FINAL RISK ANALYSIS REPORT

APPLICATION A385

Food derived from insect-protected Bt-176 corn

Note:

This report is the "Inquiry" as referred to in Section 16 of the *Australia New Zealand Food Authority Act (1991)* and sets out the reasons for making a recommendation to the Australia New Zealand Food Standards Council under Section 18 of the Act..

TABLE OF CONTENTS

INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) received an application from Novartis Seeds Pty Ltd on 30 April 1999 for the approval of food from insect-protected corn lines containing the Bt-176 transformation event, under Standard A18 – Food Produced using Gene Technology. The modified corn is protected from attack by lepidopteran pests, particularly the European corn borer and is known commercially as Bt-176 corn.

CONCLUSIONS

ANZFA has conducted a comprehensive assessment of the application according to its *Guidelines for the safety assessment of foods to be included in Standard A18 – food produced using gene technology*. These guidelines are based on upon internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

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.

RECOMMENDATION

Based on the data submitted in the application, ANZFA concludes that food derived from insect-protected Bt-176 corn is as safe for human consumption as food from other commercial corn varieties, and therefore recommends that the Australian *Food Standards Code* (Volume 1) and the recently adopted joint *Australia New Zealand Food Standards Code* (Volume 2) be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard A18 and Standard 1.5.2 is provided in Attachment 1.

BACKGROUND TO THE APPLICATION

Bt-176 corn is protected against lepidopteran pest attack through the transfer of the *cry*1Ab gene from the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki.* The *bar* gene from *Streptomyces hygroscopicus* was also transferred to the corn and is used as a selection marker. The *bar* gene encodes the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies the broad-spectrum herbicide phosphinothricin (also known as glufosinate ammonium). Bt-176 corn is not intended to be marketed as a herbicide-resistant plant as the level of PAT expression in Bt-176 corn is insufficient to confer tolerance to commercial applications of the herbicide.

Bt-176 corn was developed for cultivation in the United States and is in decline due to it no being longer marketed by Novartis. Bt-176 corn is not currently grown in either New Zealand or Australia. Corn imported into Australia and New Zealand is likely to be in the form of a small amount of imported processed 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.

ANZFA then conducted an assessment of the application, including a safety evaluation of the food, taking into account the comments received. A draft risk analysis report was released for public comment on 29 September 2000. A total of 10 submissions were subsequently received in response to the release of this report and the report for Bt-11. A summary of the second round public comment is also provided in Attachment 5.

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 (Attachment 2)

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

The *cry*1A(b) gene is one of several isolated from the bacterium *Bacillus 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*1A(b) gene is known as Cry1A(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

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

expected in the genetically modified corn, as the gene has bacterial-specific regulatory elements.

The *cry*1A(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 Cry1A(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 Cry1A(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

Cry1A(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 Cry1A(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 were submitted by the applicant and were reviewed in this assessment. The nutritional adequacy of Bt-176 corn as chicken feed 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 Bt-176 corn

Under the current Standard A18, which remains in effect until 7 December 2001, food derived from insect-protected Bt-176 corn does not require labelling as it is regarded as substantially equivalent to food derived from non-genetically modified corn varieties.

When the amended Standard (A18 in the Australian *Food Standards Code*, 1.5.2 in the Australia New Zealand Food Standards Code) comes into effect on 7 December 2001, food products made from insect-protected Bt-176 corn will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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.

Issues raised in first round of public comment (see Attachment 5 for summary)

(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.

Issues raised in second round of public comment (see Attachment 5 for summary)

(i) Safety of the synthetic gene used to produce the Bt protein

Dr Kate Clinch-Jones stated that the Bt toxin used in the corn lines (Cry1Ab) is not identical to the conventional form and that ANZFA should not extrapolate toxicity data from the conventional form to the corn-produced version, with no confirmatory testing. Robert Anderson and FE Peters also stated that the Bt toxin is not identical to the one used in organic sprays. Susie Lees stated that because the Bt spray used in organic farming is considered safe, it does not follow that the corn-produced version is safe.

