environment.

The probable degradation and removal of DNA through the processing steps for corn further mitigate against any horizontal transfer of DNA from DBT418 corn to cells in the human digestive tract.

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally-occurring toxins

There are no naturally occurring toxins known to occur at biologically significant levels in corn (Wright 1987).

4.2 Potential toxicity of novel protein

The potential toxicity of the CryIAc and PAT proteins was evaluated using acute oral toxicity in mice and in birds. The scientific basis for using an acute test is that, if toxic, proteins are known to act via acute mechanisms and laboratory animals have been shown to exhibit acute toxic effects from exposure to proteins known to be toxic to humans (Sjoblad *et al* 1992). Bacterial proteins produced by fermentation, rather than proteins purified from the transgenic plants, were used for the acute toxicity studies in mice, because of the difficulty of obtaining sufficient material from plants. As described in Section 3.2, microbial produced Cry1Ac and PAT were shown to be equivalent to the transgenic proteins produced by DBT418 corn.

Studies submitted by Monsanto:

Merriman, T.N. (1996). An acute oral toxicity study in mice with *Bacillus thuringiensis* subsp *kurstaki* Cry1Ac delta endotoxin. Performing laboratory: DEKALB Genetics Corporation and Springborn Laboratories (SLI). Study No. DEKALB – DGC-95-A17 / SLI No. 3406.1.

Merriman, T.N. (1996). An acute oral toxicity study in mice with phosphinothricin acetyltransferase (PAT) protein. Performing laboratory: DEKALB Genetics Corporation, Springborn Laboratories (SLI). Study No. DEKALB – DGC-95-A18 / SLI No. 3406.2.

Palmer, S.J. and Beavers, J.B. (1996). Lyophilized DBT418 leaf tissue: a dietary toxicity study with the northern bobwhite. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A13.

CryIAc – acute toxicity study in mice

CryIAc is insecticidal only to lepidopteran insects (MacIntosh *et al* 1990b) and its specificity of action is directly attributable to the presence of specific receptors in the target insects (Wolfersberger 1990, Ferré *et al* 1991). There are no receptors for the δ-endotoxins of *B. thuringiensis*, including CryIAc, on the surface of mammalian intestinal cells (Hofmann *et al* 1988; MacIntosh *et al* 1990b).

Young adult CD-1(ICR)BR mice (source: Charles River Laboratories, Portage, MI) were acclimatised for at least 5 days before dosing. They were housed individually in controlled conditions with free access to food and water, except for the 3–4 hours before dosing, when food was withheld. *Bacillus thuringiensis* Cry1Ac delta-endotoxin (lot CSV-102695, purity 66.5%) was administered to the mice (5/sex) at 5000 mg/kg bodyweight (bw) in a volume of 20 mL/kg bw by single oral gavage, equivalent to 3825 mg of Cry1Ac protein.

Mice were observed for clinical signs twice on the day of dosing (post dosing) and once daily after this for the 14-day duration of the test. Bodyweight was determined before fasting and before dosing on day 0 and on days 7 and 14. At the end of the study, mice were killed and examined for gross pathology. Any abnormalities were recorded.

There was one death on day 1 due to gavage error. No deaths and no clinical abnormalities were observed in the 9 remaining mice. The LD_{50} was determined to be >3825 mg/kg bw in mice.

PAT – acute toxicity study in mice

Young adult CD-1(ICR)BR mice (source: Charles River Laboratories, Portage, MI) were acclimatised for at least 5 days before dosing. They were housed individually in controlled conditions with free access to food and water, except for the 3–4 hours before dosing, when food was withheld. Histidine-tagged PAT protein (lot CSV-102695, purity >99%) was administered to the mice (5/sex) at 2500 mg/kg bodyweight (bw) in a volume of 20 mL/kg bw by single oral gavage.

Mice were observed for clinical signs three times on the day of dosing (post dosing) and once daily after this for the 14-day duration of the test. Bodyweight was determined before fasting and before dosing on day 0, and on days 7 and 14. At the end of the study, mice were killed and examined for gross pathology. Any abnormalities were recorded.

There were no deaths during the study. The only clinical abnormality observed was few faeces in one male. During the 7–14-day interval one male had a slight loss of bodyweight, the 9 other mice gained weight. No gross intestinal findings were seen on day 14. The LD_{50} was determined to be >2500 mg/kg bw in mice.

CryIAc and PAT protein – avian toxicity study

Northern bobwhite quails (14 days of age, source: Wildlife International Ltd) were acclimatised from day of hatch until initiation of testing. Each treatment or control group was made up of 10 birds uniquely identified by wing tags. The birds used in the study were immature and could not be differentiated by sex. Birds were housed in controlled conditions with free access to food and water during acclimatisation and during the test. The applicant provided test and control corn leaf protein to the testing laboratory. The control lyophilised corn leaf material was received in three shipments (lots 3495, 3504 and 3507) and contained 0% CryIAc protein, while the test lyophilised corn leaf material was received in two shipments (lots 3496 and 3505). The DBT418 lyophilised corn leaf material contained CryIAc protein at 150.5 ng/g dry weight, determined by ELISA, and PAT protein at 209.8 µg/g dry weight, determined by protein immunoblotting.

Three replicate groups, each containing 10 chicks, received a diet containing lyophilised DBT418 leaf tissue at 200 000 parts per million (ppm) or 20% weight per weight (w/w). One control group containing 10 chicks received lyophilised control leaf tissue at 200 000 ppm or 20% w/w. Another control group received untreated diet only. For the test, each group was fed the appropriate test or control diet for five days and then given untreated feed for three days.

During acclimatisation, all birds were observed daily. Birds exhibiting abnormal behaviour or physical injury were not used for the test. Throughout the test, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity and abnormal behaviour. Bodyweight was measured at the initiation of the test, at the end of the exposure period on day 5 and at termination of the test on day 8. Average feed consumption was determined for each group for days 0–5, and days 6–8.

No birds died during the test period and there were no abnormal clinical signs or behavioural changes in any group. There were no treatment-related effects on bodyweight or food consumption during this study. The dietary LC_{50} for northern bobwhite exposed to lyophilised DBT418 leaf tissue in the diet was determined to be greater than 200 000 ppm or 20% w/w.

Conclusion

The results do not indicate any potential toxicity from either the Cry1Ac protein or PAT.

4.3 Levels of naturally-occurring allergenic proteins

Corn does not contain any known naturally occurring allergenic proteins.

4.4 Potential allergenicity of novel protein

The concerns regarding potential allergenicity of novel proteins are two fold. Firstly, there are concerns that the ability to express new or different proteins in food will result in the transfer of allergens from one food to another, thereby causing some individuals to develop allergic reactions to food they have not previously been allergic to. Secondly, there are concerns that the transfer of novel proteins to food will lead to the development of new allergies in certain individuals. The former is more easily addressed than the latter because if an allergen is already known it is possible, using human sera or human skin tests, to test if it has been transferred. There are no reliable tests or animal models, however, which enable the prediction of the allergenic potential of novel proteins. Instead, potential allergenicity can only be indicated by examination of a number of characteristics of the novel protein, such as whether it is derived from a known allergenic source, its physical/chemical characteristics (most allergens have a molecular mass between 10 and 70 kDa, are glycosylated, and are resistant to acid and protease degradation), whether it has any sequence similarity to any known allergens, and whether it is likely to be present in large amounts in the food as consumed and therefore have potential for allergic sensitisation.

Studies submitted by Monsanto:

Walters, D.S. and Adams, W. (1996). *In vitro* digestibility of CryIAc and PAT proteins. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-96-A22.

Appendix 2: CryIAc and phosphinothricin acetyl transferase proteins show no homology to allergenic proteins. (Appendices to submission to ANZFA for the inclusion of corn containing the DBT418 gene by Monsanto in Standard A18 – Food derived from gene technology.)

Digestibility of CryIAc and PAT

E. coli produced CryIAc and PAT as well as PAT protein purified directly from DBT418 corn were subject to digestion under simulated gastric conditions. The microbial produced CryIAc protein had been experimentally determined to be equivalent to that which is expressed in DBT418 plants (see Section 3.2). The bacterially produced proteins were used to spike protein extracts from leaf tissue from non-transgenic plants. The proteins were added to leaf extract to give a concentration of 500 ng CryIAc/5 µL extract and 1900 ng PAT/5 µL extract.

The extracts were added to simulated gastric fluid (SGF) containing 3.2 mg/mL pepsin (1X), no pepsin or a 100 fold dilution of pepsin (0.01X). Samples were taken at 0, 2, 5, 15 and 30 minutes and analysed using immunoblotting.

CryIAc degraded rapidly in 1X SGF. No trace of CryIAc was detectable by immunoblot at 0 or 2 minutes. CryIAc was not degraded in SGF lacking pepsin. In 0.01X SGF, significant degradation of CryIAc was seen at 0 and 2 minutes incubation, and after 5 minutes no CryIAc protein could be detected.

PAT protein also degraded rapidly in 1X SGF. Significant degradation occurred at 0 minutes, and after 2 minutes only trace amounts of PAT protein were detectable. No degradation was seen in SGF lacking pepsin.

In 0.01X SGF, significant degradation of PAT was seen at 0 and 2 minutes incubation, and after 5 minutes no PAT protein could be detected. DBT418 leaf protein extract was also added to 0.01X SGF. PAT was visible at the time 0 point but was not detectable after 2 minutes incubation in 0.01X SGF.

The results demonstrate that both CryIAc and PAT are digested as normal dietary protein, both being rapidly degraded in the proteolytic and acid conditions of simulated gastric fluid suggesting they would not survive mammalian digestion.

