07/01
4 October 2000

DRAFT RISK ANALYSIS REPORT

APPLICATION A385

Food derived from insect-protected Bt-176 corn

Note:

This report is the "Full Assessment" as referred to in Section 15 of the *Australia New Zealand Food Authority Act (1991)*.

Public comments are now sought before completion of a Final Risk Analysis Report (referred to as the "Inquiry" in Section 16 of the Act). See under 'Invitation for Public Submissions' for details.

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EXECUTIVE SUMMARY

Background

ANZFA received an application from Novartis Seeds Pty. Ltd. Australia on 30 April 1999 for the assessment and approval of food derived from insect-protected Bt-176 corn. This corn has been genetically modified to confer protection against lepidopteran pests, especially the European corn borer. This report describes the risk analysis including the scientific assessment of the application.

Issues addressed during assessment

(i) Safety Evaluation

Bt-176 corn has been evaluated according to ANZFA's safety assessment guidelines. The process involves an extensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the new GM food. This approach is used to establish whether or not foods produced from GM corn and its progeny are as safe and nutritious as their conventional non-GM equivalent.

The detailed information available on the genetic modification used to produce Bt-176 corn indicates that no unintentional changes have taken place at the molecular level and that the novel genetic material (the *cry*1A(b), *bar* and *bla* genes) is stably inserted and maintained over several generations.

Data on the potential toxicity and allergenicity of the proteins encoded by the transferred genes as well as potential changes to naturally occurring toxins or anti-nutrients have been reviewed. Corn has no endogenous toxins or anti-nutrients. The studies, including acute oral toxicity tests and model digestion system studies, indicate that the new proteins expressed in Bt-176 corn are non-toxic and unlikely to have allergenic effects. No protein product from the antibiotic resistance *bla* gene is expected in the genetically modified corn, as the gene has bacterial-specific regulatory elements. From these data, it can be concluded that the food products derived from insect-protected Bt-176 corn should pose no greater threat as a toxin or source of allergic reaction than food products from conventional corn.

Compositional analyses demonstrate no significant differences between Bt-176 corn kernels and its conventional counterparts supporting that Bt-176 corn kernels are compositionally and nutritionally equivalent to conventionally produced corn. This constitutes further evidence that no unintentional effects have occurred as a result of the genetic modification.

In assessing all of the above data, ANZFA has concluded that food derived from insect-protected Bt-176 corn does not raise any public health and safety concerns.

(ii) Labelling

On the basis of the data considered in the safety evaluation, food derived from insect-protected Bt-176 corn was found to be substantially equivalent to food derived from non-GM corn therefore no mandatory labelling is required.

It should be noted that on 28 July 2000 the Australia New Zealand Food Standards 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. This requirement will come into effect 12 months after the date of gazettal and may result in changes to the way in which GM foods, including those derived from insect-protected Bt-176 corn, are labelled.

(iii) Public Submissions

Forty-five public submissions were received in relation to this application, of which only four were supportive. Those opposing the application did so primarily on the basis that they perceive GM food to be unsafe. The food safety concerns raised in submissions have been addressed by the safety assessment carried out by ANZFA, the details of which can be seen in Attachment 2.

Conclusion

ANZFA considers that food derived from insect-protected, herbicide-tolerant corn line Bt-176 is as safe for human consumption as food from other commercial corn varieties, and is therefore proposing an amendment to the Australian *Food Standards Code* to give approval to such food. Based on the data submitted in the present application, food derived from Bt-176 corn can be regarded as substantially equivalent to non-GM corn, therefore no mandatory labelling is required, although this may change when the proposed changes to the labelling provisions of Standard A18 have been finalised.

ANZFA now seeks public comment on the proposed amendment to Standard A18 of the *Food Standards Code,* in accordance with the procedures described in Section 17 of the *Australia New Zealand Food Authority Act 1991*.

INVITATION FOR PUBLIC SUBMISSIONS

The Authority has completed a Draft Risk Analysis Report on this application, (referred to as the 'Full Assessment' in section 15 of the Act), which includes a draft Safety Assessment report and a draft variation to the Australian *Food Standards Code.* The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the *Food Standard Code,* and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' in section 16 of the Act).

Written submissions containing technical or other relevant information, which will assist the Authority in preparing the Final Risk Analysis Report for this application, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. The Authority's policy on the management of submissions is available from the Standards Liaison Officer upon request.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. The *Australia New Zealand Food Authority Act 1991* requires the Authority to treat in confidence trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A385** at one of the following addresses:

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Submissions should be received by the Authority by **15 November 2000.**

General queries on this matter and other Authority business can be directed to the Standards Liaison Officer at the above address or by Email on slo@anzfa.gov.au. Submissions should not be sent by Email as the Authority cannot guarantee receipt. Requests for more general information on the Authority can be directed to the Information Officer at the above addresses.

INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a bi-national statutory body responsible for making recommendations on food standards which, when approved by the Australia New Zealand Food Standards Council (ANZFSC), are adopted by reference and without amendment into food law. ANZFA is currently working to establish a joint Australia New Zealand Food Standards Code that will apply in both countries. In the interim, a system of dual standards operates for the majority of the food standards. Standard A18 has been accepted by New Zealand, and currently applies in both countries.

Standard A18 was adopted by ANZFSC as a joint Australia/New Zealand standard in July 1998 and came into force on 13 May 1999. Under this standard, the sale of food produced using gene technology is prohibited unless the food is included in the Table to clause 2 of the standard. The standard requires that a pre-market safety assessment be conducted on all foods produced using gene technology. However, the standard provides interim arrangements for those foods currently on the market 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 ANZFSC has not become aware of evidence that the food poses a significant risk to public health and safety.

BACKGROUND TO THE APPLICATION

ANZFA received an application from Novartis Seeds Pty. Limited on 30 April 1999 to amend the Australian *Food Standards Code* to include food produced from insect-protected corn lines containing the Bt-176 transformation event in the Table to clause 2 of Standard A18 – Food Produced using Gene Technology.

The modified corn under consideration is known commercially as Bt-176 corn and is protected from attack by lepidopteran pests, particularly the European corn borer. The corn was developed for cultivation in the United States.

The modification involves the incorporation of two novel genes. The first is a truncated and modified version of *cry*1A(b), from the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki.* This gene codes for a protein that is toxic to Lepidopteran insects, and the modified corn is thus protected from attack by this type of insect pest. The second is the *bar* gene. This gene was used as a selection marker and encodes the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies the broad-spectrum herbicide phosphinothricin (also known as glufosinate ammonium). The level of expression of PAT was sufficient to enable phosphinothricin-tolerance to be used in the selection of transformed plants, however the level of PAT expression in Bt-176 corn is insufficient to confer tolerance to commercial applications of the herbicide. Bt-176 corn is not intended to be marketed as a herbicide-resistant plant.

Bt-176 corn is not currently grown in either New Zealand or Australia. However, domestic production of corn in both countries is supplemented by a small amount of imported corn-based products. The major imported corn commodity 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.

According to the applicant, the novel genes present in Bt-176 have only been bred into field corn varieties and there is no intention to breed them into sweet corn varieties. Therefore, grain harvested from Bt-176 corn will enter the food chain only after processing.

The main benefits of Bt-176 corn are agronomic in nature, and are therefore likely to accrue mainly to the primary producer. It is envisaged that target pests, in particular the European corn borer, should be easier to control, with lower expenditure on labour and pesticides and higher overall crop yields. More general benefits may flow to the community as a result of reduced primary production costs.

PUBLIC CONSULTATION

ANZFA completed a Notice of Application (formally referred to as the Preliminary Assessment Report) upon receipt of the application and called for public comment on 3 November 1999. A total of 45 submissions were subsequently received. Attachment 5 contains a summary of the submissions.

NOTIFICATION OF 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 Technological Barriers to Trade (TBT Agreements) (for further details on WTO, see Attachment 4). 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 these foods, the proposed changes to Standard A18 are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters and will therefore be notified to the WTO.

ISSUES ADDRESSED DURING ASSESSMENT

1. Safety assessment

The safety assessment was performed according to the safety assessment guidelines prepared by ANZFA[1] and 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.

Nature of the genetic modification

Bt-176 corn was generated by the transfer of three new genes, a truncated *cry*1A(b) gene, the *bar* gene and the *bla* gene. All three genes were derived from bacteria. The *cry*1A(b)gene was modified at the DNA sequence level to increase its level of expression in the plant. The modification to the DNA sequence of each gene did not result in any changes to the amino

[1] ANZFA (1999) Guidelines for the safety assessment of foods to be included in Standard A18 – Food Produced Using Gene Technology.

acid sequence of the protein. The corn transformation was carried out using microprojectile bombardment of immature embryos.

The *cry*1(A)b gene is one of several isolated from the bacterium *B. thuringiensis*, which encode a group of toxins known as the *Bt* toxins. These toxins are selectively active against several groups of insects such as moths and butterflies, beetles, and flies and mosquitos. The *Bt* toxin produced by the *cry*1(A)b gene is known as Cry1(A)b and is selectively active against lepidopteran insects. The protein becomes active against the target insect upon ingestion. The protein binds to specific receptors on the insect midgut, inserts into the cell membrane and ultimately disrupts the digestive processes resulting in the death of the insect.

The *bar* gene is derived from the bacterium *Streptomyces hygroscopicus*, and codes for the enzyme phosphinothricin acetyl transferase (PAT). PAT inactivates the herbicide phosphinothricin (glufosinate ammonium), and its presence thus confers tolerance to the plant. The *bar* gene was used only as a selectable marker to distinguish genetically modified plant cells from unmodified cells. The level of PAT expression in Bt-176 corn is insufficient to confer tolerance to commercial applications of glufosinate ammonium.

The *bla* gene was derived from the bacterium *Escherichia coli* and encodes β-lactamase, which confers resistance to the antibiotic, ampicillin. It was used as a marker to allow for selection of bacteria containing the plasmids carrying the *cry1A(b)*and *bar* genes prior to transformation of the plant cells. No protein product from the antibiotic resistance *bla* gene is expected in the genetically modified corn, as the gene has bacterial-specific regulatory elements.

The *cry*1(A)b, *pat* and *bla* genes were found to be stably integrated at a single chromosomal location and were maintained in corn plants over multiple generations. They were also found to be inherited in a Mendelian manner, and always segregated together.

General safety issues

Corn represents a staple food for a significant proportion of the world's population. Corn-based products are routinely used in a wide range of foods, and have a long history of safe use. Sweet corn varieties are grown largely for human consumption, although corn grain is also widely used as an animal feedstuff.

The Bt-toxin expressed in the modified corn, though in truncated form, was found to be equivalent to that occurring naturally, and equivalent to that produced for use as the biopesticide that is widely used by the organic food industry. The Cry1A(b) protein was targeted to pollen and green tissues and thus the level of protein present in the kernel was, as expected, detectable but below the limit of quantification (>5 ng/g fresh weight).

Phosphinothricin acetyl transferase (PAT) is specific for the herbicide phosphinothricin (as well as the natural substrate bialaphos produced by *S. hygroscopicus*), neither of which are found in the human body. The PAT protein was not detectable in kernels or pollen and was detected in, but below the limit of quantification in leaves, roots, pith and whole plants. Although the level of expression of the enzyme in Bt-176 corn is sufficient to allow selection of modified plant cells, it is not sufficient to confer tolerance to field applications of the herbicide and is not regarded as herbicide tolerant.

The impact on human health from potential transfer of novel genetic material from Bt-176 to cells in the human digestive tract was evaluated. It was concluded that transfer was extremely unlikely to occur, and unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods. In the case of the *bla* antibiotic resistance gene, it was concluded that even should transfer occur, the health impacts would be negligible because this antibiotic resistance gene is already commonly carried by bacteria found in the environment as well as inhabiting the human digestive tract.

Toxicological issues

The presence of naturally-occurring toxins and allergens in Bt-176 corn was investigated, as well as the potential toxicity and allergenicity of the Cry1(A)b and PAT proteins.

Corn contains no naturally-occurring toxins or allergens and has a long history of safe use.

Biochemical studies confirmed the equivalence of the truncated Bt-toxin to that produced naturally. The novel protein, which is equivalent to that present in *B. thuringiensis* formulations, has been used commercially for many years to control insect pests. These formulations have been used extensively with no evidence of toxicity to humans, or to non-target species of insects, birds, fish or mammals. The potential acute oral toxicity of Cry1(A)b was assessed in mice, using protein extracts from Bt-176 corn. No adverse findings were seen in the animal studies. On the basis of this evidence, it can be concluded that Cry1(A)b, as expressed in insect-protected Bt-176 corn, is non-toxic to humans. The toxicity of PAT protein was assessed using similar studies. Results from acute oral toxicity testing in mice did not indicate any toxic effects. In addition, the substrate for the enzyme is not found in humans and PAT shows no amino acid similarity to known toxins.

The potential for the novel proteins to be allergenic was investigated using a number of criteria, including amino acid sequence similarity to known allergens, history of use and common physicochemical properties of allergens, including the sensitivity to digestion by digestive enzymes. The Cry1(A)b has a long history of safe use, and shares no characteristics or amino acid similarity with known allergens. In laboratory tests it was found to be rapidly digested in conditions that mimic human digestion, and was found to be identical to the microbially-produced protein in terms of immunoreactivity, molecular weight, trypsin resistance, glycosylation and bioactivity. The PAT protein was also found to be rapidly digested in conditions that mimic human digestion.

In addition, in the kernel, which is the only part used for human consumption, the Cry1A(b) protein is detectable but only at levels which are below the limit of quantification (>5 ng/g fresh tissue). The PAT protein was not detected in kernels.

Nutritional issues

Detailed compositional analyses were carried out to establish the nutritional adequacy of Bt-176 corn, and to look for any unintended effects by comparing it to non-modified control lines. Samples were taken from trials in both Europe and the USA. Composition in terms of key chemical components, including fatty acids, amino acids and carotenoids were investigated.