Response

The Bt toxin produced by the corn plants has been assessed as safe by consideration of a number of factors, including the history of safe use of Bt as a biopesticide. The Bt pesticide sprays may consist of a number of Bt proteins including the Cry1Ab protein. The plant produced Bt protein has an identical amino acid sequence to the one used in biopesticide formulations, except that it is shorter, i.e. the 3' end of the gene has been truncated so that only approximately the first half of the protein is translated. So even while the nucleotide sequence of the synthetic Bt gene transferred to the corn lines differs to that of the "native" gene sequence in the soil bacterium, this difference does not result in any changes to the amino acid sequence of the encoded protein.

Experiments are done to show that the plant and bacterially produced proteins are equivalent, as discussed in the safety assessment. Thus in ANZFA's assessment process, the history of

safe use of Bt is but one in several steps that support the conclusion that the Bt protein in corn is safe.

Changes to the DNA sequence of a gene between bacteria and plants are often required because these organisms have slightly different DNA sequence preferences for protein production: in the case of the two Bt corn lines, the Bt gene was originally derived from a soil bacterium and was completely re-synthesised to facilitate the production of higher levels of the Bt protein in corn cells. It has been found that many bacterial genes are poorly expressed in plant cells, meaning that they do not produce high levels of protein. The re-synthesised Bt gene expresses a Bt protein (Cry1Ab) that is identical to the first half of the protein that is produced in nature by soil bacterium.

Another important step in assessing the safety of novel proteins is an analysis of the toxicity of the protein itself. The plant-produced version of the Bt protein is shortened but is known to have the same amino acid sequence as the active part of the Bt protein, which is considered to be non-toxic. No adverse effects were found in acute toxicity studies using the Bt protein produced from the resynthesised gene.

Finally, the protein is present at very low levels in corn kernels: it represents 0.02% of total protein in Bt-11 kernels and it is negligible in Bt-176 corn kernels (i.e. it was at the limit of detection - <5ppb).

(ii) Data from acute oral toxicity studies

The Ministry of Health and Kate Clinch-Jones commented that the evidence presented in the safety assessment report did not support conclusions drawn from the acute oral toxicity studies with the Bt and PAT proteins in the two corn lines. Kate Clinch-Jones was concerned that the feeding studies were conducted using poor scientific methodology and had not been peer reviewed. Both submitters commented that the tests should be repeated given that some adverse effects had been observed. The Ministry of Health also stated that histopathological examinations should have been done on relevant tissues.

Response

ANZFA has taken an inherently cautious approach in its assessment and approval processes for genetically modified food. Each applicant has to prove to ANZFA's satisfaction that their genetically modified food product is safe for human consumption before they can be legally sold in Australia.

It is now recognised that the safety assessment process for genetically modified foods established by ANZFA is one of the most scientifically rigorous and comprehensive systems in place anywhere in the world. Wherever the application covers a gene or a commodity not previously assessed by ANZFA, the safety assessments also undergo peer review by independent external experts who are considered leaders in this field.

All data is evaluated by ANZFA's own senior scientists with expertise in this area. They follow guidelines and best-practice principles of assessing genetically modified foods developed by the FAO/WHO and OECD. These processes have also been adopted in countries such as Canada, Japan and members of the European Union. These stringent

mandatory requirements go much further than the mainly voluntary system used by the United States Food and Drug Administration.

ANZFA will not accept an application unless adequate robust scientific data is provided by the applicant that allows a comprehensive assessment of the safety of the product. For this data to be accepted as reliable, the relevant studies must have been conducted using internationally accepted protocols for research. ANZFA receives the raw data from every experiment under the strict guidelines outlined above. This enables a more rigorous analysis of experimental outcomes than the summary data of the type submitted in support of publication of a scientific article in a peer reviewed journal.

Large amounts of raw data for the acute oral toxicity studies for the native and Bt-176 produced Cry1Ab and the PAT proteins have been submitted by the applicant. This data is held on file at ANZFA and is available both for inspection as well as copying by any member of the public. From the evidence presented, ANZFA concluded that there are no human health and safety risks with the use of these foods.