Comparison of CryIAc and PAT amino acid sequence with known allergens

The amino acid sequences of CryIAc and PAT proteins in DBT418 corn and those of known allergens were compared. A significant sequence similarity was defined as a sequence identity of eight or more contiguous amino acids. A database of known allergenic proteins was assembled from the public domain genetic databases including GenPept, PIR and SwissProt. After eliminating duplicated and irrelevant sequences, a database of 276 known allergens remained. The database was searched for sequences similar to CryIAc and PAT proteins using the program FASTA.

The search did not identify any allergens with significant amino acid sequence similarity to either CryIAc or PAT.

Conclusion

As CryIAc and PAT are present at very low levels in the kernel, are easily digested in conditions mimicking mammalian digestion and do not show any significant amino acid sequence similarity with known allergens, they have limited potential to become a food allergen.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

There are concerns that genetic modification will affect the overall nutritional composition of a food, or cause unintended changes that could adversely affect the safety of the product. Therefore a safety assessment of food produced from transgenic plants must include analysis of the composition of the food, based on a comparison with other commercial varieties of the crop. Generally, comparisons are made not only with the parental line but also with other non-transformed lines. If the parameter for the transformed line is within the normal range for non-transformed lines, this is considered acceptable (Hammond and Fuchs 1998).

To determine whether unexpected changes had occurred in the nutritional composition of corn as a result of the DBT418 insertion event, and to assess the nutritional adequacy of the corn, compositional analyses of forage and grain were undertaken. Material was collected for analysis from DTB418 corn grown in field trials in the United States in 1995. The genotype tested was the same as used for the molecular characterisation and the protein expression analyses – that is, DK.DL(DBT418). This hybrid was sold commercially as DK566-DBT418. A non-transformed version of the same hybrid was used as a control.

Major components of the grain and forage (proximates) were determined using conventional chemical methods (Association of Official Analytical Chemists, AOAC; and American Oil Chemists Society, AOCS) and near infrared transmission (NIT) spectroscopy. Analysis included protein, oil, fibre, ash, starch and moisture. Chemical determination of composition was determined on samples collected from ten locations. NIT data was derived from samples collected from nine locations. Amino acid composition of DBT418 and control grain was determined by acid hydrolysis of corn meal and reverse phase high-performance liquid chromatography. Fatty acid composition was determined by trans-esterification and gas chromatography. Nine samples, representing three locations, were tested, except for fatty acid analysis where only three samples, representing one location, were tested.

Results are shown in Tables 10 – 13 below.

Table 10: Proximate analysis[1] of grain from DBT418 corn

Constituent analysed	Chemical analysis[2]		NIT analysis[3]		Literature
	DBT418	control	DBT418	control	Range[4]
Protein	9.02 ± 0.22	8.56 ± 0.16	9.06 ± 0.19	9.09 ± 0.17	6.0 – 12.0
Oil	4.05 ± 0.05	3.92 ± 0.04	4.16 ± 0.04	4.12 ± 0.04	3.1 – 5.7
Fibre	1.96 ± 0.03	2.02 ± 0.03	Not done	Not done	2.0 – 5.5
Ash	1.32 ± 0.01	1.30 ± 0.02	Not done	Not done	1.1 – 3.9
Moisture	8.14 ± 0.04	8.22 ± 0.04	70.61 ± 0.20	70.62 ± 0.17	7 – 23
Starch	Not done	Not done	5.63[5] ± 0.17	5.57[5] ± 0.11	-

[1] values are expressed as a % (dry weight basis) and are the mean ± S.E.
[2] sample size of 30
[3] sample size of 27
[4] Watson 1987
[5] samples were artificially dried

The proximate composition of grain from DBT418 corn is equivalent to that of the control hybrid lacking the DBT418 insertion event and the levels are also comparable to the available literature ranges for these constituents.

Table 11: Proximate analysis[1] of forage from DBT418 plants

Constituent	DBT418 hybrid[2]	Control[3]	Literature range[4]
Protein	6.81 ± 0.23	7.12 ± 0.29	3.5 – 15.9
Oil	2.77 ± 0.07	2.82 ± 0.06	0.7 – 6.7
Fibre	20.56 ± 0.03	20.57 ± 0.38	-
Ash	4.33 ± 0.15	4.28 ± 0.13	1.3 – 10.5
Moisture	66.68 ± 0.04	66.96 ± 0.04	-

[1] values are expressed as a % (dry weight basis) and are the mean ± S.E.
[2] sample size = 24
[3] sample size = 30
[4] Watson 1987

No significant differences were observed in proximate composition of forage between the DBT418 hybrid line and the control hybrid line. The proximate levels were also comparable to the literature reported range, where these were available.

Table 12: Amino acid composition of grain from DBT418 and control hybrids

	mg amino acid / g dry weight	
Amino acid[1]	**DBT418 hybrid**	**Control hybrid**
Aspartate + asparagine	2.58 ± 0.14	2.29 ± 0.15
Glutamate + glutamine	12.95 ± 0.64	13.75 ± 0.54
Serine	2.84 ± 0.26	2.99 ± 0.32
Glycine	3.08 ± 0.06	3.12 ± 0.06
Threonine	2.11 ± 0.09	2.1 ± 0.11
Arginine	3.95 ± 017	3.98 ± 0.21
Alanine	4.59 ± 0.17	4.85 ± 0.13
Valine	3.14 ± 0.11	3.22 ± 0.12
Phenylalanine	3.19 ± 0.16	3.43 ± 0.14
Isoleucine	2.39 ± 0.09	2.46 ± 0.10
Leucine	7.33 ± 0.42	7.91 ± 0.34
Lysine	2.68 ± 0.06	2.70 ± 0.06

[1] Tryptophan, cysteine and proline are acid labile therefore no values are reported. Methionine, histidine and tyrosine levels were not determined.

No significant differences in the amino acid composition of grain were observed between the DBT418 hybrid line and the control hybrid line.

Table 13: Fatty acid composition of grain from DBT418 and control hybrids

	% Total fatty acids		
Fatty acid[1]	**DBT418 hybrid**[2]	**Control hybrid**[3]	**Literature Range**[4]
Palmitic (16:0)	13.8	13.7	7 - 19
Stearic (18:0)	4.1	4.0	1 - 3
Oleic (18:1)	27.2	28.2	20 - 46
Linoleic (18:2)	53.4	52.6	35 - 70
Linolenic (18:3)	1.2	1.2	0.8 – 2.0
Eicosenoic (20:0)	0.5	0.6	

[1] Other fatty acids were below the limit of detection.
[2] The values for DBT418 corn are the means of three samples from one location.
[3] The values for the control are the means of nine samples, three from each of three locations.
[4] Watson 1982.

No significant differences in the fatty acid composition of grain were observed between the DBT418 hybrid line and the control hybrid line.

Compositional analysis of commercial hybrids

In addition to the above data, compositional data was also generated during the period of product use, 1997-1999, prior to the discontinued marketing of the product beginning in 2000. This data was generated from the analysis of commercial hybrid corn seed lots, archived material, and field samples. In these analyses, a number of hybrids, including all five hybrids that were sold commercially in 1999, were analysed for nutritional composition including proximate, amino acid content and calcium and phosphorus content. The results of these analyses are presented in Tables 14 – 16 below.

Table 14: Proximate analysis[1] of commercial F1 DBT418 and control hybrid corn seed

	DBT418 hybrids[2]						
	DK493-DBT418	**DK566-DBT418**	**DK580-DBT418**	**DK595-DBT418**	**DK626-DBT418**	**Control hybrids[3]**	**Literature values[4]**
Protein	9.8 (9.7-10.0)	9.6 (9.1-9.9)	9.5 (9.2-10.2)	10.4 (10.0-10.9)	11.7 (11.5-12.0)	10.2 (9.0-11.8)	9.5 (6.0-12.0)
Oil	4.1 (4.0-4.2)	4.8 (4.8-4.9)	4.2 (4.1-4.3)	3.9 (3.8-4.0)	4.4 (4.2-4.5)	4.2 (3.7-4.9)	4.3 (3.1-5.7)
Ash	1.3 (1.3-1.3)	1.3 (1.2-1.3)	1.3 (1.2-1.3)	1.3 (1.3-1.4)	1.3 (1.3-1.3)	1.3 (0.5-1.5)	1.4 (1.1-3.9)
Fibre	2.7 (2.6-2.8)	2.6 (2.4-2.8)	4.2 (3.9-4.3)	3.1 (2.7-3.5)	3.0 (2.8-3.2)	3.0 (2.0-4.1)	3.3 (3.3-4.3)
Moisture	11.1 (10.6-11.8)	11.9 (11.7-12.1)	11.9 (11.6-12.1)	11.4 (11.4-11.4)	11.7 (11.5-11.9)	11.6 (10.7-12.6)	(7 – 23)

[1] Mean and (range) reported as percent on a dry weight basis (except for moisture)
[2] Values derived from three lots tested for each hybrid (n=3) except for DK595-DBT418 (n=2).
[3] Analysis based on three lots each of DK493, DK566, DK580, DK595, and DK626. Values for individual controls were also provided by the applicant.
[4] Values for grain purchased off the open market (Watson 1987).

No major differences in proximate of grain were observed between the commercial DBT418 hybrids line and the control hybrids. The values reported were comparable to the literature reported ranges.

No significant differences in amino acid content of grain were observed between the commercial DBT418 hybrids line and the control hybrids. Except for tyrosine, the values reported were comparable to the literature reported ranges. The values for tyrosine for both the DBT418 and control hybrids were low compared to the literature reported ranges.