There were no significant compositional differences between Bt-176 corn kernels and control samples, confirming that insect-protected Bt-176 corn kernels are compositionally equivalent to kernels from other commercial corn lines. Similarly, there were no significant differences in composition in kernels from glufosinate ammonium treated samples.

Animal feeding studies were not considered essential because sufficient information had been provided about the genetic modification and the composition of the food. However, data on feeding studies conducted on chickens was submitted by the applicant and was reviewed in this assessment as additional supporting data. The nutritional adequacy of Bt-176 corn in chickens was found to be equivalent to that of conventional corn.

Conclusion

On the basis of the data submitted in the present application, insect-protected, herbicide-tolerant corn line Bt-176 is equivalent to other commercially available corn in terms of its safety and nutritional adequacy.

2. Labelling of food produced from insect-protected corn

Clause 3 of Standard A18 prescribes mandatory labelling of a food produced using gene technology when it contains new or altered genetic material and where it is not substantially equivalent in any characteristic or property of the food. As Bt-176 corn has been found, on the basis of data submitted with the present application, to be equivalent to other commercial varieties of corn there is no requirement for mandatory labelling under the current standard.

It should be noted that on 28 July 2000 the Australia New Zealand Food Standards 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. This requirement will come into effect 12 months after the date of gazettal and may result in changes to the way in which GM foods, including those derived from Bt-176 corn, are labelled.

3. Issues arising from public submissions

3.1 General issues

Of the 45 submissions received, 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 had already been addressed in the safety assessment report (see Attachment 2). A discussion of some of the general issues in relation to gene technology that were raised in public submissions can be found in Attachment 6.

3.2 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) Use of Bt toxins – toxicity and allergenicity concerns

Mr Arnold Ward, the National Council of Women of Australia and the Health Department of Western Australia raised 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 (NZMH) 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. NZMH also suggested that the dietary intake of Bt-toxin should be calculated.

Response

The toxicity and allergenicity of the *Bt* toxin are reviewed in the draft 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 Cry1A(b)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 it can therefore be inferred that the *Bt* toxins are highly unlikely to exert any toxic effects in mammals, including humans. The Cry1A(b) protein does not share the biochemical properties common to known allergens.

The applicant provided direct experimental evidence of the absence of acute toxicity in mice and birds, with doses of up to 5050 mg protein/kg, far higher than those estimated to be ingested through normal dietary intake. No adverse effects were observed in six week feeding study in chickens, in which Bt-176 corn formed the major portion (greater than 60%) of the diet. The level of the Cry1A(b) protein in corn kernels, the only part of the plant used for human food, is very low – less than 5 ng/g fresh weight or 5 parts per billion, which is at the limit of quantification. The dietary exposure will be lower than that experienced through eating products sprayed with *Bt*-based insecticides. The processing steps for corn would be expected to remove and/or destroy the Cry1A(b) protein. Thus the level of Cry1A(b) protein present in processed products derived from Bt-176 corn would be extremely low.

It is therefore concluded that consuming food products derived from corn containing these proteins is extremely unlikely to result in adverse effects in humans.

4. Risk management

Under Standard A18, a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines. The requirement for the food to be labelled must also be assessed in accordance with the labelling criteria specified in clause 3 of the standard.

On the basis of the conclusions from the safety assessment report, together with a consideration of the public submissions, it is proposed that Table 1 to clause 2 of Standard A18 be amended to include food from insect-protected Bt-176 corn. The proposed amendment is provided in Attachment 1.

In relation to labelling of the food, the safety assessment report found, on the basis of the data provided in the application, that food from insect-protected Bt-176 corn is substantially equivalent to that from other commercially available corn in terms of its safety and nutritional adequacy. Therefore, under the current standard, no mandatory labelling is required, although this may change when the proposed changes to the labelling provisions of Standard A18 have been finalised.

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. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

REGULATORY IMPACT ASSESSMENT

The benefits and costs associated with the proposed amendment to Standard A18 have been analysed in a draft Regulatory Impact Statement (Attachment 3). The benefits of the proposed Standard A18 amendment to approve food from insect-protected Bt-176 corn primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

CONCLUSIONS

It is concluded that:

- the introduced genes in food derived from insect-protected Bt-176 corn are not considered to produce any increased public health and safety risk;
- on the basis of the data provided in the application, food derived from insect-protected Bt-176 corn is equivalent to food derived from other commercial varieties of corn in terms of its safety and nutritional adequacy;
- food derived from insect-protected Bt-176 corn does not require labelling under the current provisions of Standard A18 as it is substantially equivalent to food derived from non-GM corn. Recently agreed amendments to the labelling

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

provision of Standard A18 may result in some Bt-176 corn food products being labelled in the future; and

- the benefits to government, consumers and industry associated with the proposed amendment outweigh the costs.

ATTACHMENTS

1. Draft variation to the Australian *Food Standards Code*
2. Draft safety assessment report
3. Draft regulatory impact assessment
4. World Trade Organization Agreements
5. Summary of public comments
6. General issues raised in public comments

ATTACHMENT 1

DRAFT VARIATION TO THE FOOD STANDARDS CODE

A385 - FOODS FROM Bt-176 CORN

Standard A18 *is varied by inserting into Column 1 of the Table to clause 2 -*

Food derived from insect-protected Bt-176 corn.

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT REPORT

A385 – FOOD PRODUCED FROM INSECT-PROTECTED Bt-176 CORN

SUMMARY AND CONCLUSIONS

Insect-protected Bt-176 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 unintended changes; and the potential of the newly expressed proteins to be allergenic or toxic.

Nature of the genetic modification

Insect-protected Bt-176 corn was generated by the transfer of the *cry1A(b)* gene derived from *Bacillus thuringiensis* subsp *kurstaki* which confers protection against attack by insects. The Cry1A(b) protein is an insecticidal crystal protein, whose toxic effect is specific to Lepidopteran insects, including the European Corn Borer. The introduced gene for *cry1A(b)* was found to be stably integrated into the corn plant genome and is phenotypically and genetically stable over multiple generations.

Other genes transferred with the *cry1A(b)* gene were the *bar* gene and the *bla* gene. The *bar* gene was used as a marker to select transformed plant cells during the corn transformation procedure. It codes for the enzyme phosphinothricin acetyltransferase (PAT) and is derived from the bacterium *Streptomyces hygroscopicus*. It confers resistance to the herbicide phosphinothricin (glufosinate ammonium) and was used as a selectable marker for transformed plants. The level of PAT expressed in Bt-176 corn was at least thirty times less than in phosphinothricin-tolerant corn lines developed by Novartis for agronomic use, and Bt-176 corn is not intended to be marketed as a herbicide-resistant plant. The *bla* gene was used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken prior to transformation of the plant cells. It codes for the enzyme □-lactamase and confers resistance to the antibiotic ampicillin.

The molecular and genetic analyses indicated that the introduced genes have been stably integrated into the genome of insect-protected Bt-176 corn and were stably inherited for multiple generations.

General safety issues

Corn represents a staple food for 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 Bt-176 corn hybrids may include highly processed corn products such as flour, breakfast cereals, high fructose corn syrup and other starch products as well as fresh sweet corn and associated products.

The transformed corn produces two new proteins: Cry1A(b) and phosphinothricin acetyltransferase (PAT). In kernels, the expression of Cry1A(b) was detected but was below the limit of quantification of 5 ng/g fresh weight and the PAT protein was not detected. The bla gene was not expressed in plants.

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 or bacteria in the human digestive tract. Much of the concern in this regard is with the presence of antibiotic

resistance genes in genetically modified foods. In the case of the insect-protected Bt-176 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 novel genetic material from the insect-protected Bt-176 corn to human cells via the digestive tract was also considered to be equally unlikely.

The level of DIMBOA (2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one), a naturally occurring plant defense compound, was unaltered in Bt-176 corn indicating that the genetic modification has not altered the levels of these compounds.

Toxicological issues

Corn does not have any naturally-occurring toxins or allergens and has a long history of safe use. Cry proteins from *B. thuringiensis* have a long history of safe use as insecticides.

In Bt-176 corn kernels, the Cry1A(b) protein is detectable but below the limit of quantification. The PAT protein is not detectable in Bt-176 kernels. The *bla* gene is not expressed in Bt-176 corn.

Data for the newly expressed Cry1A(b) and PAT proteins in Bt-176 corn have been evaluated for their potential toxicity to humans. Studies showed no signs of toxicity among mice following acute oral doses up to 3535 mg/kg for Cry1A(b) and 2575 mg/kg for PAT. No significant similarity to the amino acid sequences of known toxins was identified for either protein.

Neither of the expressed proteins exhibits characteristics of known allergens. Both proteins have been shown to be rapidly digested in simulated mammalian digestive systems. Amino acid sequence analyses did not reveal any similarities to known allergens.

Therefore, the evidence does not indicate that there is any potential for either protein to be toxic or allergenic to humans.

Nutritional issues

The compositional analyses were comprehensive and demonstrated that there are no substantial differences in the levels of major constituents or nutrients, between Bt-176 corn and conventional corn lines. The components measured were proximate (protein, fat, moisture, fibre, ash, carbohydrates and calories), fatty acids and, amino acids.

The nutritional adequacy of Bt-176 corn was found to be equivalent to that of conventional corn in a feeding study with chickens.

These analyses confirm that insect protected Bt-176 corn is nutritionally and compositionally comparable to other corn lines and that no health or safety risks are posed by consuming food derived from the genetically modified corn.

Conclusion

No potential public health and safety concerns have been identified in the assessment of insect-protected Bt-176 corn. On the basis of the data provided in the present application foods derived from Bt-176 corn can be regarded as equivalent to foods derived from conventional corn in terms of their safety and nutritional adequacy.

1 BACKGROUND

Novartis Seeds Pty Ltd have made an application to ANZFA to vary Standard A18 to include food derived from insect-protected corn in the Table to the standard.

The corn referred to as '*Bt-176* corn' has been modified to confer protection against insect attack by the production of an insecticidal protein representing the active portion of the Cry1A(b) protein that occurs naturally in *Bacillus thuringiensis* subsp. *kurstaki* strain HD1. The insect-protected corn plants are protected against attack from Lepidopteran insects, particularly by the European Corn Borer (ECB). The *bar* gene, which confers increased tolerance to the herbicide phosphinothricin, has also been introduced to Bt-176 corn, but it does not confer protection to commercial applications of the herbicide.

The corn lines containing the Bt-176 transformation event were developed by Novartis Pty Ltd for cultivation in the United States.

The Bt-176 corn described in this application includes insect-protected (dent) corn hybrids into which the Bt-176 transformation event has been introduced. According to the applicant, grain harvested from Bt-176 corn will enter the food chain only after processing. It should be noted that two other varieties of corn are cultivated: sweet corn and popping corn. Sweet corn is the variety grown for use as a fresh vegetable or in canned form. Popping corn is grown for use in the production of popcorn. While the current application refers to the insect-protection trait in dent corn hybrids the germplasm from the Bt-176 event may subsequently be introduced into commercial sweet and popping corn varieties.

Following assessment by the United States Department of Agriculture (USDA) in 1995 hybrids incorporating the Bt-176 event were commercialised in the USA (USDA 1995). The US Food and Drug Administration (US FDA) approved the use of Bt-176 corn in human food in 1995 (US FDA 1999). In 1997 the United States Environmental Protection Agency (US EPA) exempted phosphinothricin acetyl transferase (PAT, and the genetic material, ie the *bar* gene, necessary for its production in all plants) from "the requirement of a tolerance on all raw agricultural commodities" (US EPA 1997). Approvals for environmental release and use in human food and animal feed in Canada were given in 1995 and 1996 respectively (Canadian Food Inspection Agency 1995, 1996).

Corn harvested from these plants or processed products containing Bt-176 corn components may have been imported into Australia and New Zealand since 1995. The Genetic Manipulation Advisory Committee (GMAC) and Environmental Risk Management Authority (ERMA) in New Zealand have not received an application from Novartis Seeds for commercial release of Bt-176 corn for cultivation in Australia and New Zealand.

Domestic production of corn in both countries is supplemented by a small amount of imported corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Other products include maize starch which is used by the food industry for the manufacture of dessert mixes and canned foods and corn-based ingredients processed into breakfast cereals, baking products, extruded confectionary and corn chips.

The data regarding the generation and characterisation of Bt-176 corn and backcross hybrids have been published in peer-reviewed scientific literature (Koziel *et al* 1993, Fearing *et al* 1997, Brake and Vlachos 1998).

2 DESCRIPTION OF THE MODIFICATION

2.1 Methods used in the genetic modification

The *Bt-176* corn was produced by simultaneous introduction of plasmids pCIB3064 and pCIB4331 (Figures 1a & b) into immature embryos of proprietary inbred corn line CG00526 (*Zea mays* L.) via microprojectile bombardment.

pCIB3064 contains the *bar* gene for herbicide resistance and the *bla* gene for antibiotic resistance. Schematic maps of the two plasmids are shown in Figures 1a and 1b. pCIB4331 contains two copies of the *cry*1A(b) gene for insect resistance and a single copy of the *bla* gene for antibiotic resistance.

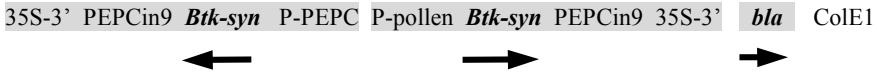
Transformed plants were selected on the basis of their ability to grow in the presence of phosphinothricin conferred by the transfer of the *bar* gene.

Until recently, transformation of cereals has been achieved by use of the particle bombardment technique rather than the *Agrobacterium*-mediated DNA transformation system (Komari *et al* 1998). Introduction of DNA into the plant is achieved by bombarding tissues with microscopic particles (commonly tungsten or gold) coated with the DNA of interest (Klein *et al* 1992).

Figure 1a Schematic diagram of pCIB3064[1]

[1] See Table 1 for an explanation of the abbreviations. Not to scale.

Figure 1b Schematic diagram of pCIB4431[1]



[1] See Table 2 for an explanation of the abbreviations. Not to scale.