In relation to the specific concerns raised about the cause of death of animals (1 control and 2 test animals) during the acute toxicity test using the Bt-176 protein extracts, the three deaths were not attributed to the test material. The reason for this conclusion is explained more fully. The death of one test animal was clearly a result of an injury caused during the dosing procedure, i.e. a punctured oesophagus that occurred when the protein extract was administered by gavage to the mouse. The deaths of the remaining animals were not considered to be related to the Bt protein because both a test and control animal died. However, it was considered possible that some other component in the leaf extracts (i.e. both GM and control corn plants) may have caused the deaths in this study. Upon gross necropsy however, no abnormalities were found in the other animals and thus additional tests were not considered necessary.

The adverse effects that were noted in some animals (piloerection, and decreased activity), were not attributed to the Bt protein because they occurred randomly across all groups, that is, they also occurred in animals that had not been dosed with any of the Bt protein. Some effects that were noted in the test group (lacrimation (crying), polyuria and ptosis) were not consistent across the animals in the group and were resolved by day 4 of the test. Since the deaths and adverse effects were not concluded to be a result of the Bt protein, no additional studies were considered necessary.

Adverse effects (ptosis, piloerection, and decreased activity) were noted only in one animal during the acute toxicity test using the PAT protein. This animal died on day 8 of the study and upon necropsy, was found to have material blocking its oesophagus proximal to the stomach preventing passage of food or water into the stomach. This animal had lost a large amount of its body weight which supports the conclusion that the blockage was the likely cause of death.

(iii) long term/chronic toxicity studies

The New Zealand Ministry of Health stated that an investigation of the combined chronic toxicity/carcinogenicity of newly expressed proteins would strengthen the safety assessment report. Robert Anderson was concerned that the foods had not been subjected to long term testing.

Response

Several types of data are required to provide a reasonable certainty that no harm will result from exposure to novel proteins such as the Cry1Ab and PAT proteins. A structured, case-by-case assessment approach is used that involves a decision tree analysis, taking account of the nature of the food, its dietary role and consequent intake and the target population. Additional tests are required if adverse effects are observed in the initial (or previous) test. This information is intended to show that the proteins behave as would be expected of ordinary dietary protein, are not structurally related to any known toxins (or allergens) and do not display any oral toxicity when ingested at very high doses.

Acute oral toxicity tests are the first stage of toxicity testing and have been designed to permit determination of toxic effects associated with a single exposure to a potential toxin. Data from this type of study are also useful in predicting potentially important toxicity endpoints, identifying potential target organs and systems and in establishing the dose regimen which might be used in chronic exposure studies. Thus these tests are the essential first step in determining the likelihood of toxicity of a particular protein and are used as one step in the process to predict whether other toxicity tests are required.

ANZFA considers that the use of acute toxicity tests, combined with other information about the protein, such as its digestibility and structural similarity to known protein toxins, should enable the identification of any potential toxicity (or allergenicity). Various expert groups[2] have considered the issue of whether there is a need for long-term toxicity testing of novel proteins. The overwhelming international consensus on the need for animal toxicity studies as part of the assessment process for the safety of genetically modified foods (Codex, FAO/WHO and OECD) is that acute toxicity testing is sufficient in most circumstances as most ingested proteins have a predictable metabolic fate. It is only where adverse effects are observed in acute toxicity tests or where the novel protein does not behave as ordinary dietary protein that more extensive analyses may be warranted.

Conventional toxicity testing procedures are generally not ideally suited to the safety evaluation of the products of biotechnology. A holistic approached that integrates nutritional and safety evaluation processes is used which enables a complete assessment of the "wholesomeness" of the food. Based on the evidence supplied, ANZFA did not consider that further toxicological studies were warranted. This is based on the data on the molecular characterisation, compositional and nutritional analyses and potential for toxicity or allergenicity that was sufficiently robust to conclude that the genetically modified food was as safe as its conventional counterpart.

(iv) The Ministry of Health stated that the safety assessment of A385 should be based on the corn kernels and not any downstream processing products.

Response

[2] OECD (2000). Report of the Task Force for the Safety of Novel Foods and Feeds, WHO (2000). Safety aspects of genetically modified foods of plant origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology.

The safety of food derived from Bt-176 corn is based on the corn kernels and additional information about downstream processing products has been considered as additional supporting information but is not essential to the assessment of the application.