Table 15: Amino acid analysis[1] of commercial F1 DBT418 and control hybrid corn seed

	DBT418 hybrids[2]						
	DK493-DBT418	**DK566-DBT418**	**DK580-DBT418**	**DK595-DBT418**	**DK626-DBT418**	**Control hybrids[3]**	**Literature values[4]**
Lysine	2.8 (2.8.3.0)	2.7 (2.7-2.7)	2.9 (2.6-3.1)	2.5 (2.4-2.6)	2.3 (2.2-2.3)	2.7 (2.3-3.1)	2.5 (2.0-3.8)
Threonine	3.1 (3.0-3.1)	3.1 (3.1-3.2)	3.6 (3.4-3.7)	3.3 (3.2-3.4)	3.0 (3.0-3.0)	3.4 (2.9-3.7)	3.8 (2.9-3.9)
Isoleucine	2.6 (2.6-2.7)	2.7 (2.6-2.7)	2.7 (2.6-2.8)	2.7 (2.7-2.8)	2.7 (2.7-2.7)	2.8 (2.5-3.2)	4.2 (2.6-4.0)
Histidine	2.5 (2.5-2.6)	2.5 (2.4-2.5)	2.5 (2.3-2.6)	2.4 (2.4-2.5)	2.3 (2.2-2.3)	2.5 (2.2-2.7)	2.1 (2.0-2.8)
Valine	3.7 (3.7-3.8)	3.7 (3.5-3.7)	3.7 (3.5-3.8)	3.7 (3.7-3.8)	3.5 (3.4-3.6)	3.8 (3.4-4.3)	4.7 (2.1-5.2)
Leucine	10.7 (10.5-10.8)	11.2 (11.0-11.3)	11.1 (11.0-11.4)	12.2 (12.0-12.3)	12.2 (12.0-12.5)	11.3 (10.4-12.6)	11.2 (7.8-15.2)
Arginine	3.8 (3.7-3.9)	3.7 (3.6-3.7)	4.0 (3.7-4.1)	3.5 (3.4-3.6)	3.2 (3.1-3.4)	3.6 (3.2-4.2)	5.8 (2.9-5.9)
Phenylalanine	4.1 (4.1-4.2)	4.3 (4.2-4.4)	4.5 (4.4-4.5)	4.6 (4.5-4.6)	4.6 (4.5-4.7)	4.3 (4.1-4.7)	4.9 (2.9-5.7)
Glycine	3.7 (3.6-3.7)	3.6 (3.5-3.7)	3.6 (3.3-3.8)	3.4 (3.2-3.5)	3.3 (3.2-3.4)	3.5 (3.2-3.8)	3.7 (2.6-4.7)
Alanine	6.9 (6.8-7.0)	7.2 (7.1-7.2)	6.9 (6.8-7.0)	7.4 (7.4-7.4)	7.4 (7.3-7.5)	7.0 (6.6-7.4)	7.8 (6.4-9.9)
Aspartic acid	6.6 (6.6-6.7)	6.7 (6.7-6.7)	6.7 (6.5-6.9)	6.6 (6.4-6.8)	6.2 (6.1-6.2)	6.5 (6.0-6.9)	6.8 (5.8-7.2)

Glutamic acid	16.3 (16.1-16.5)	17.0 (16.9-17.0)	17.4 (17.2-17.8)	18.1 (17.7-18.5)	17.3 (17.0-17.6)	19.2 (16.6-24.3)	17.7 (12.4-19.6)
Proline	8.3 (8.0-8.5)	8.6 (8.5-8.7)	8.0 (7.7-8.2)	8.8 (8.8-8.8)	9.1 (8.9-9.1)	8.5 (8.1-9.1)	8.4 (6.6-10.3)
Serine	5.6 (5.6-5.7)	5.9 (5.8-5.9)	5.1 (5.1-5.1)	5.5 (5.1-5.9)	5.9 (5.8-6.0)	5.2 (4.5-5.9)	4.6 (4.2-5.5)
Tyrosine	1.7 (1.6-1.7)	1.7 (1.7-1.8)	1.7 (1.6-1.8)	1.8 (1.7-1.8)	1.6 (1.7-1.8)	1.7 (1.5-1.8)	4.7 (2.9-4.7)

[1] Mean and (range) reported as percent total protein. Analyses for methionine, cystine and tryptophan not included.
[2] Values derived from three lots tested for each hybrid (n=3) except for DK595-DBT418 (n=2).
[3] Analysis based on three lots each of DK493, DK566, DK580, DK595, and DK626. Values for individual controls were also provided by the applicant.
[4] Values for grain (Watson 1982).

Table 16: Calcium and phosphorus content[1] of commercial F1 DBT418 and control hybrid corn seed[2]

	Hybrid						
	DK493-DBT418	**DK493**	**DK595-DBT418**	**DK595**	**DK626-DBT418**	**DK626**	**Literature[3]**
Ca	ND-0.007	ND-0.004	ND-0.003	ND-0.003	0.003 (0.003-0.003)	0.003 (0.003-0.003)	0.03 (0.01-0.1)
P	0.26 (0.24-0.27)	0.26 (0.25-0.28)	0.29 (0.29-0.30)	0.25 (0.24-0.26)	0.25 (0.23-0.27)	0.31 (0.28-0.32)	0.27 (0.26-0.75)

[1] Mean and (range) reported as percent total calcium or phosphorus.
[2] Phosphorus and calcium content were determined through analysis of randomly chosen archived commercial F1 hybrid seed lots. Because the samples were not drawn from a controlled trial, the results are not suitable for statistical analysis.
[3] Values for grain purchased from the open market (Watson 1982).

No meaningful differences were observed between the DBT418 and control hybrids. The values for phosphorus were also comparable to the literature reported values. The values for calcium, for both the DBT418 hybrids and the control hybrids, were low when compared to the literature reported values. This is most likely due to environmental factors and is not considered to be treatment related.

Conclusion

Grain from hybrid corn lines containing the DBT418 event is compositionally no different to grain from hybrid corn lacking the DBT418 event.

5.2 Levels of anti-nutrients

Corn contains few natural toxins or anti-nutrients. The anti-nutrients trypsin and chymotrypsin inhibitors are present in corn at very low levels and are not considered nutritionally significant (Wright 1987).

5.3 Ability to support typical growth and well-being

In assessing the safety of food produced using gene technology, 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 the case of DBT418 corn, the extent of the compositional and other data provided in this application is considered adequate to establish the safety of the food.

REFERENCES

Adang, M.J., Staver, M.J., Rocheleau, T.A., Leighton, J., Barker, R.F. and Thompson, D.V. (1985). Characterised full-length and truncated plasmid clones of the crystal protein of *Bacillus thuringiensis* subsp. *kurstaki* HD73 and their toxicity to *Manduca sexta*. *Gene* **36:** 289.

Alexander, R.J. (1987) Corn dry milling: processes, products and applications. In: *Corn: Chemistry and Technology*. Watson, S.A. and Ramstead, P.E. (eds), American Association of Cereal Chemists Inc, St Paul, Minnesota. pp 351-376

Benfey, P.N. and Chua, N. (1990). The cauliflower mosaic virus 35S promoter: combinatorial regulation of transcription in plants. *Science* **250:** 959-966.

Bietlot, H., Carey, P.R., Choma, C., Kaplan, H., Lessard, T. and Pozsgay, M. (1989). Facile preparation and characterisation of the toxin from *Bacillus thuringiensis* var. *kurstaki*. *Biochem. J*. **260:** 87-91.

Bouchez, D., Tokuhisa, J.G., Llewellyn, D.J., Dennis, E.S. and Ellis, J.G. (1989). The OCS-element is a component of the promoters of several T-DNA and plant viral genes. *EMBO J.* **8:** 4197-4204.

Calva, J.J., Sifuentes-Osbornio, J. and Ceron, C. (1996) Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. *Antimicrobial Agents and Chemotherapy* **40**: 1699-1701.

Comai, L. and Stalker, D. (1986). Mechanism of action of herbicides and their molecular manipulation. *Oxford Surveys of Plant Molecular and Cell Biology* **3:** 166-195.

De Block, M., Botterman, J., Vanderwiele, M., Dockx, J., Thoen, C., Gossele, V., Rao Movva, N., Thompson, C., Van Montagu, M., and Leemans, J. (1987). Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO J* **6**: 2513-2518.

Dennis, E.S., Gerlach, W.L., Pryor, A.J., Bennetzen, J.L., Inglis, A., Llewellyn, D., Sachs, M.M., Ferl, R.J. and Peacock, W.J. (1984). Molecular analysis of the alcohol dehydrogenase (*adhI*) gene of maize. *Nucl. Acids Res.* **12:** 3983-4000.

Dhaese, P., De Greve, H., Gielen, J., Seurinck, J., Van Montague, M. and Schell, J. (1983). Identification of sequences involved in polyadenylation of higher plant nuclear transcripts using *Agrobacterium* T-DNA genes as models. *EMBO J*. **2:** 419-426.

Ferré, J. *et al* (1991). *Proc. Natl. Acad. Sci. USA* **88:** 5119-5123.

Gordon-Kamm, W.J., Spencer, T.M., Mangano, M.L., Adams, T.R., Daines, R.J., Start, W., O'Brien, J.V., Chambers, S.A., Adams Jr, W.R., Willets, N.G., Rice, T.B., Mackey, C.J., Krueger, R.W., Kausch, A.P. and Lemaux, P.G. (1990). Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell* **2:** 603-618.

Hammond, B.G. and Fuchs, R.L (1998). Safety evaluation for new varieties of food crops developed through biotechnology. In: *Biotechnology and safety assessment*, Thomas, J.A (ed.). Taylor and Francis, Philadelphia.

Hofmann, C, Vanderbruggen, H.V., Hofte, H, Van Rie, J, Jansens, S. and Van Mellaert, H, (1988). Specificity of *B. thuringiensis* delta-toxins is correlated with the presence of high affinity binding sites in the brush border membrane of target insects midguts. *Proceedings of the National Academy of Sciences USA*. **85:** 7844–7848.

Hofte, H. and Whiteley, H.R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.* **53:** 242-255.

Johnson, R., Narvaez, J., An, G. and Ryan, C. (1989). Expression of proteinase inhibitors I and II in transgenic tobacco plants: Effects on natural defense against *Manduca Sexta* larvae. *Proc. Natl. Acd. Sci. USA* **86:** 9871-9875.