2.2 Function and regulation of the introduced genes

A total of four genes were transferred to the corn line. Plasmid pCIB4431 contained two constructs of the *cry1A(b)* gene for expression in corn plants. Plasmid pCIB3064 contained one construct of the *bar* gene for expression in corn plants as well as the *lacZ* gene. The *bla* gene was present on both plasmids. The genetic elements in pCIB3064 and pCIB4431 are shown in Tables 1 and 2 respectively.

The cry1A(b) gene

The *cry1A(b)* gene used to generate Bt-176 corn was derived from the soil bacterium *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*) strain HD1 (Geiser *et al* 1986). The gene encodes a δ-

endotoxin that protects against certain species of Lepidopteran insects including the European corn borer (ECB, *Ostrinia nubilalis*). The *cry*1A(b) gene introduced into Bt-176 corn was truncated version of the native *cry*1A(b) gene equivalent to the coding region for the N-terminal 648 amino acids of the 1155 amino acid full length native Cry1A(b) protein. This 648 amino acid peptide includes the portion responsible for insecticidal activity, and is processed by proteases in the lepidopteran gut to yield an insecticidal protein of 564–578 amino acids.

Until recently δ-endotoxins present in *B. thuringiensis* subspecies were classified into four groups on the basis of their insecticidal range: CryI, Lepidoptera specific; CryII, Lepidoptera and Diptera specific; CryIII, Coleoptera specific; and CryIV, Diptera specific (Hofte and Whitely 1989). Since that time over 100 *cry* genes have been sequenced. Characterisation of many of these *cry* genes is inconsistent or anomalous with the original system and a revised nomenclature was recently proposed (Crickmore *et al* 1998). Under the new nomenclature *cry*1A(b) would be referred to as *cry*1Ab4. However, to retain consistency with the data provided by the applicant, the former nomenclature of *cry*1A(b) has been retained in this assessment.

The native *cry*1A(b) gene contains codons that are not frequently used in plant genes as well as A+T rich regions that could be potential polyadenylation sites thus impairing its expression in the plant. The *cry*1A(b) gene used to transform corn was modified to reflect plant codon usage to allow efficient expression in the corn plant (Perlak *et al* 1991, Koziel *et al* 1993). The synthetic *cry*1A(b) DNA sequence is 65% identical to the native gene, however the encoded amino acid sequence of the resultant Cry1A(b) protein is identical to that of the native toxin (Koziel *et al* 1993, Koziel *et al* 1996).

Plasmid pC1B4431 contains two copies of the synthetic *cry*1A(b) gene; one controlled by the promoter from the corn phosphoenolpyruvate carboxylase (PEPC) gene, specific for expression in the green tissue of the plant (Hudspeth and Grula 1989), the other controlled by a corn calcium-dependent protein kinase gene (P-pollen), resulting in pollen-specific expression (Estruch *et al* 1994). Transcription termination and polyadenylation of mRNA of both copies of *cry1A(b)* are controlled by the 3' untranslated 35S sequence from cauliflower mosaic virus (CaMV). Both *cry1A(b)* gene constructs also contain intron #9 from the corn PEPC gene, which stimulates expression of the gene encoding the truncated Cry1A(b) protein (Callis *et al* 1987).

The bar gene

The *bar* gene is derived from the soil microorganism *Streptomyces hygroscopicus* and confers resistance to the herbicide phosphinothricin.

The *bar* gene was used as a selectable marker to distinguish transformed (ie genetically modified) corn cells from unmodified cells. To ensure the expression of the *bar* gene in plant cells it was fused to the 35S promoter and 3' polyadenylation sequences from cauliflower mosaic virus (CaMV, Benfey and Chua 1990) to direct initiation and termination of transcription and polyadenylation of the mRNA transcript.
Transformed callus tissue was selected for on the basis of phosphinothricin tolerance. Putative transformants were further selected by amplification of transferred sequences using the polymerase chain reaction (PCR).

Table 1. Genetic Elements contained in pCIB3064

Genetic element	**Region**	**Name**	**Function**	**Source**
bla		*bla*	Ampicillin resistance in bacterial cells	*Eschericia coli*
lacZ		*lacZ*	partial coding sequence of lacZ *lacZ* encodes β-galactosidase	*Eschericia coli*
bar	Promoter *bar* 3' untranslated	P-35S *bar* Tr7	drives expression in plant cells Phosphinothricin acetyl transferase signals termination of transcription	Cauliflower Mosaic Virus *Streptomyces hygroscopicus* Cauliflower Mosaic Virus
ColE1		ColE1	origin of plasmid replication in *Eschericia coli*	*Eschericia coli*

Table 2. Genetic Elements contained in pCIB4431

Genetic element	**Region**	**Name**	**Function**	**Source**
Btk	Promoter Btk-syn Enhancer 3' untranslated	PEP-C *cry1A(b)* PEP-C Intron #9 35S 3'	drives expression in green plant cells Bt toxin enhances transcription signals stop point of transcription and initiation of polyadenylation	*Zea mays* *Bacillus thuringiensis* subsp *kurstaki* *Zea mays* Cauliflower Mosaic Virus
Btk	Promoter Btk-syn Enhancer 3' untranslated	P-Pollen *cry1A(b)* PEP-C Intron #9 35S 3'	drives expression in pollen Bt toxin enhances transcription signals stop point of transcription and initiation of polyadenylation	*Zea mays* *Bacillus thuringiensis* subsp *kurstaki* *Zea mays* Cauliflower Mosaic Virus
bla		*bla*	Ampicillin resistance in bacterial cells	*Eschericia coli*
lacZ		*lacZ*	partial coding sequence of *lacZ* *lacZ* encodes β-galactosidase	*Eschericia coli*
ColE1		ColE1	origin of plasmid replication in *Eschericia coli*	*Eschericia coli*

The *bla* gene

The *bla* gene is derived from *Eschericia coli* and encodes β-lactamase which confers resistance to 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 pCIB3064 and pCIB4431 prior to transformation of the plant cells. The *bla* gene has no plant regulatory sequences and is unlikely to be expressed in plant tissues.

2.3 Characterisation of the genes in the plant

Studies submitted by Novartis:

Privalle, L. 1994 Quantification of Cry1A(b) and PAT proteins in *Bt* corn (corn) tissues, whole plants and silage. Performing laboratory: Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No CAB-009-94

Molecular characterisation of the integrated DNA present in glufosinate ammonium-tolerant Bt-176 corn was performed using DNA from untransformed corn CG00526 and plasmids pCIB3064 and pCIB4431 as reference material.

Southern blotting experiments confirmed the presence in Bt-176 corn of the *cry1A(b)* (Koziel *et al* 1993), *bar* and *bla* genes. The data indicate that there may be as many as six copies of the *cry1A(b)* and *bla* genes present in Bt-176, and at least two of the *bar* gene (together with the 35S promoter) as determined by the number of hybridizing bands in DNA isolated from Bt-176 corn and digested with restriction enzymes which do not cut inside the gene sequence(s). A summary of the genes transferred to Bt-176 corn is shown in Table 3.

The presence of at least one functional copy of each of the *cry1A(b)* genes under the control of the leaf-specific corn PEPC promoter and the pollen-specific promoter respectively was confirmed from the expression of Cry1A(b) protein in these tissues (see Section 3.3 below).

Table 3. Genes transferred to Bt-176 Corn

Gene	Copy number	Function	Source
cry1A(b)	**≥5**	Gene encoding Cry1A(b) Bt toxin, insect resistance.	*Bacillus thuringiensis*
bar	**≥2**	Gene encoding PAT protein, phosphinothricin tolerance.	*Streptomyces hygroscopicus*
P-35S (*bar*)	**≥2**	Direct transcription of *bar* in plant cells.	Cauliflower mosaic virus
bla	**≥5**	Ampicillin resistance in bacteria.	*Eschericia coli*

2.4 Stability of the genetic changes

Studies submitted by Novartis:

Privalle, L. 1994 Genetic stability of the modified corn plants: segregation analyses and Southern blots. Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Appendix B to Study No CAB-009-94

The inheritance of the phosphinothricin-tolerant and ECB-protected phenotypes of Bt-176 corn was analysed by backcrossing experiments. In typical studies, 887 plants comprising 12 three-way cross populations, 1273 plants comprising 11 BC1 (backcross) populations and 699 plants comprising 7 F2 populations were evaluated for phosphinothricin tolerance. Similarly, 207 plants from 5 BC1 populations and 899 plants from 13 BC2 populations were evaluated for protection against ECB. The analysis showed a 1:1 phenotypic segregation ratio for phosphinothiricn tolerance and ECB tolerance. These data showed that phosphinothricin tolerance and insect protection co-segregate as tightly linked Mendelian traits, suggesting a single site of insertion of the transgenes with a few copies of each gene (Koziel *et al* 1993).

The low number of recombinants identified further suggested a tight linkage of the transgenic traits: of 3240 plants evaluated from 1993 field trials, only 5 (0.15%) were found to exhibit only one of the introduced traits.

Inbred progeny plants also exhibited the same banding pattern over four generations as the original Bt-176 as demonstrated in Southern blots probed with either the *cry1A(b)* or the *bar* sequences. Restriction Fragment Length Polymorphism (RFLP) analysis of phosphinothricin-tolerant corn plants indicated that the active *bar* gene acts as a single locus and maps between probes CG320 and CG378 (with known map locations) of Chromosome 1 of maize. Probing with the *cry1A(b)* gene indicated that it maps to the same locus as that determined for *bar*.

Levels of expression of the Cry1A(b) protein in leaves and pollen of anthesis stage plants, determined by enzyme-linked immunosorbent assay (ELISA), were stable over four successive backcross generations for two different *Bt* corn lines (original transformant CG00526-176 x CG00642, CG00526-176 x CG00554), with no indication of reduced expression. These data are shown in Table 4.

From these data it can be concluded that the introduced genes have been stably integrated into the corn genome and are stably inherited over four generations.

2.5 Conclusions regarding the genetic modification

The *cry1A(b)*, *bar* and *bla* genes were transferred to corn via a microprojectile bombardment transformation system resulting in the generation of the ECB-protected and phosphinothricin-tolerant Bt-176 corn. Segregation analyses indicate that the DNA was integrated into the genome of Bt-176 corn as a single and stable insert.

Table 4. Cry1A(b) and PAT expression over four backcross generations of hybrids of CG00526-176 (Bt-176)

Generation		Cry1A(b) µg/g dry wt		PAT µg/g dry wt
CG00526-176 x	n	Leaves	Pollen	Leaves
BC1				
CG00642	8	6.42 (4.36-9.24)	3.43 (2.90-4.01)	lod*
CG00554	2	5.13 (4.70-5.56)	3.82 (3.81-3.84)	lod*
BC2				
CG00642	3	10.24 (8.52-11.11)	4.25 (3.51-4.66)	lod*
CG00554	5	7.81 (4.5-9.1)	5.86 (4.38-7.02)	lod*
BC3				
CG00642	4	8.89 (7.25-12.18)	4.47 (3.27-6.49)	lod*
CG00554	2	6.58 (4.06-10.3)	6.68 (5.46-7.90)	lod*
BC4				
CG00642	3	7.77 (6.61-8.83)	4.45 (3.90-5.51)	lod*
CG00554	5	11.08 (7.0-15.76)	5.23 (4.74-5.63)	lod*

*lod: limit of detection = trace PAT activity was detectable, but below the limit of quantitation (0.75 µg/g dry wt)
range shown in parentheses.
CG00526-176 x CG00642 and CG00526-176 x CG00554

3 GENERAL SAFETY ISSUES

Bt-176 corn is grown in the USA for both domestic use and for export. Bt-176 corn was approved for environmental release and use in human food in the USA in 1995 (USDA 1995, US FDA 1999) in food in Canada in 1996 and 1995 (CFIA 1995, 1996). According to the applicant, grain harvested from Bt-176 corn will enter the food chain only after processing. Processed foods, including imported processed foods may contain genetically modified Bt-176 corn.

The Bt-176 corn has been assessed according to the safety assessment guidelines developed by ANZFA, relating to Group D foods, i.e. plants or animals that contain new or altered genetic material (ANZFA 1999a).

3.1 History of use of corn as food

Corn has been cultivated for centuries and is used as a basic food item by people throughout the world (Wright, 1987). The grain is widely used as a feedstuff, although a large part of corn production is also used for human food products, and a wide variety of food products are derived from corn kernels.

Two milling procedures are used for 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. Corn flakes are produced by a process that involves high temperatures and pressures, grits are prepared by boiling.

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). These treatments would be expected to degrade and remove proteins (May 1987). 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 (Rogers 1990).

According to the applicant, grain harvested from *Bt-176* dent corn varieties will enter the food chain only after processing. There is the potential that the novel traits could be bred into sweet corn varieties, in the future, and therefore could be consumed as fresh, frozen or canned corn.

3.2 Nature of novel proteins

Two new proteins are expressed in *Bt*-176 corn: a truncated form of the insecticidal protein Cry1A(b), and phosphinothricin acetyl transferase (PAT). No β-lactamase enzyme expression is expected in Bt-176 corn as the *bla* gene does not have any regulatory sequences that would be recognized in the plant background.

cry1A(b)

The insecticidal δ-endotoxins referred to as Cry proteins are produced by the aerobic, spore-forming soil bacterium *Bacillus thuringiensis* (Bt) (Schnepf *et al* 1998). There are a multitude of Cry proteins, with particular Cry proteins being toxic to only certain insects. During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 μm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxin–containing crystals. The protoxin is then activated by trypsin–like gut proteases which cleave off domains from the carboxy– and amino–termini leaving a protease–resistant core which is the active toxin. The active toxin binds to a highly specific glycoprotein receptor on the surface of midgut epithelial cells in the insect (Gill 1995, Rajamohan *et al* 1998). Aggregation of the core toxins 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 synthetic *cry1A(b)* gene encodes a Cry1A(b) protein of 648 amino acids with a predicted molecular weight of 65 kD. The predicted amino acid sequence includes the sequence equivalent to that of the natural Cry1A(b) tryptic fragment (607 amino acids, 60 kD).