The processing steps in the production of such food or food ingredients often denature or remove proteins from the final product (eg. heating, pressing, oil extraction, refining etc). The applicant has indicated that corn products from Bt-176 are likely to enter the food chain only after processing because it has been bred only into processing corn varieties. Thus, corn products derived from Bt-176 are even less likely to contain novel protein due to its likely removal or degradation upon processing.

(v) Use of proteome analysis

Kate Clinch-Jones comments that full proteome analysis could and should be done on any transgenic food

Response

ANZFA actively keeps abreast of new technologies that may be important in the assessment of genetically modified foods. Proteomics is the comprehensive analysis of proteins present in a cell, tissue and/or organism. It combines a range of molecular, biochemical and analytical techniques that separate, identify and characterise proteins. As proteomic analysis develops, there will be an increase in our understanding of protein production and interactions in a cell and in an organism. Combining this information with improvements in databasing and analytical software is likely to permit a greater understanding of biology at a biochemical and molecular level.

Techniques such as proteomics may in the future play a significant role in the safety assessment process, for example, in the determination of substantial equivalence. However, the consensus in the international community (i.e. the Joint WHO/FAO Expert Consultation on Foods Derived from Biotechnology and the OECD Task Force for the Safety of Novel Foods and Feeds), is that such techniques certainly hold a lot of promise but need further development and validation before they may be used on a routine basis for screening for unintended effects in transgenic plants.

(vi) Feeding studies

The Canberra Consumer was concerned that there were no rat feeding studies. Kate Clinch-Jones suggested that an expert team of advisors be established to design scientifically sound feeding studies that also consider the ethics of such studies.

Response

The purpose of the animal feeding studies is not to determine if there are any toxicological effects associated with consumption of the food, but rather to confirm that a food is nutritionally adequate and will support typical growth and well being. The requirement for feeding studies is assessed on a case-by-case basis. Several international organisations have convened a panel of experts (Codex, FAO/WHO and OECD) to consider the issue of the safety assessment of genetically modified foods including long term testing. ANZFA actively

participates at international forums on these issues and the contribution of ANZFA's experts on the expert consultations continues to be recognised at the international level.

The consensus of the international standard setting bodies (Codes, FAO/WHO) is that animal feeding studies using whole foods at an appropriate range of doses are technically difficult to design and may not achieve meaningful information. Whole foods are complex mixtures of substances, varying widely in both their composition and nutritional value. Due to their bulk and effect on satiety, they can usually only be fed to animals at low levels. Feeding studies using whole foods may result in changes to balanced diets, leading to a whole range of adverse effects which are not related to any specific component in the food.

(vii) Estimation of dietary intakes of novel proteins

The New Zealand Ministry of Health submitted that the dietary intakes of the novel proteins present in Bt-11 corn line should be estimated.

Response

When food substances are known to be hazardous, an estimate is made of the dietary intake to determine the likely human exposure to the hazard. If exposure is likely to be low there may be less cause for concern than if exposure is likely to be high. In Bt-11 corn, the dietary exposure estimate has been calculated for the Cry1Ab protein and was not determined for the PAT protein because it was at the limit of detection in corn kernels.

The *Bt* protein is not considered hazardous, that is, it is non-toxic to mammals, including humans. Because of the absence of any hazard, an estimate of the dietary intake of the *Bt* protein was not considered essential for the safety assessment. However, it is recognised that such information may be useful in providing reassurance to the community that exposure to a novel protein is low and/or that the novel protein is likely to be present in the diet at levels well below those found to be safe in animal toxicity studies.

Cry1Ab is expressed in Bt-11 corn kernels at levels ranging from 0.78 to 3.80 µg protein/g fresh weight. Therefore, if certain assumptions are made about market penetration of the Bt-11 corn products, it is possible to estimate the dietary intake of the *Bt* protein.