Jones, D.D. and Maryanski, J.H. (1991). Safety considerations in the evaluation of transgenic plants for human food. In: *Risk assessment in genetic engineering*, Levin, M.A. and Strauss, H.S. (eds). New York: McGraw-Hill.

MacIntosh, S.C., Kishore, G.M., Perlak, F.J., Marrone, P.G., Stone, T.B., Sims, S.R. and Fuchs, R.L. (1990a). Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. *J. Agric. Food Chem.* **38:** 1145-1152.

MacIntosh, S.C., Stone, T.B., Sims, S.R., Hunst, P., Greenplate, J.T., Marrone, P.G., Perlak, F.J., Fischhoff, D.A. and Fuchs, R.L. (1990b). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *J. Insect Pathol.* **56:** 258-266.

May, J.B. (1987) Wet milling: processes and products. In: *Corn: Chemistry and Technology*. Watson, S.A. and Ramstead, P.E. (eds), American Association of Cereal Chemists Inc, St Paul, Minnesota. pp 377-397.

Neu, H.C. (1992) The crisis in antibiotic resistance. *Science* **257**:1064-1073.

Odell, J.T., Nagy, F. and Chua, N-H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* **313:** 810-812.

Plunkett G., Senear D.F., Zuroske G., Ryan C.A. (1982) Proteinase inhibitors I and II from leaves of wounded tomato plants: purification and properties. Arch Biochem Biophys 1982 Feb;213(2):463-72

Rajamohan, F., Lee, M.K., and Dean, D.H. (1998) *Bacillus thuringiensis* insecticidal proteins: molecular mode of action. *Prog Nucleic Acid Res Mol Biol* **60**: 1-27.

Ryan, C. (1978). *Trends Biochem*. *Sci*. **7:** 148-150.

Ryan, C.A. (1990). Protease inhibitors in plants: genes for improving defences against insects and pathogens. *Ann. Rev. Phytopathol*. **28:** 425-449.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual, 2nd Edition*. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H. (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol Mol Biol Rev* **62**: 775-806.

Sjoblad, RD, JT McClintock and R Engler. 1992. Toxicological considerations for protein components of biological pesticide products. Regulatory Toxicol. and Pharmacol. 15:3-9.

Sprague, G.F. and Dudley, J.W. (eds) (1988). *Corn and Corn Improvement, Third Edition*. Number 18 in the Series, Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WI.

Stewart, G.J. and Carlson C.A. (1986). The biology of natural transformation. *Ann. Rev. of Microbiol*. **40:** 211-235.

Sutcliffe, J.G. (1978). Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. *Symposia on Quantitative Biology* **43**: 77-103.

Thompson, C.J., Movva, N.A., Tizard, R., Crameri, R., Davies, J.E., Lauwereys, M. and Botterman, J. (1987). Characterisation of the herbicide resistance gene bar from *Streptomyces hygroscopicus*. *EMBO J.* **6:** 2519-2523.

Thornburg, R.W., An, G., Cleveland, T.E., Johnson, R. and Ryan, C.A. (1987). Wound-inducible expression of a potato inhibitor II-chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA*. **84:** 744-748.

Watson, S.A. (1982). Corn: Amazing Maize: General Properties. In: *CRC Handbook of Processing and Utilization in Agriculture, Volume II*: Part 1 Plant Products. I.A. Wolff (ed.) CRC Press Inc., Florida, USA.

Watson, S.A. (1987). Corn marketing, processing and utilisation. In: Sprague, G.F. and Dudley, J.W. (eds.) *Corn and Corn Improvement*, pp 885–940. American Society of Agronomy, Madison, Wisconsin.

White, J., Chang, S.-Y.P., Bibb, M.J. and Bibb, M.J. (1990). A cassette containing the bar gene of *Streptomyces hygroscopicus*; a selectable marker for plant transformation. *Nucl. Acids Res.* **18:** 1062.

WHO (1991) Strategies for assessing the safety of foods produced by biotechnology. Report of a joint FAO/WHO Consultation. World Health Organization, Geneva, 59 pp.

WHO (1993) Health aspects of marker genes in genetically modified plants. Report of a WHO Workshop. World Health Organization, Geneva, 32 pp.

Wohlleben, W., Arnold, W., Broer, J., Hillemann, D., Strauch, E. and Puehler, A. (1988). Nucleotide sequence of phosphinothricin-N-acetyltransferase gene from *Streptomyces viridochromogenes* Tue H94 and its expression in *Nicotiana tabacum*. *Gene* **70:** 25-37.

Wolfersberger, M.G. (1990). Specificity and mode of action of *Bacillus thuringiensis* insecticidal crystal proteins toxic to lepidopteran larvae: Recent insights from studies utilising midgut brush border membrane vesicles. *Proc. Vth Int. Colloq. Invertebr. Pathol*. August 20-24, 1990, Adelaide, pp. 278-282.

Wright, K.N. (1987). Nutritional properties and feeding value of corn and its by-products. In: *Corn: Chemistry and Technology*. S.A. Watson and P.E. Ramsted (eds.), American Association of Cereal Chemists, Inc. St. Paul, MN., USA.

ATTACHMENT 3

SUMMARY OF PUBLIC SUBMISSIONS

A. First round submissions

1. **National Genetic Awareness Alliance (Australia)**
   - Believes that the patenting of life-forms and living processes represents a violation of human rights, threat to food security, impediment to medical research and a threat to animal welfare
   - Believes that current GM techniques are inherently hazardous, and have been shown recently to offer no benefits
     - Lower yields with high pesticide input
     - Intensification of the corporate monopoly on food
     - Spread of antibiotic resistance marker genes and promoter sequences
     - Possible increase of allergenicity due to spread of transgenic pollen
   - Urges governments to use precautionary principle and carry out research into sustainable agricultural methods
   - Calls for suspension of trials and sale of GM products and public inquiry.

2. **Pola Lekstan and Anna Clements (Australia)**
   - Are concerned that approval without long-term testing may pose a health threat, that more GM food means less choice for those wanting to avoid it, that Bt may affect non-target organisms, and that herbicide resistance may lead to overuse of chemicals.

3. **Arnold Ward (Australia)**
   - Questions the system of MRL-setting in light of the levels of high glyphosate residues in Roundup Ready soybeans and of other chemicals (including the Bt toxin) in GM crops
   - Is concerned about detrimental effect of Bt on non-target (beneficial) organisms and on humans, and believes that genetic engineering is imprecise with uncertainties in outcomes
   - Believes that the concept of substantial equivalence is inadequate and should not be used to avoid more rigorous testing, and that commercial factors are overriding need for basic research. Also believes that ANZFA's arguments defend the needs of biotechnology companies and food processing industry, and that since ANZFA does no testing itself, the results can't be trusted.

4. **Australian GeneEthics Network**
   - Believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
     - Direct health effects of pesticide residues
     - Possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
     - The possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
     - Insertion of viral DNA could create new and virulent viruses
     - The possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
     - The threat to GE-free status export markets
   - Believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

5. **Public and Environmental Health Service (Australia)**
   - Believes that the data provided should cover both the intentional and unintentional effects of the genetic modification. The unintended consequences of random insertion of new genetic material into the host genome could include loss or change of function of gene or controlling element, disregulation or amended regulation of the gene or controlling element, or production of a novel hybrid protein which could occur in an unregulated manner. They should also cover any compositional changes e.g. nutrients, antinutritional factors, natural toxicants, and define when a change would be considered 'significant'

- Potential effect of introduced proteins on metabolic pathways should be addressed e.g. over-expression or inhibition of enzymes
- Data should include details of whether introduced proteins are detectable in whole commodities, processed products and highly processed derivatives
- Data should include details of toxicity and allergenicity tests to prove that food is safe, as well as address issues of specificity and potency of proteins. It should also address the ability to support typical growth and well-being
- Data for herbicide-tolerant plants should be derived from studies performed on plants treated with herbicide. They should address the human toxicity of the herbicide and whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.

6. David Grundy (Australia)

- Considers that the expression of Bt toxins and other chemicals in plant tissues removes the choice of washing chemicals off fruit and vegetables. Believes that Roundup Ready crops have glyphosate or glufosinate molecules genetically attached
- Believes that GM crops should not be used for feed given to animals bound for human consumption, that products encouraging antibiotic resistance should not be used, and that labelling should be mandatory for all products containing GM ingredients

7. Leesa Daniels (Australia) Member of the Genetic Engineering Action Group

- Believes that:
  - Scientific research although limited, has brought concerns to light
  - Substantial equivalence is a subjective principal
  - Comprehensive and mandatory labelling must be urgently implemented
  - The cauliflower mosaic virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
  - Antibiotic marker genes could lead to increase in antibiotic resistance
  - Several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

8. Australian Food And Grocery Council

- Fully endorses the policy of minimum affective regulation, supports these applications, and considers that food manufacturers should make their own choice with regard to use of GM crops or products derived from them
- Believes that since the growth of GM crops has been approved overseas, they would support their growth in Australia if approved through the GTAC/GMAC/OGTR process
- Considers it unfortunate that ANZFA has not negotiated "equivalence" agreements for products already approved overseas to enable approval without having to carry out its own safety assessment. In the absence of such an agreement it supports the ANZFA safety assessment process.
- Believes that an appropriate information and labelling scheme would enable consumers to make an informed choice.

9. New Zealand Ministry of Health

- Referred preliminary report to New Zealand Health Research Council, who stated concern that all safety aspects should be carefully considered in the ANZFA process.

10. Nestlé Australia Ltd.

- Supports the continued approval of glufosinate ammonium-tolerant canola, and believes that manufacturers would be disadvantaged were approval not to be granted.