PAT

S. hygroscopicus (and other *Streptomyces* spp.) produce the tripeptide antibiotic bialaphos (phosphinothricin alanyl alanine) which consists of phosphinothricin (glufosinate

ammonium), an analogue of L-glutamic acid, and two alanine residues. The *bar* (bialaphos antibiotic resistance) gene encodes the enzyme phosphinothricin acetyl transferase (PAT) which breaks down bialaphos thus giving *Streptomyces* protection from the toxicity of the antibiotic it produces (Thompson *et al* 1987, Kumada *et al* 1988).

Phosphinothricin is used as a broad spectrum herbicide and is a potent inhibitor of glutamine synthetase (GS), the key enzyme in ammonia metabolism in plants. Phosphinothricin application to plants results in a rapid (< 2 hours) increase in the level of free ammonium resulting in cell death (De Block *et al* 1987). The *bar* gene, derived from either *S. hygroscopicus* or *S. viridochromogenes*, has been transferred to a number of plant species other than corn, including tobacco (De Block *et al* 1987, Wohlleben *et a*l 1988), canola (Beriault *et al* 1999), sugar cane (Gallo-Meagher and Irvine 1996) and rice (Cao *et al* 1992) to confer tolerance to glufosinate ammonium.

3.3 Expression of novel protein in the plant

Studies submitted by Novartis:

Privalle, L. 1994 Characterisation of Cry1A(b) protein produced in *Bt* corn (corn) Event 176 and comparison with native Cry1A(b) protein produced by *Bacillus thuringiensis* subsp. *kurstaki* strain HD1-9. Performing laboratory: Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No CAB-006-94

Privalle L. 1994 Quantification of Cry1A(b) and PAT proteins in *Bt* corn (corn) tissues, whole plants and silage. Performing laboratory: Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No CAB-009-94

Ciba Seeds petition for the Determination of Nonregulated Status for Insect-Resistant Event 176 Corn. Submitted to the USDA/APHIS on November 15, 1994. Reference number 94-319-01. Chapter 6.

The expression of Cry1A(b) and PAT in corn plants derived from *Bt*- 176 corn was determined for various plant tissues and developmental stages in three corn lines from field tests carried out in the United States in 1993, and in selected tissues of mature greenhouse-grown inbred plants representing four additional genotypes and four backcross generations of two genotypes.

Cry1A(b)

The presence of Cry1A(b) protein in leaves and pollen of *Bt*-176 corn was demonstrated by Western blotting with an antibody specific to the native Cry1A(b) protein. There were two major immuno-reactive protein bands with apparent molecular weights of approximately 65 kD, as predicted from the *cry1A(b)* gene sequence (see Section 3.2 above). The bands correspond to the predicted sizes of the 648 amino acid truncated Cry1A(b) protein with and without the first 24-28 amino acids. Three other immuno-reactive bands of lower molecular weight were observed in Western blots of leaf extracts: 60kD; 40kD; and 36kD. The tryptic fragment of the Cry1A(b) protein has a molecular weight of 60kD. It was demonstrated that the presence of these bands of less than 65kD were not artifacts of the extraction procedure.

The 65kD and 36kD immunoreactive protein bands from leaf tissue were subjected to N-terminal amino acid sequencing. The N-terminus of the 65kD band corresponded with amino acid 25 of the predicted sequence and the sequence was identical to that expected over the 10 amino acids sequenced. The N-terminus of the 36kD protein corresponded with amino acid

31 of the predicted sequence and the sequence was identical to that expected over the 15 amino acids sequenced. This identified the 36kD protein as the N-terminal fragment that extends to amino acid 350-400 as encoded by the native and synthetic cry1A(b) genes. From these data the applicant reasoned that the N-terminal processing of the 65kD protein, and the bands of less than 65kD, resulted from the action of intrinsic corn proteases rather than the presence of truncated gene sequences.

Immuno-purified Cry1A(b) protein from Bt-176 corn was tested for post-translational modifications. No evidence was found of acetylation, glycosylation or phosphorylation.

The functionality of the Cry1A(b) protein expressed in Bt-176 corn and hybrids was demonstrated by resistance to attack by ECB. The Cry1A(b) immunoreactive proteins of less than 60kD are not expected to contribute to insecticidal activity as they are below the minimum size needed for bioactivity.

The Cry1A(b) protein was detected by ELISA in significant quantities in leaves and pollen, as expected given the tissue specificity of the promoters (Table 5a). Trace amounts of the Cry1A(b) protein were detected in kernels as well as in other plant tissues (roots and pith) but were below the limit of quantification. Whole plants selected at various stages throughout the growing season were assessed for their level of Cry1A(b) protein which was measured to be highest in plants (per gram dry weight) selected at seedling stage and decreased during the rest of the growing season (Table 5a).

When considered as a proportion of total plant protein, the highest mean level of Cry1A(b) protein was measured in whole plants - 14.4 μg/g total protein. This was observed in homozygous *Bt*-176 corn (inbred) plants taken at anthesis. This represents 0.00144% of the total protein. Trace levels of Cry1A(b) protein were detected in fresh kernels, however the levels were below the limit of quantification of 5 ng/g fresh weight kernels (5 ppb). Consistent results have been determined in several genetic backgrounds including hybrids 176 x 554 and 176 x 564 (Tables 5a and 5b).

The presence of the Cry1A(b) protein in fresh kernels was verified by a bioassay of insecticidal activity against ECB larvae. There was no significant insecticidal activity against ECB in dried and re-hydrated kernels. The data are shown in Table 6.

PAT protein levels

Expression of functional PAT protein in Bt-176 corn was evidenced by increased tolerance to phosphinothricin (glufosinate ammonium herbicide). PAT was detected by ELISA in trace quantities in leaves, roots, pith and whole plants, but the levels were below the limit of quantification (Table 5a). No PAT was detected in either kernels or pollen. The level of PAT expressed in Bt-176 corn was at least thirty times less than in phosphinothricin-tolerant corn lines developed by Novartis for agronomic use, and Bt-176 corn is not intended for use as a herbicide-resistant plant.

β-lactamase

The *bla* gene introduced into Bt-176 corn is under the control of a bacterial promoter and would not be expected to be expressed in plant tissues.

Expression of the *bla* gene in Bt-176 corn was investigated by assay of β-lactamase activity and Northern blotting. No β-lactamase activity was detected in protein extracts of either leaves or pollen of Bt-176 corn. Northern blotting of total RNA from leaves of Bt-176 corn did not detect any *bla* mRNA transcripts. These results confirm, as predicted, that there is no β-lactamase expression in Bt-176 corn.

Table 5a: Cry1A(b) and PAT Protein levels in Bt176 corn and hybrid lines during development.

	Stage of development µg/g dry weight (n)							
	Seedling		**Anthesis**		**Seed Maturity**		**Senescence**	
Leaves	Cry1A(b)	PAT	Cry1A(b)	PAT	Cry1A(b)	PAT	Cry1A(b)	PAT
Bt176[1]	10.5 (3)	<1.5	3.04 (3)	<0.75	1.43 (3)	<0.40	0.10 (2)	nd
176 x 554[2]	4.78 (5)	<1.5	2.70 (3)	nd	1.65 (3)	nd	0.12 (3)	nd
176 x 564[3]	7.56 (3)	<1.5	13.37 (2)	nd	1.52 (2)	<0.40	0.30 (3)	<0.30
Whole Plant								
Bt176	4.19 (3)	nd	1.44 (5)	<1.20	0.29 (4)	<0.50	<0.02 (5)	<0.35
176 x 554	2.85 (6)	<2.30	0.20 (3)	nd	0.15 (4)	<0.50	<0.02 (4)	<0.35
176 x 564	3.40 (2)	<2.30	0.74 (3)	nd	0.26 (4)	nd	<0.02 (3)	<0.35
Kernels								
Bt176	---	---	---	---	<0.01 (4)	nd	<0.01 (5)	nd
176 x 554	---	---	---	---	<0.01 (3)	nd	<0.01 (3)	nd
176 x 564	---	---	---	---	<0.01 (2)	nd	<0.01 (3)	nd
Pollen[4]								
Bt176	---	---	4.32 (4)	nd	---	---	---	---
176 x 554	---	---	2.34 (3)	na	---	---	---	---
176 x 564	---	---	5.01 (3)	nd	---	---	---	---
Roots								
Bt176	<0.1 (2)	nd	<0.04 (4)	<0.90	<0.04 (4)	<0.90	na	na
176 x 554	<0.1 (6)	nd	<0.04 (3)	<0.90	<0.04 (3)	<0.90	na	na
176 x 564	<0.1 (1)	nd	<0.04 (3)	nd	<0.04 (2)	<0.90	na	na
Pith								
Bt176	na	na	<0.07 (4)	<1.60	<0.04 (4)	<0.85	na	na
176 x 554	na	na	<0.07 (3)	nd	<0.04 (3)	<0.85	na	na
176 x 564	na	na	<0.07 (3)	na	<0.04 (2)	nd	na	na

---: not relevant at this development stage; na: not analysed; nd: not detectable = the mean ELISA absorbance did not exceed that of the control equating to 0 ng protein;
[1]Genotype Bt176 refers to the genetically modified corn line CG00526-176 which is homozygous for both the *cry1A(b)* and *bar* genes;
[2]Genotype 176 x 554 refers to the hybrid corn line developed by the cross of CG00526-176 and untransformed line CG00554 and is hemizygous for the introduced genes.
[3]Genotype 176 x 564 refers to the hybrid corn line developed by the cross of CG00526-176 and untransformed line CG00564 and is hemizygous for the introduced genes
[4]Pollen values were determined on dry pollen samples and extrapolated to fresh weight.

Table 5b: Cry1A(b) levels in Bt-176 corn hybrids[1]

Line	Kernels at seed maturity	Whole plants at seed maturity	Whole plants at anthesis
µg Cry1A(b)/g total protein[2]			
526	0	0	0
Bt-176	<0.09[3]	3.63	14.40
526 x 554	0	0	0
Bt-176 x 554	<0.09[3]	2.14	2.50
526 x 564	0	0	0
Bt-176 x 564	<0.10[3]	3.71	7.40
Cry1A(b) as % total protein			
526	0	0	0
Bt-176	0.000009%	0.000363%	0.00144%
526 x 554	0	0	0
Bt-176 x 554	0.000009%	0.000214%	0.00025%
526 x 564	0	0	0
Bt-176 x 564	0.00001%	0.000371%	0.00074%

[1]Bt-176 = CG00526-176 homozygous inbred line, 554 = CG00554, 564 = CG00564, 526 = CG00526.
[2]µg/g protein values derived by calculation from values in Table 5a, plants grown in 1993 field trials in Hawaii
[3]Below the limit of quantification, --: not determined,

Table 6. Presence of Cry1A(b) protein in kernels: bioassay of insecticidal activity on European Corn Borer larvae.

Kernels	% mortalilty mean ± standard deviation
Fresh kernels	76.5 ± 10.9
Dry kernels	13.3 ± 4.7
Rehydrated kernels	3.3 ± 4.7

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

Study submitted by Novartis:

Duck, N. and Peters, C. 1995 Attempts to select ampicillin-resistant E.coli by transformation with DNA from the genetically modified maize. Performing laboratory: Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA.

The human health considerations in this regard 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 FAO[3]/WHO Expert Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). It was concluded by that consultation 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 cells in the human digestive tract 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). There have been concerns expressed, however, that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of 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-protected corn to microorganisms present in the human digestive tract.

The two plasmids used to transform corn line CG00526 - pCIB4331 and pCIB3064 - both contained a copy 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. Analysis of the Bt-176 corn and its hybrids confirmed the presence of as many as six intact copies of the *bla* gene along with its bacterial promoter. The *bla* gene is not expected to be expressed in the Bt-176 corn lines because it is under the control of a bacterial promoter and lacks regulatory sequences that would be recognized in plants. Experimental evidence discussed in Section 3.3 demonstrated no expression of the *bla* gene, as expected.

Potential for horizontal gene transfer

The first issue that must be considered in relation to the presence of an intact *bla* gene in Bt-176 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;
- 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 *bla* gene by the bacteria.

[3] Food and Agriculture Organization.

The transfer of a functional *bla* gene to microorganisms in the human digestive tract is therefore highly unlikely because of the number and complexity of the steps that would need to take place consecutively.

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

In the case of transfer of the *bla* gene from Bt-176 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 Bt-176 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.

The transfer of novel genetic material from genetically modified food to human cells via the digestive tract is also unlikely to occur. 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 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.

The applicant assessed the possibility of transfer of the *bla* gene from Bt-176 corn to bacteria by attempts to transfer ampicillin resistance through transformation of *E. coli* bacteria with total DNA extracted from Bt-176 corn plants. No ampicillin resistant colonies were isolated from either transformationally competent or non-competent *E. coli* cells treated with either intact or degraded DNA from Bt-176 corn.

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) which are responsible for degrading DNA into smaller fragments.

3.5 Other relevant data

Privalle, L. 1994 Assessment of DIMBOA levels in transgenic *Bt* corn (corn) and nontransgenic corn. Ciba Seeds, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Report No CAB-015-94.

To determine whether the transformation process had caused unintended changes in endogenous corn gene expression, levels of DIMBOA (2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one), were measured in transgenic *Bt* corn and compared to levels in isogenic control plants. DIMBOA is a natural plant defense compound (Gierl and Frey 1999) and has been correlated with natural resistance to ECB larvae (Klun and Brindley 1966). Analyses were conducted by HPLC after conversion of DIMBOA to the more stable MBOA (6-methoxy-2(3*H*)-bezoxazolone). The data are shown in Table 7. No significant differences were observed between transgenic and control plants, although considerable plant to plant

variation was observed. The data support the conclusion that the introduction of the Cry1A(b) gene has not resulted in any unintended perturbation of endogenous gene expression related to natural plant defence mechanisms.