Australian and New Zealand consumption data is available for maize flour and products in which maize flour is an ingredient (corn flour, corn meal: raw, cooked with water and cooked with milk, custard powder, breakfast flakes, breakfast puffed, tortilla, taco shells, pasta). Although other corn products exist, the above corn products represent the major processed corn products available on the market and are also more likely to be present in the corn based food or food ingredients imported from the USA and Canada (eg corn flour). It should be noted that these estimates assume that all corn products consumed in Australia and New Zealand are made using Bt-11 corn and will therefore be an overestimate of the true content of Bt-11 corn. Data on the dietary intake of other processed corn products is not available (eg high fructose corn syrup).

Excluding other corn products, the average total consumption[3] of processed corn products per person is 3.48 g/day in Australia, and 3.23 g/day in New Zealand. If, however, the

[3] Calculated for all respondents

consumption figures are based only on those in the population who report consuming such corn products, then the average total consumption is 20.0 g/day and 14.1 g/day in Australia and New Zealand respectively and the 97.5th percentile consumption is 90 g/day and 68 g/day in Australia and New Zealand respectively.

For calculation of the dietary intake of the novel proteins, the highest corn product consumption figure (90 g/day) and the highest protein concentration of both corn lines (3.80 µg protein/g fresh weight in Bt-11 corn) was used. This represents a 'worst-case' estimate.

To do the calculation, assumptions about the proportion of processed corn products derived from Bt-11 and Bt-176 corn must be made. In 2000, Bt-11 and Bt-176 comprised less than 6% (4.2 and 1.4% respectively) of the total United States corn acreage (NASS, USDA 2000[4]). It is possible therefore to make two dietary intake estimates — one using a very worst case estimate where it is assumed that all corn products on the market are derived entirely from the Bt corn lines and the other, more realistic but still conservative estimate, where it is assumed that 10% of corn products are derived from Bt-11 and Bt-176 corn. The dietary intake estimates are provided in the table below:

Theoretical	**Estimated dietary intake of Cry1Ab**	
Market penetration	**µg /day**	**µg/kg BW/day[1]**
100 %	342	5.10
10 %	34.2	0.510

[1] assuming a body weight of 67 kg.

The worst-case estimate of dietary exposure is at least 0.7 million times less than the dose found to have no adverse effects in mice (3535 mg Cry1Ab/kg body weight). Therefore, even if all processed corn products were to be derived from Bt-11 and Bt-176 corn, a very large margin of safety exists.

(viii) Human health consequences of animal feeding

The New Zealand Ministry of Health stated that it would strengthen the safety assessment report if there was inclusion of the measures taken, if any, to assess the possible consequences for human health of consumption of genetically modified feed products by farm animals.

Response

Many animal feeds are derived from the same GM crops used for human food and concerns are occasionally expressed about whether such practices may pose an indirect risk to humans through the consumption of products derived from such animals, such as meat, milk and eggs.

ANZFA considers that the human health consequences, if any, of the feeding of GM foods to animals should be assessed on a case-by-case basis, taking into account any potential hazards identified for the novel proteins present in the food and changes to the composition of the

[4] Crop Production, 9 November 2000. National Agricultural Statistics Service, US Department of Agriculture.

food combined with a consideration of the animal feeding practices used for the particular food/feed in question.

Maize is widely used as a feed stuff and worldwide, approximately 72% of the grain is used as animal feed, 8% is used in food production and 20% is used in starch production. In Australia, a larger percentage of the grain produced locally directly enters the food chain (38%) and in New Zealand, about 1% of local production is used in food production.

The requirement for these studies is assessed on a case-by-case basis and in the case of Bt-11 and Bt-176 corn lines evaluated by ANZFA, such an assessment was considered unnecessary for the following reasons. No hazards (toxicity or allergenicity) were identified as associated with any of the novel proteins expressed in the Bt-11 or Bt-176 corn lines. Additionally, it is known that the Cry1Ab and PAT proteins behave as normal dietary protein in conditions that mimic mammalian digestion therefore even in cases where animals are fed concentrated protein extracts there is no reason to expect that significant residues of novel protein would remain in the animal.

Although these studies were not considered essential to the assessment of this application, Novartis submitted one study that assessed animal products derived from animals fed genetically modified stockfeed. This study was done on laying hens fed the two Bt corn lines and supports the safety of chicken products (meat and egg) derived from animals fed genetically modified feedstock. This study is presented in Section 5.3 of the Safety Assessment Report (Attachment 2).