11. Consumers' Association of South Australia Inc. & National Council of Women of Australia (CASA supports submission of NCWA)

- Believe that current testing procedure is inadequate and that human trials are the only adequate method, as with testing of new drugs. Also that physiological and neurological effects as well as the toxicological and allergenic effects should be looked at, and that an independent body should be responsible for testing
- Do not support the use of antibiotic markers, since they believe they may pose a threat to efficacy of antibiotics in humans

- State that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans confirming the inadequacy of the term 'substantial equivalence'
- Raise the point that although these crops have been approved elsewhere, this situation may change with consumer pressure
- Do not accept that it is impossible to source food to ascertain whether or not it contains GM ingredients. Believe that if McCain and Sanitarium can do it, then others should also be able to
- State general concern about the risk that MRLs will be raised as a result of herbicide-tolerant crops being developed, and feel that the calculations used are flawed and are not based on safety criteria
- Believe that the use of GM crops in animal feed should also be regulated. A378
- State concern over possible increase in glyphosate use (it is apparently confirmed in one reference that herbicide use increases with herbicide resistant crops), referring to studies that link the chemical to Hodgkin's lymphoma, and the possibility that Europe may ban it due to adverse effects on beneficial insects**.** They are particularly concerned that glyphosate is not looked at by the same regulatory body as that looking at GM foods

A379, A388

- State concern over the persistence and toxicity of bromoxynil, and consider that these have not been adequately assessed by the US FDA. They understand that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself, and believe that a safety assessment needs to be done on this too. This is apparently the main residue, and they believe that this may appear in cotton oil and linters.

A372, A375, A380, A381, A386

- With respect to glufosinate ammonium, state concern about toxicity, neurotoxicity, teratogenicity and residues in food, soil and water. They believe that Monsanto is likely to apply for an increase in the MRL, and that such increases are likely to constitute a health hazard

A380, A382, A383, A384, A385, A386

- Raise issues of adverse effects of Bt toxins on non-target insects and think that it needs more study.

A387

- Believe that raising the amount of a nutrient in a food may have unknown drawbacks e.g. affecting the efficacy of other nutrients.

12. Health Department of Western Australia

- Highlights various health and environmental concerns:
  - the use of antibiotic resistance genes as markers may transfer resistance to animals via gut bacteria
  - the possibility that microbial gene sequences may contain fragments of other virulent genes, and also that ingesting Bt toxins may be harmful to humans
  - the possibility that insects may be more prone to developing resistance to Bt, since Bt toxins have been found to be released into the soil
- Believes that both safety data and gene sequences should be available for public scrutiny.

13. Meat New Zealand

A379

- Concerned at how labelling regulations will apply to sausage casings that may contain cotton linters even if they are not to be eaten, i.e. are effectively a processing aid. Think that labelling should only be used to advise the sausage manufacturer not consumers.

14. BRI Australia

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety.

15. Food Technology Association of Victoria Inc.

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety.

16. Diane Davie (Australia)

- Believes all 13 applications should be rejected, since they have not undergone human safety testing here or overseas, and have not been assessed on their ethical merits
- Believes that risks include:
  - Bacterial and viral vectors which could affect human physiology
  - Herbicide and insect-resistance genes, which could increase allergies and antibiotic resistance
  - Environmental risks

- Also believes that ANZFA must heed the concerns of consumers opposed to GM foods.

17. Martin Hurley, David Hook, Ian Smillie, Margaret Dawson, Tee Rodgers-Hayden, David Lovell-Smith (Natural Law Party), Barbara Brown, Ngaire Mason, Robert Anderson (member, Physicians and Scientists for Responsible Genetics), Louise Carroll, Gilbert Urquart, Caroline Allinson-Dunn, Megan Lewis, Peter Barnes, James Harlow, Gabrielle Dewan, Scott Young, Virginia Murray, Stephanie Chambers, Kay Dyson, Peter Fenwick, Joanne Xerri, Paul True, Josh Gill, James & Peysha Charlwood, Mitta Hirsch, Alan Florence, Nicole Paul, Lawrence Clarke, David Snowman, Reg Paling, Mark and Johanna Blows, David and Bev Semour, Richard and Sharon Moreham (see also below), Stuart Drury and Helen Murphy (All Australia), Brennan Henderson (New Zealand)

- Believe that most Australians and New Zealanders do not want GM foods, there are no benefits, and deferral would not be disadvantageous. Approval should be delayed until they are proven safe.
- Feel that there is insufficient time to assess these applications thoroughly, and there are so many products under development that there is a high overall risk of a major disaster
- Believe that GM foods encourage pesticide use, and applications have made for commercial purposes only, and also that here could be commercial benefit to Australia and New Zealand in remaining GM-free.

18. Richard and Sharon Moreham (see also above)

- In addition to the points above, also think that it is unfortunate that the NZ government agreed to joint approval of food, as the Australian public are less educated about the issues surrounding GM foods
- Think that approval would only prove that ANZFA serves the interests of large multinational companies rather than those of the public.

19. Vicky Solah (Australia)

- Is for GM foods if the safety evaluation is carry out using approved, validated methods by an independent body, if the results are made available to consumers, and if all GM food is labelled
- Is concerned that transformation may lead to disruption of another gene, and that more research is needed before it is clear whether the process is safe
- With regard to herbicide tolerant crops, is concerned that consumers may not be aware of the need to wash products that have been sprayed, and that this therefore impacts on food safety. Also concerned about environmental impact of these chemicals, and of the possibility of resistance necessitating higher pesticide use in the future.

20. Dr Rosemary Keighley (Australia)

- Will not purchase foods unless they are certified GM-free. Believes that Australian producers who do not actually use GM products, but who fail to label them as such, will suffer.

21. Nicola Roil (Australia)

- Believes that GM foods pose health threats and may contaminate non-modified crops

22. Ian and Fran Fergusson (Australia)

- Believe there has been inadequate testing, and are concerned about possible side-effects.

23. Lyndal Vincent (Australia)

- Urges delay of approval until proven safe by extensive testing. Considers that genetic material is being released without knowing what the effects are, and cannot be recalled.
- Believes that there is no benefit to the consumer, and that national economic interests are best served by maintaining a GM-free market.

24. Fay Andary (Australia)

- Does not want any of the 13 products covered by the applications to be approved for inclusion in the food supply.

25. John and Francesca Irving (Australia)

- Thinks that no GE foods should be approved for inclusion in the food chain.

26. Diana Killen (Australia)

- Believes that there is no proven benefit to consumers and in many instances nutritional value is actually lower in GM crops, and it is therefore irresponsible to push through approval without thorough assessment of their long-term safety for public health.
- Suggests that research has highlighted adverse allergic reactions and a lowered immune response in some individuals, and that there are health implications with crops designed to be grown with greater concentrations of pesticides
- Thinks that labelling is essential for consumers to discriminate in purchasing, and that Australia has a unique opportunity in supply of organic and GM-free food.

27. Sheila Annesley (Australia)

- Does not want any of the 13 foods included in the food supply.

28. David and Edwina Ross (Australia)

- State concern for the future food supplies and well-being of their grandchildren.

29. Beth Schurr (Australia)

- Wishes to protest against the threat of GM foods, the possible future detrimental effects and the further endangering of the planet.

30. Beth Eager (Australia)

- As a parent is concerned that neither the long-term effects on health nor the environment are being considered.

31. Bruce Pont and Ljiljiana Kuzic-Pont (Australia)

- Believe that safety has not been, and cannot be satisfactorily determined, and that any party associated with GM foods could be legally liable should adverse health effects be seen. Thalidomide, smoking, 'Agent Orange' and asbestos all show that such things can affect subsequent generations
- Believe that an increase in use of pesticides will result from pesticide-tolerant crops, and that the emphasis should be on organic and/or safe agriculture
- Believe that GM-food is a retrograde step, contrary to nature and has the potential to destroy the human race.

32. Chitta Mylvaganum (Australia)

- Wishes to know what tests were done to assess negative effects on human and environmental health, how thorough they were, what the outcomes were, are the results publicly available, and what further avenues of inquiry are open to the public
- Requests the prevention of the import or release of any products until tests are carried out by unbiased scientists in order to prove the lack of health or environmental effects.

33. John Stevens (Australia)

- Would be concerned if approval were granted before sufficient research had been completed on potential impacts on human health and gene pools of nearby crops. Once grown, spread via pollen would be impossible to stop, and labelling would not prevent exposure by this route
- Considers that utmost caution should be exercised and import approval denied indefinitely.

34. Tim Carr (Convenor of the Emergency Committee against GE Foods)(Australia)

- Believes that GM-foods are produced using a radical and unpredictable new technology so should be subject to more rigorous testing
- States that it is unknown how the introduced gene will interact with and influence genetic expression in the host genome, and could change the chemical nature of the food
- Considers that health risks could result from the increased use of pesticides, and also that ANZFA should consider wider environmental, ethical and socio-economic issues.

35. Jan Kingsbury (Australia)

- Believes that GM-foods could result in loss of economic advantage for Australia and New Zealand since they are known internationally for pure and safe products

- Believes that foods are being complicated and pushed by big internationals, and organic farmers are being contaminated by cross-pollination.

36. Teresa Sackett (Australia)

- Believes that:
  - The KPMG report on labelling was prepared in a ridiculously short time and provided limited analysis
  - The proposal of 'no label' for foods which 'may contain' or in which there is 'no evidence' of GM material is inadequate
  - Inadequate testing procedures should not be used to declare a product is GM-free just because material can't be detected. In fact testing methods have been developed that can be used to work out the GM content
  - Government and industry seem to be favouring the introduction of GM foods. This will result in the increased use of chemicals and the destruction of soil life
  - Organic farming pay high costs for producing healthy plants, while conventional farmers have little restriction on pollution of air, soil and water. Salinity problems, the death of the Great Barrier Reef, rivers and streams has resulted from ignorance in farming and broader community. Such problems will increase with GM foods.
  - The implication that the public will not understand the issues is wrong. Everyone needs to be fully informed.
- Asks the question of whether workers in the food industry are to be better informed, and also why no 'verification documents' are to be required by retailers? Believes that certification schemes should be on a par with those for Kosher foods and organics.