Table 7. Levels of MBOA in Bt-176 corn leaves

Genotype	N	MBOA µg/g fr wt ± standard deviation
Control CG00526 inbred	10	0.86 ± 0.38
CG00526-Bt 176 inbred	10	0.84 ± 0.36

3.6 Conclusions regarding general safety issues

The *cry1A(b)* and *bar* genes are expressed in insect-protected corn containing the Bt-176 transformation event. The Cry1A(b) protein is expressed at the highest levels in leaves and pollen, as expected from the tissue specificity of the PEP-C and P-pollen promoters, but below the level of quantification in the kernel. The PAT protein is also expressed in trace amounts in all tissues except kernels, where it was undetectable. The levels of protein and DNA in highly processed corn products such as corn oil, high fructose corn syrup and corn starch is considered negligible, and therefore the level of Cry1A(b) and PAT genes and proteins would be vanishingly small. The Cry1A(b) and PAT genes and proteins have been well characterised. The transfer of these genes to corn is not considered to be a risk public health and safety.

It is extremely unlikely that the ampicillin resistance gene will transfer from foods derived from insect-protected Bt-176 corn to bacteria 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 to bacteria in the human digestive tract the human health impacts would be negligible because ampicillin-resistant bacteria are already commonly found in the human gut and in the environment.

It is also equally unlikely that novel genetic material from the insect-protected Bt-176 corn will be transferred to human cells via the digestive tract. The novel genetic material comprises only a minute fraction of the total DNA in the insect-protected Bt-176 corn therefore it is unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

The probable degradation and removal of DNA through the processing steps for corn further mitigate against any horizontal transfer of DNA from insect-protected Bt-176 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 proteins

Reports submitted by Novartis:

Kuhn, J.O. 1994a Cry1A(b) *B.t.k.* delta-endotoxin. Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Study No 1238-94Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC, USA.

Kuhn, J.O. 1994b *Bt* Corn Leaf Protein Lot: LP176-0194 and Control Corn Leaf Protein Lot: LP176-0194C. Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Study No 1443-94 Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC, USA.

Campbell, S.M. 1994 Cry1A(b) enriched corn leaf protein: An acute oral toxicity study with the northern bobwhite (*Colinus virginianus)*. Performing lab. Wildlife International Ltd. Project No 108-371 Sponsor: Ciba Seeds, Research Triangle Park, NC, USA.

Kuhn, J.O. 1995 Phosphinothricin acetyltransferase (Sample PAT-0195) Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Study No 1910-95 Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC, USA

Privalle, L. 1994 Characterisation of Cry1A(b) protein produced in *Bt* corn (corn) Event 176 and comparison with native Cry1A(b) protein produced by *Bacillus thuringiensis* subsp. *kurstaki* strain HD1-9. Performing laboratory: Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No CAB-006-94

Neither of the newly expressed proteins was found to have any similarity to a database of 2632 sequences of known toxins. The potential toxicity of the Cry1A(b) and PAT proteins was assessed by Novartis by evaluating acute oral toxicity in mice and in birds. The scientific basis for using an acute test is that known protein toxins generally act via acute mechanisms (Jones and Maryanski 1991).

Novartis carried out four acute toxicity studies: three in mice, using corn-expressed Cry1A(b), native Cry1A(b) and PAT; and one in the northern bobwhite quail using corn-expressed Cry1A(b).

Cry1A(b)

Cry proteins from *B. thuringiensis* have a long history of safe use as insecticides. There is no evidence from this history of use that there is any associated toxicity to humans. The toxicity of these proteins is very specific to Lepidopteran insects and there is no evidence that they are active against non–target insects, birds, fish or mammals (Hadley *et al* 1987, Drummond and Pinnock 1991). This lack of activity against non–target species appears to be due to a number of factors including physical differences in the gut environment and an absence of specific gut receptors in other organisms (Frick 1995). The binding of the δ–endotoxin to specific gut receptors appears to be a pre–requisite for toxicity (Cooper 1991, Schnepf *et a*l 1998). *In vivo* studies with rats given Cry1A(b) orally, and *in vitro* studies with rats, mice, rhesus monkeys and humans did not reveal receptors for the protein (Noteborn *et al* 1995).

(i) Equivalence of the plant Cry1A(b) protein to the native protein.

Cry1A(b) protein derived from *Bacillus thuringiensis* subsp. *kurstaki* strain HD1-9 as well as plant derived protein was used in acute toxicity studies. Characterisation of the Cry1A(b)

proteins from Bt-176 corn and from *B. thuringiensis* confirmed that the proteins were comparable in physical and chemical properties (see Section 3.2 above).

(ii) Acute oral toxicity in mice – native Cry1A(b)

Cry1A(b) δ-endotoxin purified from *Bacillus thuringiensis* (purity 70%) was administered to the mice (5/sex) at 5050 mg total protein/kg body weight by single oral gavage. There were no adverse effects from the dosing volume.

Mice were observed for clinical signs at least 3 times on the day of dosing and once daily for 14 days. Bodyweight was determined predosing (day 0) and on days 7 and 14. At the end of the study, mice were killed for postmortem examination of gross pathology.

No deaths occurred during the study. The only abnormal clinical sign observed was piloerection (raised hair), which occurred only on day 1. During the second week after dosing, one female lost weight; all other mice showed normal body weight gains for their age and sex. No abnormalities were detected on necropsy. Taking account of the purity of the protein preparation, the acute oral LD_{50} for Cry1A(b) δ-endotoxin was therefore concluded to be >3535 mg/kg bw in mice.

(iii) Acute oral toxicity in mice – Bt-176 Cry1A(b)

A protein extract from Bt-176 corn leaves enriched for Cry1A(b) (0.07%) or control corn leaf protein was administered to the mice (5/sex) at a dose of 5050 mg leaf protein /kg bw by single oral gavage, corresponding to a dose of plant-derived Cry1A(b) protein of 3.54 mg/kg bw.

Mice were observed for clinical signs at least 3 times on the day of dosing and once daily for 14 days. Bodyweight was determined predosing (day 0) and on days 7 and 14. At the end of the study, mice were killed for postmortem examination of gross pathology.

One test male died due to an injury sustained during the dosing procedure. One female mouse receiving the test corn leaf protein and one control female died during the study.

There were no significant differences in clinical findings or body weight gain between the group receiving leaf protein containing Cry1A(b) protein and that receiving control leaf protein. Clinical signs in the test group included piloerection, lacrimation (crying), polyuria, ptosis and decreased activity. Except for piloerection, these were seen at a low frequency, and all clinical signs had resolved by day 4. In the control group, piloerection and decreased activity were seen until day 5. One female dosed with the test material, and two females dosed with the control material lost weight during the second week of the study; bodyweight in the remaining animals was considered normal for their age and sex. On gross postmortem examination, there were no treatment-related abnormalities.

The applicant concluded that it could not be categorically ruled out that a maize protein component present in both the control and Cry1A(b) protein preparations was responsible for the mortality observed in this study. On the basis of the data presented, ANZFA concurs with this conclusion. These mortalities do not appear to be due to the Cry1A(b) protein as the female mice were from both the test and control groups thus suggesting some other component of the leaf extract. It should also be noted that there was no incremental increase

in mortality throughout the study as might be expected if the observed deaths were due to a component of the corn leaf extracts. The acute oral LD_{50} of Bt-176 corn leaf protein was therefore determined to be > 5050 mg/kg bw in mice, corresponding to 3.54 mg Cry1A(b) protein /kg bw.

(iv) Acute oral toxicity in birds – Bt-176 Cry1A(b)

Bt-176 corn leaf protein (0.07% Cry1A(b)) or control leaf protein was administered to 8 week old Northern bobwhite quail (5/sex) at 2000 mg protein/kg bw by single oral gavage corresponding to a dose of plant-derived Cry1A(b) protein of 1.4 mg/kg bw.

Following dosing, all birds were observed at least twice daily for mortality, signs of toxicity or abnormal behaviour. Bodyweight was measured one to two days before dosing and on days 3, 7 and 14. Average feed consumption was determined for each group for days 0–3, 4–7 and 8–14. At the end of the observation period the birds were killed and postmortem examinations conducted.

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 and no abnormalities were detected on post-mortem examination. The acute oral LD_{50} for northern bobwhite quail exposed to modified corn leaf protein (0.07% Cry1A(b) protein) was therefore concluded to be >2000 mg protein/kg bw, corresponding to 1.4 mg Cry1A(b) protein /kg bw.

PAT protein

An exemption from requirement to establish a maximum permissible level for residues of PAT and the genetic material necessary for its production was granted by the United States Environmental Protection Agency in April 1997 (US EPA 1997). Data demonstrating the absence of acute oral toxicity of PAT in mice have been evaluated by ANZFA for another application (Application A380 DBT-418 corn, Merriman 1996).

The PAT protein was expressed in trace amounts in Bt-176 corn but was at the limit of quantitation (see 3.2 above). This level of PAT expression would have been insufficient to allow extraction of adequate quantities for use in toxicity or digestive lability experiments. PAT protein was therefore derived from expression of the recombinant protein in *E. coli*.

Groups (5/sex) of mice were given a single oral dose (gavage) of either PAT protein (purity 51%) in carboxymethyl cellulose; heat inactivated PAT (52% purity) in carboxymethyl cellulose; or carboxymethyl cellulose control to a total dose of 5050 protein mg/kg bw, or adjusted for purity, 2575 mg PAT protein/kg bw.

Mice were observed for clinical signs at least 3 times on the day of dosing and once daily after this for a 14-day observation period. Bodyweight was determined predosing (day 0) and on days 7 and 14. At the end of the study, mice were killed for postmortem examination of gross pathology.

One male receiving the test substance died during the study as a result of material lodged in the oesophagus. The only notable clinical signs were decreased activity, piloerection and ptosis on days 6–8 in the male that died. One male receiving the reference substance showed

slight piloerection on the day of dosing. Bodyweight gain was unaffected by treatment, except in the male that died. There were no abnormal findings on postmortem of animals surviving until the end of the study. The acute oral LD_{50} of PAT protein was concluded to be >2575 mg/kg bw.

4.3 Potential allergenicity of existing proteins

There are no naturally occurring allergenic proteins known to occur in corn (Wright, 1987).

4.4 Potential allergenicity of novel proteins

Studies submitted by Novartis:

Privalle, L. 1994 Characterisation of Cry1A(b) protein produced in *Bt* corn (corn) Event 176 and comparison with native Cry1A(b) protein produced by *Bacillus thuringiensis* subsp *kurstaki* strain HD1-9. Ciba Seeds Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Report No. CAB-006-94

Privalle, L 1994 *In vitro* digestibility of CryIA (b) protein from Bt corn (corn) and *Bacillus thuringiensis* subspecies *kurstaki* under simulated mammalian gastric conditions. Ciba Seeds. Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No. CAB-007-94

Privalle, L. 1994 *In vitro* digestibility and inactivation of the *bar* marker gene product phosphinothricin acetyltransferase (PAT) under simulated mammalian gastric conditions. Ciba Seeds. Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Study No. CAB-008-94.

Although there are no predictive assays available to definitively assess the allergic potential of proteins, a number of characteristics are common among many of the allergens that have been characterized (Lehrer and Reese 1998, Jones and Maryanski 1991). Known allergens tend to be glycosylated proteins with a molecular weight of 10–70 kD (Lehrer *et al* 1996**)**. Protein allergens also tend to be heat stable as well as resistant to peptic and tryptic digestion and the acidic conditions of the stomach. Consequently, many allergenic factors tend to be resistant to proteolytic digestion (Taylor and Lehrer, 1996). Amino acid sequence similarity with known allergens may be a useful gauge of allergenic potential. A string of 8-12 consecutive amino acid residues in common with known allergens could be an indicator for allergenicity given that many T-cell epitopes of allergenic proteins are that length (Taylor and Lehrer, 1996).

The Cry1A(b) and PAT proteins were evaluated for potential allergenicity against these criteria: size; glycosylation; resistance to heat (PAT), digestive degradation and sequence similarity to known allergens.

Cry1A(b) protein

The *cry1A(b)* gene was derived from *B. thuringiensis* subsp. *kurstaki*. *B. thuringiensis* is not a food source but is a common soil bacterium that may be found on or around plant produce. Cry proteins have been used extensively as insecticides for decades and there are no reports of allergic reactions from either occupational exposure or ingestion of fresh produced sprayed with such insecticides.

The molecular weight of the Cry1A(b) protein expressed in Bt-176 corn is 65 kD, and thus within the size range of typical allergens. As described above (Sections 2.1 and 3.3) the

synthetic cry1A(b) gene encodes a protein identical to that of the native tryptic fragment, and the N-terminal amino acid sequence of the Cry1A(b) protein produced in Bt-176 corn was determined to match that of the native protein. The Cry1A(b) protein sequence contains six potential N-glycosylation sites. Direct testing of Cry1A(b) immunopurified from Bt-176 corn was negative for glycosylation and acetylation. Direct testing for phosphorylation of the native tryptic fragment of Cry1A(b) from *B. thuringiensis* was also negative. Given the equivalence of the corn-produced and native Cry1A(b) proteins it is also unlikely that the Cry1A(b) protein in Bt-176 corn is phosphorylated. Western blots indicated that the relative mobility of Cry1A(b) protein from Bt-176 corn did not differ from the predicted molecular weight. These data support the conclusion that the Cry1A(b) is not subject to post-translational modifcation *in planta*.

(i) Digestibility of Cry1A(b) protein under simulated gastric conditions

The digestibility of Cry1A(b) protein obtained from both genetically modified corn and from *Bacillus thuringiensis* subsp *kurstaki* (*Btk*) was assessed in simulated gastric conditions. Both proteins yield the same active fraction following proteolytic cleavage in the alkaline gut of Lepidopteran insects.

The digestive lability of Cry1A(b) protein extracted from leaves of mature field grown hybrid plants of Bt-176 corn and native Cry1A(b) protein extracted from *Bacillus thuringiensis* subsp *kurstaki* strain HDI-9 was assessed in simulated gastric fluid (SGF, 3.2 mg/ml pepsin at 1x, 0.1x, 0.01x and 0.001x).

The Cry1A(b) protein derived from Bt-176 corn was rapidly degraded in 1x SGF such that no immunoreactive Cry1A(b) polypeptides were detectable by Western blot upon immediate sampling and was undetectable after 10 minutes incubation with 0.001x SGF. The native Cry1A(b) protein was almost all degraded after 2 minutes in 1x SGF and was undetectable after 5 minutes with 0.01X SGF.

These data demonstrate that the Cry1A(b) protein expressed in Bt-176 corn and its hybrids is rapidly degraded in simulated digestive conditions. These results are consistent with published studies (Noteborn *et al* 1995, Sanders *et al* 1998).

Comparisons of the Cry1A(b) protein sequence from *B. thuringiensis* subsp *kurstaki* against sequences present in public domain databases (GenBank, EMBL, PIR and SwissProt) by Monsanto Pty Ltd, as part of Application A346 for Bt corn MON810, revealed no biologically significant homology with sequences other than Bt insecticidal proteins (Astwood 1995).

PAT protein

The *bar* gene encoding PAT was derived from *Streptomyces hygroscopicus*. The PAT protein is not found in plants or animals and is therefore not a normal component of food. However *Streptomyces* is a common soil bacterium which may be found on and around plant produce.

(i) Digestibility of PAT protein under simulated gastric conditions

The digestive lability of PAT protein was assessed in simulated gastric fluid (SGF, 3.2 mg/ml pepsin at 1x, 0.1x, 0.01x and 0.001x). The presence of PAT in the fluid following incubation was determined by SDS-PAGE analysis. The PAT activity was also determined after incubation in SGF at the pH optimum for the enzyme, at gastric pH and following serial incubation with a gastric solution containing 0.0032 mg/ml pepsin.

The PAT protein was rapidly degraded in 1x SGF such that no polypeptides were detectable by SDS-PAGE upon immediate sampling and was undetectable after 2 minutes incubation in 0.01x SGF. The apparent half-life of PAT in 0.001x SGF was between 1 and 2 minutes.

Incubation of PAT protein at 37°C for 10 minutes resulted in a 44% loss in activity. The heat sensitivity of PAT to temperatures above 35°C has previously been reported (Botterman *et al* 1991). PAT activity was not detected after 1 minute incubation in SGF without pepsin (pH1.0). Activity was not restored by neutralisation.

The amino acid sequence of the PAT protein was compared to the amino acid sequences of known allergens present in the GenBank public domain databases. No biologically significant homology was found with any known allergens or toxins.

These data indicate that the PAT protein will be destroyed upon exposure to the temperature, acid and peptidases of the mammalian gastric system and therefore is unlikely to act as an allergen.

4.5 Other relevant data

(i) Residues of glufosinate or its metabolites

Glufosinate is a herbicide commonly used on crops in the USA. No maximum residue limits have been set for glufosinate in grain crops in Australia (Standard A14 – Maximum Residue Limits, ANZFA 1999b). Glufosinate is not considered to be toxic to mammals at the levels applied in agriculture (Ebert *et al* 1990, Hack *et al* 1994), although ingestion of large amounts of the herbicide can result in severe pathology including neurological effects (Watanabe and Sano 1998).

However Bt-176 corn is not intended for use as a herbicide-resistant crop because the very low level of PAT expression (see Section 3.3 above) is insufficient to confer resistance to commercial doses of glufosinate ammonium. As there will be no herbicide application there will be no glufosinate residues present in Bt-176 corn.

4.6 Conclusions regarding toxicological issues

In all studies the acute oral toxicity of Cry1A(b) and PAT proteins was low. In mice the LD_{50} of the native Cry1A(b) protein was >3535 mg/kg bodyweight. The LD_{50} of Cry1A(b)-containing leaf extracts of Bt-176 corn was >5050 mg/kg bodyweight in mice and and >2000 mg/kg bodyweight in the northern bobwhite quail. These results are consistent with other studies on the acute toxicity of Cry1A(b) in mice and in rabbits (Noteborn *et al* 1995, Sanders *et al* 1998). The LD_{50} of PAT in mice was >2575 mg/kg bodyweight.

The data and analyses on the potential for toxicity or allergenicity of the Cry1A(b) or PAT proteins support the conclusions that neither protein is derived from an allergenic or toxic food source nor exhibits the characteristics of known protein allergens. Neither protein exhibits sequence similarity with known toxins or allergens. Furthermore, the Cry1A(b) and PAT proteins are present at very low abundance in corn kernels and both have been shown to be degraded in conditions that mimic human digestion. In addition, the activity of the PAT protein was shown to be destroyed by temperatures in excess of 37°C and by acid pH that would be encountered in the digestive system.

From these data it can be concluded that the food products derived from insect-protected Bt-176 corn should pose no greater threat as a source of allergic reaction than food products from conventional corn.

5 NUTRITIONAL ISSUES

Study submitted by Novartis:

Privalle, L. 1994 Compositional analysis of kernels from transgenic *Bt* corn (corn) as compared with nontransgenic control corn. Ciba Seeds, Ciba-Geigy Corporation, Research Triangle Park, NC, USA. Report No CAB-016-94.

5.1 Compositional analysis

Corn used for compositional analyses was derived from field trial and glasshouse experiments conducted in 1993 and 1994 (see Table 8). A range of analyses were performed on grain of Bt-176 corn and hybrids containing the Bt-176 transformation event. The components measured included proximates (protein, fat, ash, starch, moisture, fibre), amino acid composition, fatty acids profile and carotenoids. In the Hawaiian field trial, five random 100g samples of kernels of each genotype were taken from a pooled sample representing multiple plants. These samples were used for all analyses except fatty acids. One analysis was done on each of the five samples. In the French field trial, two 12.5g samples of kernels of each genotype were taken from a pooled sample representing multiple plants. These samples were used for all analyses except fatty and amino acids. Two analyses were done on each of the two samples. Genetically modified inbred and hybrid lines were compared to their corresponding non-genetically modified controls by t-tests. Significance was judged at the level of $p = 0.05$.

All analyses of the genetically modified and control corn kernels were conducted by Southern Testing and Research Laboratories Inc. (Wilson, NC) using recognised published methods in accordance with either the Association of Official Analytical Chemists (AOAC), the American Association of Cereal Chemists (AACC) or the American Oil Chemists Society (AOCS). .

(i) Proximate analysis

The proximate analyses of kernels from corn lines containing the Bt-176 transformation event did not vary markedly from the isogenic control lines, suggesting that there were no unintentional changes to grain composition due to the genetic transformation. However some statistically significant differences were observed (5% level using a pairwise T test). The data are shown in Tables 9a and 9b.

The protein levels of some hybrid Bt-176 lines varied from the isogenic controls: CG00554 x CG00526-176 (9% lower); CG00637 x CG00526-176 (12% higher); CG00615-176 inbred (17% higher). All the protein levels observed for the various lines, both modified and unmodified, were comparable to values reported by Watson (1987) and Wright (1987).

Table 8. Inbred and hybrid lines of Bt-176 corn used in compositional analyses

	Genotype	**Location**	**Harvest**
Control Bt	inbred CG00526 inbred CG00526-176	field, Hawaii, USA	1993
Control Bt	hybrid CG00554 x CG00526 hybrid CG00554 x CG00526-176	field, Hawaii, USA	1993
Control Bt	hybrid CG00637 x CG00526 hybrid CG00637 x CG00526-176	field, Hawaii, USA	1993
Control Bt	hybrid CG00684 x CG00526 hybrid CG00684 x CG00526-176	field, Hawaii, USA	1993
Control Bt	inbred CG00615 inbred CG00615-176	field, France	1994
Control Bt	hybrid CG00635 x XG00615 hybrid CG00635 x XG00615-176	glasshouse, France	1994

Table 9a. Compositional analysis of kernels from inbred CG00526-176 Corn[1]

Component	**Control CG00526**	**Bt CG00526-176**	**Literature Range[2](%)**
Protein %	12.21 ± 0.43 (11.60-12.74)	11.71 ± 0.35 (11.23-12.05)	6-12
Total fat %	4.74 ± 0.80 (3.84-5.65)	4.07 ± 0.80 (3.30-5.38)	3.1-5.7
Ash %	1.44 ± 0.03 (1.38-1.46)	1.41 ± 0.04 (1.35-1.45)	1.1-3.9
Starch %	65.85 ± 4.17 (60.86-72.08)	69.05 ± 0.88 (68.69-70.84)	65.3-83
Fibre %	1.95 ± 0.08 (1.85-2.05)	1.86 ± 0.13 (1.69-2.03)	2.5[3]
Moisture %	11.94 ± 0.44	12.38 ± 0.34	7-23

[1]n=5, Mean ± standard deviation (range), plants from 1993 field trials in Hawaii
[2]Watson 1987
[3]average value

The fat levels of some hybrid Bt-176 lines varied from the isogenic controls: CG00554 x CG00526-176 (65% higher); and CG00684 x CG00526-176 (44% less). Fat levels for all lines were comparable to reported values (Watson 1987) and the fat content was less than 5% of kernel in all cases.

The starch level of the hybrid CG00684 x CG00526-176 was 17% higher than the CG00684 x CG00526 control. The moisture content of some hybrid lines varied from the isogenic controls: CG00554 x CG00526-176 (27% higher); and CG00684 x CG00526-176 (26% less).

It should also be noted that there was considerable variability between lines for all of the components tested and that the differences observed in some lines were not evident in all lines, suggesting that the differences are not a result of the genetic modification.

(ii) Amino acid composition of Bt-176 corn

Sixteen individual amino acids were quantified. The levels of glutamine, asparagine, cysteine and tryptophan were not determined. The data are shown in Tables 10a and 10b. Some small, but statistically significant (5% level in a pairwise T-test), differences were observed for some amino acids. However, the overall amino acid profile was similar for transgenic and isogenic corn. The values for all lines were comparable to typical values reported in the literature (Wright 1987).

Table 9b. Proximate analysis of hybrid and inbred lines containing the Bt-176 transformation event, % component

Genotype	n	Protein	Fat	Ash	*Starch*	*Fibre*	Moisture
Control 526	5	12.21 ± 0.43	4.74 ± 0.80	1.44 ± 0.03	65.85 ± 4.17	1.95 ± 0.08	11.94 ± 0.44
526-Bt 176	5	11.71 ± 0.35	4.07 ± 0.80	1.41 ± 0.04	69.95 + 0.88	1.86 ± 0.13	12.38 ± 0.34
Control 554x526	5	11.96 ± 0.35	2.55 ± 1.14	1.30 ± 0.05	68.29 ± 10.06	1.50 ± 0.13	9.64 ± 0.40
554xBt-176 hybrid	5	***10.88 ± 0.17****	***4.21 ± 0.79****	1.27 ± 0.03	72.19 ± 2.56	1.41 ± 0.12	***12.23 ± 0.30****
Control 637x526 hybrid	5	12.13 ± 0.48	4.07 ± 1.12	1.63 ± 0.25	66.84 ± 2.97	1.97 ± 0.10	12.17 ± 0.49
637x526-Bt-176 hybrid	5	***13.62 ± 0.48****	3.49 ± 1.62	1.68 ± 0.23	68.85 ± 2.29	1.77 ± 0.32	10.24 ± 1.88
Control 684x526 hybrid	5	12.85 ± 0.39	3.66 ± 0.96	1.73 ± 0.16	58.23 ± 7.19	1.56 ± 0.38	12.14 ± 0.28
684x526-Bt-176 hybrid	5	13.32 ± 0.37	***2.04 ± 0.60****	1.63 ± 0.16	***68.07 ± 3.01****	1.61 ± 0.16	***9.01 ± 1.27****
Control 615 inbred	2	10.07 ± 0.15	4.67 ± 0.59	1.73 ± 0.21	63.16 ± 0.93	1.84 ± 0.08	10.82 ± 0.26
615-Bt-176 inbred	2	***11.79 ± 0.07****	4.34 ± 0.13	1.82 ± 0.01	59.14 ± 0.98	1.70 ± 0.22	12.38 ± 0.04
Control 635x615 hybrid	2	11.17 ± 0.62	4.14 ± 0.10	1.93 ± 0.08	61.51 ± 0.75	1.74 ± 0.06	13.22 ± 0.27
635x615-Bt-176 hybrid	2	11.38 ± 0.33	4.05 ± 0.21	1.81 ± 0.01	61.04 ± 1.82	1.92 ± 0.23	12.06 ± 0.10

*: statistically sig difference 5% level pairwise T-test

Table 10a. Amino acid content of grain from inbred CG00526- 176 Corn % total protein

Component	CG00526 Control	CG00526-176 inbred	Typical literature values[1]
Glutamate	15.74 ± 0.63	15.32 ± 1.06	18.63
Leucine	10.69 ± 0.57	11.08 ± 0.42	11.05
Proline	7.49 ± 0.22	***8.50 ± 0.41****	8.84
Alanine	6.29 ± 0.24	6.47 ± 0.24±	8.21
Aspartate	5.67 ± 0.32	5.07 ± 0.47	7.16
Phenylalanine	4.75 ± 1.04	3.87 ± 0.32	4.42
Serine	3.92 ± 0.16	4.03 ± 0.24	4.63
Valine	3.81 ± 0.20	3.70 ± 0.19	4.0
Arginine	3.46 ± 0.28	3.54 ± 0.21	4.42
Glycine	2.96 ± 0.14	***3.30 ± 0.16****	3.89
Threonine	2.95 ± 0.15	3.09 ± 0.12	3.26
Tyrosine	2.94 ± 0.46	2.92 ± 0.20	3.47
Isoleucine	2.88 ± 0.33	2.59 ± 0.08	3.58
Lysine	2.34 ± 0.28	2.48 ± 0.29	2.32
Histidine	2.25 ± 0.10	2.23 ± 0.11	2.63
Methionine	1.92 ± 0.10	1.76 ± 0.15	1.58

n= 5, replicate samples from a pooled sample representing multiple plants. ± standard deviation 1: Wright 1987, *: statistically significant difference at 5% level in a pairwise T-test