37. John and Sandy Price (Australia)

- Approval of GM foods and seeds should not be allowed, as it is an affront to the sovereignty of Australia and the dignity of the Australian people. The results of the experiment cannot be reversed.

38. John Scott (New Zealand)

- Encloses article from The Irish Times, which describes the restrictions that have been placed by the US EPA on the cultivation of GM corn. These appear to have resulted from fears that Bt crops may be harmful to Monarch butterflies and that resistance may develop to Bt.

39. R A Randell (New Zealand)

- Believes that all GM products should be placed under a moratorium until the Royal Commission of Inquiry has considered the issue, and until all scientific, philosophical, ethical and moral issues have been looked at.

40. National Council of Women of New Zealand

- Believes that:
  - approval of all 13 applications should be rejected, and that none should be approved for planting.
  - Independently-funded body should be responsible for safety assessments
  - If it is possible to segregate high-oleic soybeans, then RoundUp Ready soybeans should be segregated too
  - Consumers should be made aware of the extent of GM ingredients in their food
  - GM foods, additives or processing aids already on the market must be labelled comprehensively and without extra cost to the consumer – suggest 'GM unknown' rather than 'may contain'
- Appreciates that rejection may contravene the WTO agreement, but consider that the primary role of ANZFA is the assurance of health and safety.

41. Safe Food Campaign (New Zealand)

- Believes that approval should be rejected, and a moratorium be put in place until after the Royal Commission of Inquiry, for various reasons:
  - Possible effects on non-target insects

- Spread of GM pollen may cause contamination of non-GM (especially organic) crops, and may result in the spread of herbicide-tolerance genes and an increase in resistance development. Cross-pollination is considered a particular risk for canola (A372 & A388). Bt resistance development is noted as being a particular risk for A382, A383 & A384
- Lack of long-term testing means health risks are not known
- Use of broad-spectrum pesticides affects wild flowers and non-target insects.

42. Jocelyn Logan, Caroline Phillips (New Zealand)

- Oppose all 13 applications for the following reasons:
  - Testing has not been long-term or independent, precautionary principle should apply. Approval can happen later if GM is proven safe.
  - No clear public benefit, and lack of opportunity for informed choice (immoral and undemocratic). Labelling regulations also unsatisfactory in this respect.
  - Environmental concerns (increase in pesticides, threat to organic farming, Bt resistance).

43. Robert Anderson (member of Physicians and Scientists for Responsible Genetics – New Zealand)

- Considers that the GM issue should be reconsidered in the light of the release of internal FDA documents made available for a recent lawsuit aimed at amending their policy. Attached document (presentation given by Steven Druker, Alliance for Bio-integrity) suggests that:
  - Scientist's warnings have been ignored
  - FDA policy may be illegal, violating the Food, Drugs and Cosmetic Act – Mr Druker believes that the term generally-regarded-as-safe (GRAS) cannot apply to foreign DNA.

44. Stephen Blackheath (New Zealand)

- Argues that ANZFA's approach to safety assessments is scientifically unsound:
  - Antibiotic resistance marker genes have been cited as being potentially dangerous by groups other than ANZFA e.g. the Royal Society
  - Unanticipated toxins and allergens are a concern, and it is suggested that the ANZFA process does not adequately consider these possibilities
  - Doesn't address the question of whether risks exist that are unique to the GM process
  - It relies on data from the manufacturers themselves, with little sway given to evidence from public submissions. Companies have vested interests the results and cannot be trusted (also gives evidence of Monsanto's past dishonesty)
- Believes that ANZFA is subject to undue influence through the directors, and is biased towards being pro-GM
- Suggests that RoundUp Ready soybeans are not substantially equivalent as the stems have been found to be more brittle than traditional lines, and may be lower in phytoestrogen content
- Also cites the lawsuit being brought by the Alliance for Bio-integrity, and the internal FDA documents that suggest concern from FDA scientists, as evidence of the FDA ignoring important evidence.

45. Claire Bleakley (New Zealand)

- Believes that approval should be rejected for various reasons:
  - They may be against Maori views
  - Further long-term trials are needed and should be carried out by ANZFA themselves - certain trials have apparently shown effects on immune system, allergies and rare syndromes
  - Health concerns of pesticide overuse
  - The possibility of horizontal gene transfer with respect to antibiotic resistance transfer
  - Lack of labelling and the use of the unsatisfactory 'substantial equivalence' concept, which makes hazard difficult to assess
  - There is no substantial gain to consumers

B. Second round submissions

1. **CSIRO Health Sciences & Nutrition (Australia)**
   - Notes that there must be an error in Table 11 of the safety assessment for A380, if the statement is correct that the level of fibre is comparable to those in the literature since the values given for fibre are 20.56, whereas the literature range is given as 2.0 – 5.5.
   - The compositional data presented indicate that DBT418 corn is not nutritionally different to control corn lacking the DBT418 insertion event, therefore from a nutritional standpoint this is acceptable.

2. **Dr Kate Clinch-Jones (Australia)**
   - Notes that 41 of the 45 submissions received opposed the introduction of this GM food. States that this percentage would be higher if the 41 submitters using a generic email objection were counted individually rather than as one body.
   - Notes that those opposed to the introduction of GM food represent a broad cross section of the community, and include individual doctors, scientists and the Health Department of Western Australia.
   - States that ANZFA does not produce any new evidence to support its arguments against the points raised in submissions and instead, ANZFA's response is based on rhetoric, and designed to forward the political stance that GM foods are safe.
   - ANZFA reiterates disputed statements from the original safety assessment and claims they provide additional reassurance that various aspects of the GM food are safe.
   - States that the public does not want reassurance but demands scientific proof that GM food are safe for consumption.
   - Scientific proof of safety simply does not exist.
   - States that in particular, no human feeding studies have ever been done and that animal studies look at gross parameters and omit vital assessments such as blood analysis and histopathology and use a small number of animals, rendering any statistical analysis virtually meaningless.
   - States it is time that those responsible for food safety assessments acknowledge that GM foods pose significant, unique health risks.
   - Submits that in the interests of public health, all GM foods already on the market should be immediately recalled until satisfactory independent safety test data is available.

3. **Ministry of Health (New Zealand)**
   - Notes that DBT418 corn has been discontinued for 3 years and is exiting the food supply.
   - Believe the data to support the safety of DBT418 corn is acceptable and agree with ANZFA's assessment that food from DBT418 corn is as safe and wholesome as food from other commercially available corn varieties.
   - Data supporting the non-expression of the *bla* and *pinII* genes are sufficient.
   - States that the event contains an unusually long insert compared with other transformation events and although the sequence around the truncated *pinII* gene was assessed for open reading frames there is no comment at the 5' end of the construct, or the segments before the non-functional bar gene. States it is highly unlikely that there is any potential for expression from these small segments but an examination of sequence data would provide additional assurance.
   - Genetic stability of the backcrossed lines has been adequately demonstrated.
   - It would have strengthened the nutritional analysis to have also examined material from plants sprayed with glufosinate ammonium, as it is possible that this could occur in farming situations. However, they consider this would be unlikely to affect the outcome of the assessment.

4. **Food Technology Association of Victoria Inc (Australia)**
   - The Technical Sub Committee have reviewed the document and accepted the application without any further comment.

5. **National Council of Women of Australia**
   - States that ANZFA shows no inclination to revisit their guidelines and update them in light of new knowledge.
   - Has repeatedly pointed out to ANZFA that it has been recommended that antibiotic resistance genes be phased out, however ANZFA continues to approved genetic modifications that use them. The argument that the antibiotic is ubiquitous is not an acceptable argument when any increase in antibiotic use will add to the problem.

- In its first submission to this application, it supplied ANZFA with evidence of the need for more research on the toxicity of *Bacillus thuringiensis* in the soil. It is not happy with ANZFA's response to this issue, stating that safety cannot automatically be extrapolated from *Bt*'s use as a spray to its use when expressed as part of the growing plant, which eventually finds its way into the environment.
- Supports the call by the NZ Ministry of Health that ANZFA should address the biochemistry of the Bt protein, and why it is unlikely to cause any harmful effects when consumed by humans and other non-target organisms.
- Points out that its submissions to ANZFA are always accompanied by the appropriate references and most often with the referenced articles enclosed and it do not understand that with so many credible scientists publicly saying the science used in assessing safety is flawed or inadequate why ANZFA continues to ignore them.
- Despite the majority of submissions being opposed to the GM applications, ANZFA inevitably approves them and the Regulatory Impact Statements shows that it is usually for reasons of industry and trade. The fact that consumers do not want this technology in their foods is never taken into consideration.
- ANZFA complains that many submissions make comments addressing ethics, the environment or religious beliefs, which do not pertain to the ANZFA objectives. The Council is of the opinion that either the ANZFA objectives need to be changed to ensure ANZFA does not have a narrow view point or they should not address these applications until those issues have been dealt with through the OGTR first.
- In relation to labelling, ANZFA's assertion that 'the Ministerial Council made a conscious decision to apply the regulations to the final food, not the process" does not absolve either ANZFA or the government from the fact that they knew this was not what the public were promised or expected. The Council will continue to make their views known and lobby for changes, which reflect their needs in relation to labelling.
- Notes that DBT418 corn has been discontinued and that ANZFA is approving a line which is no longer planted on the off chance that it may currently be in high fructose corn being imported into Australia. States that manufacturers should be able to ascertain whether food from this line is entering Australia or not. If it is entering Australia, the Council objects to approving it simply to avoid looking for it upon entry or for purposes of trade facilitation. As the line is not being grown now, there is no valid reason why it should be approved in Australia or New Zealand.
- Council has previously voiced its opinion and provided references for its concern with the use of the cauliflower mosaic virus as a promoter.
- With regard to the specific studies submitted by Monsanto, the Council would prefer that these studies be peer reviewed and/or repeated rather than just be assessed as is currently the situation.
- The Council does not subscribe to the "substantial equivalence" theory and is concerned about statements made by ANZFA to the effect that the presence of transferred DNA in food products, in itself poses no health risk to consumers and that bacterial proteins produced by fermentation, rather than proteins purified from the transgenic plants, were used for acute toxicity tests in mice.
- States that that Regulatory Impact Statement is biased and that it is too broad and general and is not specific to the application, therefore many of the statements made are incorrect. To be valid, the RIS needs to be specific to the application. States that it is misleading to be non-specific when developing a RIS.
- As there is no valid reason why manufacturers need, or consumers want, this specific GM corn line, particularly in view of the fact that it is no longer planted, the Application should be rejected. Both non-GM and other GM high fructose corn syrup is obviously readily available.
- The submission contained three enclosures – a copy of the ANZFA Media Release of 13 February 2002, an article by Barry Commoner entitled ' Unravelling the DNA Myth. The Spurious Foundation of Genetic Engineering" published in Harper's Magazine in February 2002, and an ISIS Report from 12 November 2001 on *Bt*.