Table 10b. Amino acid content of hybrid and inbred lines containing the Bt-176 transformation event

Genotype	Glu	Leu	*Pro*	Ala	Asp	Phe	Ser	Val	Arg	Gly	Thr	Tyr	Ile	Lys	His	Met
526	15.74 ±0.63	10.69 ±0.57	7.49 ± 0.22	6.29 ± 0.24	5.67 ± 0.32	4.75 ± 1.04	3.92 ± 0.16	3.81 ± 0.20	3.46 ± 0.28	2.96 ± 0.14	2.95 ± 0.15	2.94 ± 0.46	2.88 ± 0.33	2.34 ± 0.28	2.25 ± 0.10	1.92 ± 0.10
526-Bt 176	15.32 ± 1.06	11.08 ± 0.42	***8.50* ± 0.41***	6.47 ± 0.24	5.07 ± 0.47	3.87 ± 0.32	4.03 ± 0.24	3.70 ± 0.19	3.54 ± 0.21	***3.30* ± 0.16***	3.09 ± 0.12	2.92 ± 0.20	2.59 ± 0.08	2.48 ± 0.29	2.23 ± 0.11	1.76 ± 0.15
554x526	15.90 ± 0.45	10.82 ± 0.35	7.23 ± 0.23	6.77 ± 0.32	5.60 ± 0.18	4.03 ± 0.18	4.02 ± 0.14	3.66 ± 0.15	3.84 ± 0.20	3.00 ± 0.08	2.84 ± 0.05	2.85 ± 0.17	2.69 ± 0.05	2.36 ± 0.21	2.05 ± 0.05	1.70 ± 0.13
554x526-Bt-176	16.72 ± 0.68	***11.60**** ± 0.48	7.76 ± 0.30	6.82 ± 0.26	5.55 ± 0.25	***4.93* ± 0.44***	4.16 ± 0.15	3.87 ± 0.13	3.88 ± 0.18	3.14 ± 0.09	***3.06* ± 0.16***	2.84 ± 0.14	***2.93* ± 0.13***	2.55 ± 0.16	***2.20* ± 0.09***	1.72 ± 0.05
637x526	15.88 ± 1.15	10.66 ± 0.48	7.52 ± 0.51	6.43 ± 0.49	6.07 ± 0.42	5.20 ± 0.42	4.11 ± 0.27	3.83 ± 0.20	3.84 ± 0.26	3.25 ± 0.16	3.20 ± 0.13	3.06 ± 0.17	2.78 ± 0.17	2.99 ± 0.11	2.19 ± 0.15	1.51 ± 0.09
637x526-Bt-176	15.83 ± 0.69	10.84 ± 0.41	7.60 ± 0.46	6.12 ± 0.29	5.52 ± 0.32	4.82 ± 0.44	3.97 ± 0.17	3.50 ± 0.15	***3.09* ± 0.19***	3.05 ± 0.19	3.21 ± 0.18	2.87 ± 0.08	2.66 ± 0.19	***2.20* ± 0.21***	***1.91* ± 0.09***	***1.35* ± 0.08***
684x526	16.34 ± 1.15	11.34 ± 0.89	7.96 ± 0.78	6.25 ± 0.35	5.20 ± 0.31	5.48 ± 0.43	4.05 ± 0.28	3.41 ± 0.25	3.03 ± 0.26	2.86 ± 0.13	3.07 ± 0.24	3.23 ± 0.15	2.53 ± 0.34	1.89 ± 0.36	1.88 ± 0.16	1.52 ± 0.09
684x526-Bt-176	16.83 ± 0.88	12.02 ±0.75	7.84 ± 0.42	6.54 ± 0.36	5.02 ± 0.28	5.96 ± 0.18	4.00 ± 0.18	3.27 ± 0.18	2.71 ± 0.22	2.70 ± 0.08	2.92 ± 0.16	3.35 ± 0.18	2.55 ± 0.15	1.45 ± 0.13	1.77 ± 0.08	***1.37* ± 0.07***

* statistically sig difference 5% level pairwise T-test

(iii) Fatty acid acid composition of Bt-176 corn

The proportion of five fatty acids in kernels of hybrid and inbred corn lines containing the Bt-176 transformation event and isognenic controls was determined. The data are shown in Tables 11a and 11b. The relative proportions of the major fatty acids were similar for the transgenic and control lines and there were no statistically significant differences (5% level, pairwise T-test). The levels observed in all lines were within the ranges reported in the literature (Weber 1987).

Table 11a. Comparison of major fatty acids in kernels from control and Bt-176 corn

% of total fatty acid, mean ± standard deviation (range)

Component	CG00526 Control	CG00526-176 inbred	Literature Range[1]
Palmitic 16:0	12.71 ± 0.89 (11.77–14.33)	12.24 ± 0.57 (11.67–12.94)	6-22
Stearic 18:0	2.39 ± 0.58 (1.90–3.34)	2.20 ± 0.21 (1.95–2.48)	1-15
Oleic 18:1	27.09 ± 1.22 (24.45–28.34)	27.90 ± 0.74 (27.03–28.66)	14-64
Linoleic 18:2	55.08 ± 3.45 (50.46–59.47)	55.38 ± 2.35 (52.13–58.08)	19-71
Linolenic 18:3	0.73 ± 0.15 (0.52–0.83)	0.81 ± 0.08 (0.70–0.89)	0.5-2

n=5, replicates from pooled samples of kernels representing multiple plants, 1: Weber 1987, kernels harvested from 1993 field trials in Hawaii.

(iv) Carotenoids

The levels of the carotenoid content, specifically xanthophylls and β-carotene were determined for hybrid and inbred corn lines containing the Bt-176 transformation event and isognenic controls. The data are shown in Tables 12a and 12b. No statistically significant differences (5% level, pairwise T-test) in xanthophylls content were observed between any of the transgenic Bt-176 corn lines. There were no statistically significant differences (5% level, pairwise T-test) for β-carotene levels, except in the original CG00526-176 inbred lines, in which the level of β-carotene was higher than in the CG00526 control line. This difference could be due to differences in the length of storage time of the kernels as carotenoids have been shown to decrease with time in storage (Wright, 1987).

Table 11b. Major fatty acids in kernels of hybrid and inbred lines containing the Bt-176 transformation event % total fatty acid, mean ± standard deviation

Genotype	n	Palmitic 16:0	Stearic 18:0	Oleic 18:1	*Linoleic 18:2*	*Linolenic 18:3*
Control 526	5	12.71 ± 0.98	2.39 ± 0.58	27.09 ± 1.22	55.08 ± 3.45	0.73 ± 0.15
526-Bt 176	5	12.24 ± 0.57	2.20 ± 0.21	27.90 ± 0.74	55.38 + 2.35	0.81 ± 0.08
Control 554x526	5	14.26 ± 0.61	2.26 ± 0.16	25.76 ± 0.34	54.74 ± 1.98	0.81 ± 0.04
554xBt-176 hybrid	5	13.77 ± 0.98	2.30 ± 0.17	25.69 ± 0.74	55.25 ± 2.73	0.88 ± 0.10
Control 637x526 hybrid	5	12.92 ± 2.11	2.13 ± 0.41	30.04 ± 1.14	50.16 ± 6.92	0.84 ± 0.14
637x526-Bt-176 hybrid	5	12.72 ± 1.14	2.16 ± 0.16	29.40 ± 0.93	51.12 ± 3.65	0.81 ± 0.09
Control 684x526 hybrid	5	13.93 ± 1.65	2.15 ± 0.41	24.54 ± 1.18	55.86 ± 5.41	0.86 ± 0.13
684x526-Bt-176 hybrid	5	14.17 ± 1.69	2.54 ± 0.50	24.39 ± 0.54	54.80 ± 4.64	0.89 ± 0.15

*: statistically sig difference 5% level pairwise T-test

Table 12a. Carotenoid levels in kernels from inbred CG00526- 176 Corn

Component	CG00526 control	CG00526-176 inbred
Xanthophylls	323.6 ± 112.8 (231–512.3)	378.8 ± 37.5 (352.8–416.6)
β-carotene	15.43 ± 1.18 (14.38–17.17)	***17.38 ± 0.33**** (17.03–17.86)

n=5, Mean ± SD (μg/100g sample) (range), *: statistically significant difference in pairwise T-test at 5% level

Table 12b. Carotenoid levels in kernels from hybrid and inbred lines containing the Bt-176 transformation event

Genotype	N	Xanthophylls	*β-carotene*
Control 526 inbred	5	323.6 ± 112.8	15.43 ± 1.18
526-Bt 176 inbred	5	378.8 ± 37.5	***17.38 ± 0.33****
Control 554x526 hybrid	5	377.1 ± 116.6	15.01 ± 0.96
554xBt-176 hybrid	5	371.8 ± 50.0	15.20 ± 2.90
Control 637x526 hybrid	5	284.7 ± 51.4	4.41 ± 4.04
637x526-Bt-176 hybrid	5	180.3 ± 86.2	3.18 ± 1.24
Control 684x526 hybrid	5	237.9 ± 103.0	3.73 ± 0.80
684x526-Bt-176 hybrid	5	152.1 ± 40.8	2.80 ± 0.33
Control 615 inbred	2	3086.2 ± 67.7	60.92 ± 0.13
615-Bt-176 inbred	2	2918.8 ± 314.5	47.88 ± 2.16
Control 635x615 hybrid	2	1808.7 ± 126.3	41.43 ± 1.76
635x615-Bt-176 hybrid	2	1532.4 ± 113.0	29.25 ± 3.75

Mean ± SD (μg/100g sample) (range), *: statistically significant difference in pairwise T-test at 5% level

(v) Conclusions from compositional analyses

Comprehensive data from a range of compositional analyses conducted on kernels from Bt-176 corn and hybrids and the corresponding unmodified, isogenic control lines were presented for assessment. The compositional components measured included proximates (protein, fat,

ash, starch, fibre and moisture), amino acid composition, fatty acids profile, and carotenoid levels.

The results of the kernel compositional data do not indicate that there are any substantial differences between corn lines containing the Bt-176 transformation event and the non-transgenic control lines for any of the parameters measured. Some small statistically significant differences were observed in protein, fat, starch, moisture, amino acid content and β-carotene content for some of the Bt-176 corn lines. However these differences were not apparent in all of the transgenic lines containing the novel genes. The values were within ranges previously reported for corn and were not considered to be of either biological relevance for commercially grown corn varieties or of significance in terms of food safety. In further support of the equivalence of the nutritional adequacy of the insect protected Bt-176 corn, additional compositional data has been provided on hybrid lines of different genetic backgrounds which are consistent with data from the original transformant.

5.2 Levels of anti-nutrients

The levels of the trypsin and chymotrypsin inhibitors in corn are very low and are not considered nutritionally significant (Wright 1987).

5.3 Ability to support typical growth and well-being

Study submitted by Novartis:

Brake JT 1996 Evaluation of transgenic Event-176 *Bt* corn (corn) in broiler chickens. Performing laboratory: North Carolina State University Poultry Education Unit, Raleigh, NC. Sponsor: Ciba Seeds, Agricultural Biotechnology, Ciba-Geigy Corporation, NC, USA.

In assessing the safety of a genetically modified food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well-being. In most cases, this can be achieved through an understanding of the genetic modification and its consequences together with an extensive compositional analysis of the food. Carefully designed feeding studies in animals may provide further re-assurance 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 Bt-176 corn the extent of the compositional and other data provided in the application is considered adequate to establish the nutritional adequacy and safety of the food. The presented data indicates that the composition of Bt-176 corn lines was equivalent to, and would be expected to be equally nutritious as, the non-transgenic control lines. Nonetheless, the applicant also provided an animal feeding study to compare the wholesomeness of Bt-176 and control corn. Although not considered essential for establishing safety in this instance, this animal feeding study has been reviewed as additional supporting data.

Chicken feeding study

The data from this study have been published in the scientific literature (Brake and Vlachos 1998).

One thousand two hundred and eighty 1-day-old broiler chicks (Arbor Acres Yieldmaster) were randomly assigned to 32 single sex groups, with 40 birds/group. Transgenic corn (Ciba Seeds' *Bt*- 176 corn-derived hybrid number 5506BTX) and nontransgenic corn (Ciba Seeds' hybrid G4665) were used. Diets were prepared in both pellet and mash form with both the transgenic and nontransgenic corn, with four groups of each sex fed each type of diet (160 birds/sex/diet type). Prior to the study minor compositional differences were noted between the transgenic and nontransgenic corn and the protein content of the two diets was equalized by non-nutritional filler. Diets were formulated with corn as a base to yield standard protein percentages of 22% for starter feed and 20% for grower feed. Growth performance was measured by weight gain, the feed conversion ratio (FCR, feed:gain), and final body weight. Lower FCR values represent more efficient weight gain per unit feed. The results are summarised in Table 13 below.

Table 13. Body weight gain and Feed Conversion Ratio (FCR) in broiler chickens fed Bt-176 corn

Corn Diet	**day 1**	**day 14**	**day 28**	**day 38**
	Mean body weight			
Bt-176	41	375	1213	1825
G4665 control	41	372	1199	1802
FCR				
Bt-176		1.18	*1.51**	1.74
G4665 control		1.19	*1.55**	1.76

* statistically significant difference at 0.05 significance level with a General Linear Model analysis

During the study there were no clinical signs related to treatment with transgenic corn. Survival was very high in all groups (96–98%), with no difference between groups.

There were no significant differences in bodyweight between birds fed the transgenic corn and birds fed the nontransgenic corn at any time period. There was a slightly higher, but statistically significant ($P<0.05$), feed conversion ratio for birds fed on the transgenic corn diet than on the nontransgenic corn at 28 days, but no differences at 14 or 38 days. In birds fed Bt-176 corn there was a statistically significant increase in the amount of *Pectoralis minor* muscle and skin overlying the total breast, further demonstrating the absence of any detrimental effects.

The data demonstrate that the transgenic corn is equivalent to commercial varieties in its ability to support typical growth and well-being in chickens.

5.4 Conclusions regarding nutritional issues

The nutritional qualities of insect-protected Bt-176 corn were determined by compositional analyses of the major components of the kernels and these were found to be comparable in all respects to the conventional corn lines. Bt-176 corn was found to be equally nutritious as conventional corn when used as feed for chickens.

There is a long history of safe use of corn. Based on the data submitted in the present application, grain derived from Bt-176 corn is nutritionally and compositionally comparable to that from conventional corn and is not considered to pose a risk to human health and safety.

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ATTACHMENT 3

DRAFT REGULATORY IMPACT ASSESSMENT

The Authority is required, in the course of developing regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

Option 1–To prohibit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • no benefits were identified.	**Costs** • the governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • a prohibition on food produced using gene technology in Australia and New Zealand could result in retaliatory trade measures from other countries. • there may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	**Costs** • food manufacturers and producers will be unable to use the processed food fractions from foods produced using gene technology thus requiring the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be $A 207m in Australia and $NZ 37m in New Zealand[4]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[4] Report on the costs of labelling genetically modified foods (2000)

CONSUMERS	**Benefits** • no benefits were identified, however as some consumers perceive GM food to be unsafe, they may perceive prohibition of GM food to provide a public health and safety benefit.	**Costs** • could lead to decreased availability of certain food products. • increased costs to consumers because manufacturers and producers may have to source non-GM ingredients.

Option 2– to permit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • increased innovation and competitiveness in the food industry will benefit the economy.	**Costs** • minor costs associated with amending the *Food Standards Code.*
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • food producers and manufacturers will be able to capitalise on the latest technology. • food importers will continue to be able to import manufactured products from overseas markets including the USA and Canada where there is no restriction on the use of food produced using gene technology.	**Costs** • there may be some discrimination against Australian and New Zealand food products in overseas markets that have a preference for non-GM foods (e.g., Japan and the European Union).
CONSUMERS	**Benefits** • consumers may have access to a greater range of food products.	**Costs** • those consumers who wish to avoid GM food may experience restricted choice in food products. • those consumers who wish to avoid GM food may have to pay more for non-GM food.

Conclusion of the regulatory impact assessment

Consideration of the regulatory impact for foods produced using gene technology concludes that the benefits of permitting foods produced using gene technology primarily accrue to the government and the food industry, with potentially a small benefit to consumers. These benefits are considered to outweigh the costs to government, consumers and industry, provided the safety assessment does not identify any public health and safety concerns.

ATTACHMENT 4

WORLD TRADE ORGANIZATION AGREEMENTS

With the completion of the Uruguay Round of trade negotiations, the World Trade Organization (WTO) was created on 1 January 1995 to provide a forum for facilitating international trade.

The WTO does not engage in any standard-setting activities but is concerned with ensuring that standards and procedures for assessment of and conformity with standards do not create unnecessary obstacles to international trade.

Two agreements which comprise part of the WTO treaty are particularly important for trade in food. They are the;

Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); and
Agreement on Technical Barriers to Trade (TBT).

These agreements strongly encourage the use, where appropriate, of international standards, guidelines and recommendations, such as those established by Codex (in relation to composition, labelling, food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling) and the code and guidelines on hygienic practice.

Both Australia and New Zealand are members of the World Trade Organization (WTO) and signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS agreement) and on Technical Barriers to Trade (TBT agreement). Within Australia, the Council of Australian Governments (COAG) has put in place a Memorandum of Understanding binding all States and Territories to the agreements.

The WTO agreements are predicated on a set of underlying principles that standards and other regulatory measures should be:

- based on sound scientific principles;
- developed using consistent risk assessment practices;
- transparent;
- no more trade-restrictive than necessary to achieve a legitimate objective;
- recognise the equivalence of similar measures in other countries; and
- not used as arbitrary barriers to trade.

As members of the WTO both 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. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists). Matters raised in this proposal may be notified to the WTO as either SPS notifications or TBT notifications, or both.

SPS Notifications

These are primarily health related, and refer to any sanitary and phyto sanitary measure applied:

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;

- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

- to protect human life or health from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; and

- to prevent or limit other damage from the entry, establishment or spread of pests.

The Agreement on the Application of Sanitary or Phytosanitary Measures relates to any sanitary or phytosanitary measure applied to protect animal, plant or human life or health which may directly or indirectly affect international trade. Whether the SPS measure is in the form of a law or mandatory regulation, an advisory guideline, a code of practice or a requirement, it is the purpose of the measure that is important - not its regulatory status. Each WTO member country is entitled to apply SPS measures that are more stringent than the international standards in order to protect the health of its population. In the interests of transparency, each instance of such non-alignment which could result in an impediment to trade must be identified and justified and the documentation of that justification must be readily available

Each member country is also required to apply its methods of risk assessment and management consistently so arrangements under the SPS Agreement do not generate what may really be technical barriers to trade

Under the SPS Agreement, an exporting country can have resort to the WTO's dispute settlement procedures with respect to such a non-alignment. These arrangements mean there is potential for a code of practice to introduce an SPS measure that may bring about non-alignment with international requirements. Such non-alignment would need to be justified scientifically on the grounds that it is necessary to protect human, animal or plant life or health.

TBT Notifications

A technical barrier to trade arises when a mandatory requirement in a country's food regulatory system does not align with the international standard and it is more trade restrictive than is necessary to fulfil a legitimate objective. However, it can be acceptable for a country to have a more stringent requirement than that set internationally for reasons including:

Maintaining national security;
Preventing deceptive practices; and
Protecting human health or safety.

Instances of non-alignment with international standards which could result in trade barriers must be identified and, if questioned, justified. Voluntary codes of practice are not expected to generate technical barriers to trade except where compliance with a code of practice or some aspect of a code of practice is expected. Consequently, it is possible for a voluntary code of practice to be viewed by the WTO as mandatory and subject to all the notification and other provisions applying to mandatory regulations.

The Agreement on Technical Barrier to Trade relates to requirements covering product characteristics or their related processes and production methods. TBT covers measures that are not SPS, such as requirements relating to terminology, symbols, packaging, marking, labelling, food composition and processing methods.

ATTACHMENT 5

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS FOR APPLICATIONS A372, A375, A378, A379, A380, A381, A382, A383, A384, A385, A386, A387 & A388

National Genetic Awareness Alliance (Aus)

- 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.

Pola Lekstan and Anna Clements (Aus)

- 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.

Arnold Ward (Aus)

- 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.

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

Public and Environmental Health Service (Aus)

- 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.

David Grundy (Aus)

- 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 ingredient.

Leesa Daniels (Aus) 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.

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.

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.

Nestle Australia Ltd.

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

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

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

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.

BRI Australia

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

Food Technology Association of Victoria Inc.

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

Diane Davie (Aus)

- 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

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 Aus), Brennan Henderson (NZ) – Generic e-mail objection

- 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.

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.

Vicky Solah (Aus)

- 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.

Dr Rosemary Keighley (Aus)

- 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.

Nicola Roil (Aus)

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

Ian and Fran Fergusson (Aus) – also in generic email above

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

Lyndal Vincent (Aus)

- 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.

Fay Andary (Aus)

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

John and Francesca Irving (Aus)

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

Diana Killen (Aus)

- 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.

Sheila Annesley (Aus)

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

David and Edwina Ross (Aus)

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

Beth Schurr (Aus)

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

Beth Eager (Aus)

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

Bruce Pont and Ljiljiana Kuzic-Pont (Aus)

- 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.

Chitta Mylvaganum (Aus)

- 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.

John Stevens (Aus)

- 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

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

- 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.

Jan Kingsbury (Aus)

- 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

Teresa Sackett (Aus)

- 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:
  - Increased use of chemicals
  - 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

John and Sandy Price (Aus)

- 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.

John Scott (NZ)

- 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

R A Randell (NZ)

- 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.

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 WHO agreement, but consider that the primary role of ANZFA is the assurance of health and safety

Safe Food Campaign (NZ)

- 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.

Jocelyn Logan, Caroline Phillips (NZ)

- 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)

Robert Anderson (member of Physicians and Scientists for Responsible Genetics - NZ)

- 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

Stephen Blackheath (NZ)

- 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.

Claire Bleakley (NZ)

- 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.

ATTACHMENT 6

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, expressed general views against the use of gene technology, asserted that food produced using this technology is unsafe for human consumption and expressed opposition to the sale of the food, irrespective of the type of food concerned or the particular genetic modification. An evaluation of these general issues raised by the submissions appears below.

1. The safety of genetically modified foods for human consumption

A majority of submitters raised the issue of public health and safety in relation to food produced using gene technology. In particular, it was 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.

Evaluation

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 is to establish that the new food is at least as safe as existing foods. 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 consistent with international protocols developed by these bodies.

2. The need for long-term feeding studies

A number of submissions were concerned about the lack of long-term toxicity studies on genetically modified foods.

Evaluation

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 animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects of importance to humans. Establishing a dose-response relationship is a pivotal step in toxicological testing. In this way it is possible, in most cases, to determine the levels of exposure at which adverse effects are not present and so establish safe upper limits through applying 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 studies on foods is the need to maintain the nutritional value and balance of the diet. A diet that is poorly balanced will compromise the interpretation of any feeding study, since the effects observed will confound and usually override any small adverse effect which may be related to a component or components of the food. 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 some reason to question 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 case, studies up to 14 days have also been performed. These can provide additional re-assurance that the proteins will have no adverse effects in humans. Such experiments can provide more meaningful information than similar experiments on the whole food. Additional re-assurance regarding the safety of newly-expressed protein can be obtained by considering the digestibility of the new protein in *in vitro* assays using conditions which simulate the human gastric system.

3. Substantial equivalence

A number of submitters expressed concern regarding the use of the concept of substantial equivalence. Some rejected the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

Evaluation

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 of the new and traditionally-produced food. This can include phenotypic[2] characteristics and compositional factors, as well as 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 composition of the new food relative to the conventional food.

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 a 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*.'
The concept has been internationally recognised and embraced as a valuable tool in the safety assessment of foods produced using gene technology and was reaffirmed as an appropriate tool at the Expert Consultation held in June 2000. The OECD also advocates an approach to safety assessment based on substantial equivalence as being '*the most practical to address the safety of foods and food components derived through modern biotechnology*.'

4. The nutritional value of food produced using gene technology

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

Evaluation

The assessment of food produced using gene technology by ANZFA entails an exhaustive evaluation of technical 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.

[2] characteristics that are visible

In the future, it is proposed that genetic modification 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 issues of the community and also specific dietary problems of sub-populations.

5. Potential toxins and allergens

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

Evaluation

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.

6. Antibiotic resistance

Some submitters raised concerns about increased antibiotic resistance resulting from the use of gene technology. Some felt that it would be reassuring if independent biomedical advice were available to reassure 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.

Evaluation

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 micro-organisms 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 recent 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 unnecessary given the existence of adequate alternatives, and should be phased out.

7. Transfer of novel genes

Some submitters have expressed concern that the transfer of any novel gene may be a health concern.

Evaluation

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.

8. Viral recombination

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

Evaluation

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 will be addressed by the Genetic Manipulation Advisory Committee (GMAC) on a case–by–case basis when it assesses such plants.

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

9. Labelling of foods produced using gene technology

A majority of submissions focussed on this issue. Specifically, the submissions called for the labelling of all foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters based their demands for full labelling on the presumption that all foods produced using gene technology are unsafe and on consumer "right to know" arguments. It was stated that full labelling was the only means of identification of foods produced using gene technology available to consumers.

Evaluation

The existing Standard A18 already makes provision for mandatory labelling of genetically modified foods that are substantially different from their conventional counterparts. However, ANZFA is committed to implementing the in-principle decision of ANZFSC Health Ministers of August 1999 to require labelling of all genetically modified foods, including those that are substantially equivalent in composition to the unmodified form. In conjunction with a task force of officials from State and Territory Health Departments and the New Zealand Ministry of Health, ANZFA developed draft revision to Standard A18 in October 1999 that requires labelling of other categories of genetically modified foods. At the Ministers request this draft was circulated for public review and a cost-benefit analysis of full labelling was commissioned. The task force considered both public comments and the cost-benefit analysis in finalising their recommendations to Ministers, which were delivered in May 2000. Ministers met to resolve the issue on the 28 July 2000 following whole-of-government consideration. The 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. This requirement will come into effect 12 months after the date of gazettal which is expected to be in September 2000 and will apply to all current and subsequent applications.

10. 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.

Evaluation

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.

11. 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.

Evaluation

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 (the current Standard A18) 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, both in these submissions, and 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 is in the process of preparing a public discussion paper on the safety assessment process for GM foods. This will be widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

12. 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.

Evaluation

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.

13. 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.

Evaluation

These issues are considered in the assessment processes of GMAC in Australia and the Environmental Risk Management Authority (ERMA) in New Zealand. The Authority does not have the mandate to assess matters relating to environmental risks resulting from the release of food produced using gene technology into the environment. However, links exist between ANZFA and other regulatory agencies in both Australia and New Zealand, and a

large degree of information sharing occurs. ANZFA would not recommend the approval of a food produced using gene technology if the genetically modified organism from which it was derived did not have the appropriate clearance for general release from either GMAC (or its successor) or ERMA, as appropriate.

The regulatory system in Australia will comprise the existing regulators 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).

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

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

In Australia a new Office of the Gene Technology Regulator (OGTR) will complement the existing arrangements. OGTR will supersede the existing arrangements under the Genetic Manipulation Advisory Committee (GMAC), which advises on research and environmental release of GMOs. OGTR will regulate all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

All GM food is assessed and regulated by the Australia New Zealand Food Authority (ANZFA) under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as Australia New Zealand Food Standards Council (ANZFSC).

There will be an interface between ANZFA and OGTR. Consequential amendments proposed to the ANZFA Act arising from the draft Gene Technology Bill 2000 will establish a statutory interface between OGTR and ANZFA. This will involve amendments to the ANZFA Act requiring the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (currently Standard A 18).

14. Maximum residue levels

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.

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 (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.