6. Bruce Smith (New Zealand)

- Opposes the release of these products onto the market for growing and consumption, particularly for human consumptions.
- Finds the products unacceptable for the following reasons:
  - the right to personal choice – the majority of consumers in NZ do not want any GM products in their dietary intake. It makes no difference if ANZFA tells them they are safe as consumers consistently choose not to want to each GM products at all.

- The GM labelling that was supposed to come into force in December 2001 does not even give the public the information to reject GM products for themselves in all cases, e.g. oils.
- food safety issues – ANZFA's statement that the food safety concerns raised in submissions have been addressed by the safety assessment is at best misleading, at worst a deliberate misrepresentation of the real situation for the purposes of trying to change or obviate actual negative public opinion. The safety assessment has not addressed food quality concerns that he would have raised had he known submissions were invited, including allergenicity, nutritional aspects and agricultural chemicals.
- environmental issues – considers it incumbent on ANZFA to reject processes and foods the production of which diminishes or increases the risk of diminishing, the capacity of the environment to sustain life, especially human life, in both the short and long term. Where the likelihood of such an outcomes is unclear, ANZFA should invoke the precautionary principle and at least not allow propagation or introduction of any such materials into the human food chain until better processes and understandings are developed. DBT418 corn appears to encourage an increasing use of agrichemicals and such practices have been shown to seriously damage the structure, hence long term fertility, of the soil. Even if these GM products were considered acceptable for consumption (which they are not), but not for planting in Australasia, ANZFA's decision should have full regard for possible effects to the global environment, and again they should be rejected.
- business ethics – it is unacceptable that profit and business interests should reign paramount in the important issues of food production in society. To truly represent the peoples of Australasia, ANZFA should at least be making an assessment of human and environmental outcomes in addition to profit margins.
- the role of ANZFA – although ANZFA was set up to act in the protection of and on behalf of the people of Australia and New Zealand, ANZFA clearly works with and on behalf of the food industry as well. In the case of the release of GM material into the human food chain, these two masters are generally in direct conflict. It is clear from the ANZFA standardised reports that ANZFA effectively regards the public as its servant, or as buffoons to be told what is good for them. Consequently this means that political forces and the food industry, including the transnational food giants and the mechanisms of GATT actually act as ANZFA's master. There is no way ANZFA can try to represent the best interests of the people under such a regime. ANZFA clearly supports the release of GM products into the market for human consumption and just as enthusiastically supports a labelling regime that does not require full labelling of products.

- The public deserves the right to make their own fully informed choices about what they eat. Part of this process is recognition that a large majority of the people do not want the introduction of GM products, especially into the human food chain, and that therefore such products should not be released.

7. Consumers' Association of South Australia Inc. (Australia)

- Supports the submission of the National Council of Women of Australia.

8. Katrina Upperton for GE FREE Northland (New Zealand)

- Is concerned to hear that DBT418 corn is suitable for human consumption.
- Equivalent groups to ANZFA in the UK (the Royal Society) and France (AFSSA) have recently spoken out for the need for more robust testing and trialling of new foodstuffs before they are openly available for consumption. Believes their approach to be one of responsible caution and should be followed in this country while the verdict is still out on the potential effects in the population from consuming foodstuffs that have been genetically modified.
- New Zealand's government has given the lead for caution and the early release of insufficiently trialled food on the domestic market ignores these concerns. Once released the effects of genetic pollution, possible harmful effects and health concerns cannot be rectified.
- Strongly request that this and other genetically modified foods are not passed for released.

9. Mrs I.P. Hancox (New Zealand)

- Is greatly concerned about the genetically modified corn, which is being pushed onto us whether we like it or not.
- Does not want the herbicide glufosinate ammonium used on any corn she eat and also wants any product containing the corn to be clearly marked.

- Has already had a very bad experience with an antibiotic and does not wish the food she eats to have the same effect.
- Notes that making genetically modified food may go to a step that is impossible to turn back and may have far reaching effects on the health of New Zealand's people.

10. Australian Food and Grocery Council (Australia)

- Supports approval of the application.
- As ANZFA has concluded that the introduced genes in DBT418 corn are not considered to produce any additional public health and safety risks and that food from DBT418 corn is as safe and wholesome as food from any other commercially available corn varieties the corn should be approved for use so that food manufacturers can make their own choice with regard to its use.
- Supports the application of the labelling requirements of Standard A18/1.5.2 to DBT418 corn and the products derived from it.

11. J. E. Brooks (New Zealand)

- Is opposed to the use of GM corn for human consumption in New Zealand.
- Notes that ANZFA believes the food to be safe but is not able to guarantee its safety.
- Will only consider the use of GM food when ANZFA can guarantee its safety.
- It is far to soon to say it is safe, it often takes years to see the results of some of these experiments on human health. In the meantime it should not be allowed into New Zealand.
- Asks that we wait and see some of the results from places like Canada for the next few years first.

12. David and Darcy Jones and Family (Australia)

- The safety of GM corn is a farce as to date there has been no independent safety testing.
- Recent German research has found DNA from GM corn in chicken tissues and in cow's milk.
- Japan has started substituting non-GM modified wheat in place of GM corn and soy products.
- Researchers have found that BT corn leaches long term powerful insecticides into the soil and pollen dust from Bt corn is known to kill monarch butterfly.
- Japan's third largest beer producer has stopped using GM corn and contracts for guaranteed non-GM products have been formulated.
- The US EPA has increased the refuge area for insects to as much as 40% of cropped area because of a real concern that the very insects for which Bt corn was developed have already shown their ability to become immune to the insecticides, bringing about the possibility of super bugs that will attack both GM and non GM crops with disastrous results.
- Asks where the documentation is on insect resistance management for DBT418 corn and what are the long-term management strategies and which government body will be controlling and monitoring current and future insect resistance management for any of the current GM food crops.
- Numerous food chains in the UK have removed GM foods from the brand named food and dietary products.
- The largest baby food manufacturer in the world has said no to GM corn and the list goes on.
- Wants to know where they can view or obtain the scientific information, field data, and test results along with the independent investigation of A380 that ANZFA used to conclude that these products are safe for human consumption and are in the best interests of the Australian and New Zealand consumer.

13. Alan Willoughby (New Zealand)

- Strenuously objects to the proposal to recommend the use of GM corn for human consumption. There appear to be few valid reasons for modification except for the convenience of the decreasing number of growers who spray food crops with poisons.
- His concerns include the following:
  - food sprayed with herbicides will contain residues which will be harmful to the health of some people. As a past sufferer of pesticide poisoning, some people are more susceptible to the effects of so called safe pesticides than others. Modifying crops to be resistant to herbicides simply increases the likelihood that people eating those crops will exhibit non-specific symptoms of pesticide poisoning, reducing their quality of life and ability to contribute to their communities and the nation while impoverishing them in their vain search for cause and remedies.

- The application of additional poisons to food crops has not been shown to increase yields. The percentage losses of crops to pests since the application of pesticides became routine has increased rather than showing the expected decrease. There is no evidence to indicate that there would be any difference in this case, the only winners being the chemical companies, which produce the pesticides.
- The growing of GM crops offers the danger of cross-pollination with non-GM crops, thereby reducing or eliminating the raw species, preventing any reversion to the original.

- Calls upon his representatives in government to strenuously fight the moves currently being made to introduce they genetically modified crops.

14. Western Australia Department of Health (Australia)

- The Western Australian Food Advisory Committee agrees that the examination of the nutritional evidence supplied by Monsanto does not point to any changes in the nutritional patter of GMO material versus control material.
- Suggests it would have been useful to have some anti-nutritional tests carried out on the corn, especially for the trypsin and chymotrypsin protein inhibitors. This is suggested in consideration of the potential for the gene transformation to result in increases in these anti-nutritional components. Monsanto states that because these compounds are low in corn there is no need to test them, but this argument is not consistent with rigorous scientific evaluation.

15. John Forrest (Australia)

- Requests that ANZFA reconsider its decision to remove products containing GM corn from the list of products required to display labelling identifying its source. The assertion that this product contains no DNA is irrelevant as other substances may be produced which have not been identified and whose effects are unknown.
- The contention that the food is harmless is a hypothesis not a fact, presenting it as a fact does no service to ANZFA.
- ANZFA needs to act in the interests of consumers not industry.

16. Carl D. Kneipp (Australia)

- Wishes ANZFA to advise how he as a food consumer is being protected from potential side effects from the GM industrial production process, considering we have chosen not to enforce the disclosure of GM food to the consumer.
- If ANZFA have failed to identify how GM food could affect the society and the consumer, then ANZFA is not producing responsible public policy.
- Would prefer not to consume GM food and by ANZFA's lack of specifying that GM foods must be declared have reduced that choice for him.
- Demands his right to make the choice as to the source of food he consumes by making that decision when purchasing food for consumption, or else to hold ANZFA responsible for improperly protecting the consumers of this food from reasonable mis-consumption.
- By backing the pro-GM lobby, ANZFA is simply collapsing in the face of a strong and profitable pro-GM lobbying to preserve the mass food producers' market share and profitability and limit natural food producers' and natural food consumers' rights.
- Suggests ANZFA is shirking its responsibilities as a public regulatory as ANZFA does not know the absolute and long term effects of GM food.

17. Jon van Hoffen (Australia)

- Strongly objects to the fact that GM corn is considered to be safe for human consumption and to the fact that it will be released to be grown in Australia on a wide scale.
- Considers GM corn as a threat to the practices of organic farming because pollen from GM corn can travel extensively and that is very hard to prevent gene drift from Gm corn to non-GM corn.
- Realises its impossible to prove a new product is safe but in his view it is extremely premature to conclude that all this new technology is safe, especially seeing that most of the research into the safety of the newly created proteins in our food has been conducted by the beneficiaries of the new technologies.
- Unless there is clear scientific proof from a completely independent source that the risks are virtually non-existent, the products shouldn't be allowed in the environment. Independent research is lacking at this stage.

- Allowing the Bt gene to be incorporated in the crops might cause widespread resistance to the Bts currently being used in organic agriculture and this would rob the organic industry of a valuable tool in the fight against some insects.
- Urges ANZFA to reconsider the application and not approve the release of GM corn.

18. Dougal Crockett (New Zealand)

- Expresses deep concern about the granting of consent to farm DBT418 corn. Has concerns about what benefit they will bring to consumers and the nation as a whole.
- The only benefit he can see is that they can withstand continual exposure to the company's own herbicide.
- Is concerned that the herbicide will get into the human food chain directly through the corn itself.
- His real concern is that such continued usage of herbicides will have long term destructive effects upon the soil and local waterways.
- Knowing that countries such as the UK have supermarkets that are committed to being 100% GE free, with aims of being 100% organic by 2008, he wonders what possible benefit the introduction of such GE crops can have to New Zealand. Thinks that we would be better placed investing our farming future in organics, as this is what our export markets are demanding.
- The GE industry appears to be trying to introduce its products by stealth and he does not trust them to have the public's interest at heart.
- Hopes that ANZFA does not see fit to be bullied into accepting these crops and considers the benefit for us all in declining further GE crop licences in Australia and New Zealand.

19. J. Reeve (Australia)

- Is against any form of genetically engineered food.
- Is disturbed that modified foods have already been introduced into the open market.
- Objects most to the following:
  - lack of detailed information on packaging, which is absolutely essential to give the public the choice to eat GM foods or not;
  - the problem arising from large companies suing farmers of non-GM crops because of cross contamination;
  - the monopoly arising with large companies on the distribution of seeds.

20. Mark Neilson (Australia)

- Wants to express his deep regret and anger that ANZFA persists in forcing GM foods on Australians without requiring they be clearly labelled.
- Notes that Monsanto is being sued for their actions in the US over the contamination of a waterway in Anniston.
- Asks what effects the higher residual quantities of pesticides/herbicides have on humans.
- Wants to lodge an official objection to the approval of any GM foods until the following have been done:
  - full independent testing over a minimum of 5 years to allow any short term negative effects to be observed;
  - full independent study on the possible long term effects of cross-pollination;
  - full investigation of all unauthorised and accidental GM seed releases, with the guilty releasers receiving life bans on their product in Australia/New Zealand.
  - Knows that the makers of GM seeds will put the sale of their product before the well being of the public.
- States ANZFA must put the people of Australia and New Zealand first and must not be influenced by government or corporations in doing its job.

21. Groundswell Canterbury (Spirit of Living Trust) (New Zealand)

- Is against the application by Monsanto.
- States that genetic engineering is based on a paradigm that is fundamentally unsound which leads to GMOs being genetically unstable and dangerous to human health.
- The danger is compounded by the lack of basic human health research carried out on this GE corn on the wishful thinking of substantial equivalence.
- All of this leads Groundswell to conclude that this GE corn should not be made available for human consumption and that ANZFA should reject Monsanto's application.

22. GE Free New Zealand In Food and Environment (RAGE) Inc (New Zealand)

- Wish to state its opposition to the proposed amendment giving approval to these crops to be used for human food.
- In light of recent reports around the world on the need for more adequate regimes to determine nutritional differences, allergen and toxin identification and post sales monitoring, it is felt that no more GE foods should be approved particularly while they are still unlabelled as a result of more tardiness by manufacturers and the willingness of ANZFA to support this view.
- The foods should not be passed for the followed reasons:
  - pesticide residues – the additional burden on health from increased herbicide residues consistent with the use of pesticide resistant crops is one that most people do not want to be exposed to.
  - nature of the genetic modification – it is noted that in DBT418 corn that the *bla* gene confers resistance to a number of antibiotics including ampicillin. This is unacceptable. The potential to affect humans causing resistance is still recognised by scientists as a major problem of the use of GE foods. Any foods that potentially remove safe and effective antibiotics from use should not be approved. The use of promoter genes has not been referred to, cauliflower mosaic virus has long been used in the production of GE crops and the danger of cauliflower mosaic virus has long been pointed out. The proteins produced as a result of gene constructs are proteins which the human body has never before encountered which have not been tested to investigate toxic or allergenic reactions.
  - toxicology issues – insufficient testing has been done to prove beyond doubt that this GE food is safe.
  - nutritional issues – there was insufficient evidence to prove that there are no problems with nutritional issues.
  - conclusions of the safety assessment – safety assessments are merely an assessment, they are subjective and involve value judgements being made by the assessor. It is not sufficient to cynically point out that microbial food sources have been a component of the human diet over several thousand years and this statement does not satisfactorily take the place of real evidence to prove the safety of GE foods. No evidence has been given that has been peer reviewed of any stringent and rigorous testing having taken place and this has been so with all previous approvals prior to this.
  - labelling – a sham of a labelling system has been implemented that will undoubtedly allow much of these products in the form of corn syrup and oil to remain unlabelled and unseen.
  - public submissions – the blanket statement that describes how opposition to the foods was from those who perceived GM foods to be unsafe is a very dismissive way of treating justified concerns by members of the public. This statement demonstrates the condescending attitude held by ANZFA with regard to public submissions.
- It appears that despite the use of a science in its infancy ANZFA appears to disregard the fact that continuing evidence demonstrates that there are problems with GE foods and that consumers do not wish to eat them. Unbiased external panels may well be able to identify issues that ANZFA representatives are unable to see due to their somewhat blinkered approach to safety assessment.
- They would not have expected any other conclusion from ANZFA, who have proved them to be at the behest of corporate and government interests.

ATTACHMENT 4

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received 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. ANZFA's processes

ANZFA'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 ANZFA 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 ANZFA 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 ANZFA safety assessment process, stating that it considered it unlikely that food that has satisfied the food standard will have harmful effects. The Commission also considered that ANZFA 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 ANZFA's processes and the detailed rebuttal of those criticisms supplied to the Commission by ANZFA, 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 ANZFA assessment process that raw data from experiments supporting the safety of a GM food are submitted to ANZFA for assessment. These data are assessed in detail by ANZFA scientists and then the assessment report undergoes a robust process of internal review by ANZFA's own scientific experts and external review by ANZFA'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 ANZFA in support of applications for approval of GM foods was the subject of particularly intense scrutiny during ANZFA's evidence at the New Zealand Royal Commission on Genetic Modification. ANZFA 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 ANZFA 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 ANZFA 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. ANZFA's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard A18 of the *Food Standards Code* (Standard 1.5.2 in Volume 2) 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 ANZFA in terms of their relevance in a biological system. In order to determine if any differences have biological significance, ANZFA 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 judgment 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 would 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 ANZFA 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 ANZFA'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. This was also the conclusion of the UK Royal Society, which recently considered this issue[3]. Plant viruses are also biologically incapable of naturally infecting human or animal cells.

[3] The Royal Society (2002). Genetically modified plants for food use and human health – an update.

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 (Volume 1) / 1.5.2 (Volume 2) in 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 is present in the final food and where the food has altered characteristics.

Exempt from these requirements are:

- highly refined food, 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 prepared at point of sale (e.g. restaurants, takeaway food outlets).

In addition, the revised Standard allows for a maximum of 1 per cent of unintended presence of genetically modified product, as ascertained by laboratory testing, 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 ANZFA website (www.anzfa.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 ANZFA 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 ANZFA 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. ANZFA'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 ANZFA 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, ANZFA has prepared a public discussion paper on the safety assessment process for GM foods[4], 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 1996* requires that these matters be considered by ERMA.

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

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).

The Australia New Zealand Food Authority (ANZFA) does not have the mandate to assess matters relating to environmental risks resulting from the release of foods produced using gene technology into the environment. However, links exist between ANZFA and these other regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs.

In Australia, the current regulatory system includes a number of other agencies with a legal remit to cover some aspects of GM products (such as imports, food, agricultural and veterinary chemicals):

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

All GM foods continue to be assessed and regulated by ANZFA under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as the Australia New Zealand Food Standards Council (ANZFSC). However, an interface between ANZFA and OGTR has been established through amendments to the ANZFA Act arising from the Gene Technology Bill 2000. These amendments to the ANZFA Act require the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (Standard A18/1.5.2).

Similarly, in New Zealand various other government departments and agencies play their role in the regulatory process:

- the Ministry of Agriculture and 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 ANZFA to amend the MRLs in the Food Standards Code and the application is considered by ANZFA 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).