Recently, the Federation of Animal Science Societies (FASS) released a report of a review they conducted of all the data worldwide from research studies on the feeding of GM foods/feeds to animals in which results have been published in refereed, peer-reviewed journal articles[5]. They concluded that the results published so far indicate there are no effects of feeding GM plant material to livestock and poultry on the nutritional value or safety of the meat, milk and eggs derived from those animals. Moreover, because most components of feeds are broken down into smaller components during digestion by the animal, proteins and DNA derived from the GM plants cannot be detected in milk, meat or eggs.

(viii) Comparative analyses

Kate Clinch-Jones commented that the significant differences in the nutritional analyses had been dismissed by ANZFA in an unscientific manner and had not been regarded as indicators of unexpected effects that could be toxic. She commented that the presence of significant differences in the genetically modified lines indicated that the foods derived from them could not be regarded as substantially equivalent to their conventionally produced counterparts. The Ministry of Health commented that comparisons of nutritive values to published values are of little significance for some parameters because of the large ranges in the literature.

Response

Small but significant differences were noted in the safety assessment report (Attachment 2) and were evaluated by ANZFA in terms of whether they would affect the safety and nutritive value of the food.

[5] www.fass.org/fassfact.pdf

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. If a difference is identified in comparison with the control line, it then has to be evaluated for its biological or food safety significance. Typically, this is done by comparing the data obtained for the genetically modified food to the natural range for the particular constituent measured in conventional varieties, usually be reference to data reported in the literature. If the difference exceeds natural variation then further assessment would be required (eg. nutritional, toxicological). If the difference does not exceed natural variation, then further assessment would not normally be required. This is the standard approach used to detect unintended changes (i.e. endorsed by the recent FAO/WHO Expert Consultation). It is important to note that identification of a difference does not necessarily equate to an adverse food safety outcome. Many differences are neutral with respect to food safety and are consistent with the natural variation that occurs in all food.

This part of the assessment process uses the concept of substantial equivalence to evaluate the differences that have been observed. This approach is internationally recognised and endorsed by the FAO, WHO, Codex and OECD as a valuable tool in the safety assessment of genetically modified foods. A Joint Consultation of the FAO and WHO noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment.*' Similarly the OECD advocates an approach to safety assessment of genetically modified foods incorporating substantial equivalence to its control line as being '*the most practical to address the safety of foods and food components derived through modern biotechnology*.'

4. Risk management

Under Standard A18 (referred to as Standard 1.5.2 in the Joint Australia New Zealand Food Standards Code), 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 4 of the standard. Labelling according to the original standard A18 must be in accordance with the criteria specified in clause 2 and will be permitted until 7 December 2001. After this date, labelling will be required to comply with Standard 1.5.2 of the Australia New Zealand Food Standards Code.

On the basis of the conclusions from the safety assessment report, together with a consideration of the public submissions, it is proposed that the Table 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.

A public discussion paper on the safety assessment process for GM food[6] 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.

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

5. Regulatory impact assessment

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

ATTACHMENTS

1. Draft variation to the Australian *Food Standards Code*
2. Safety assessment report
3. 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 – FOOD DERIVED FROM INSECT-PROTECTED Bt-176 CORN

To commence : On gazettal

The Food Standards Code is varied by:

(1) inserting into Column 1 of the Table to clause 2 in Standard A18 in Volume 1 –

Food derived from insect-protected Bt-176 corn.

(2) inserting into Column 1 of the Table to clause 2 in Standard 1.5.2 in Volume 2 –

Food derived from insect-protected Bt-176 corn.

ATTACHMENT 2

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.

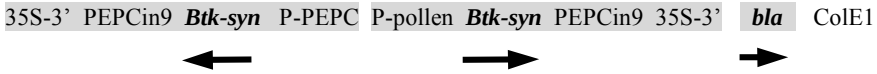
Transformed plants were selected on the basis of their ability to grow in the presence of phosphinothricin conferred by the transfer of the *bar* gene.

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 phosphinothricin 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[7]/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.

[7] 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.

Two animals that received the test material died; however one of the deaths (male on day 1) was caused by a dosing injury. One animal that received the control material died (female on day 1). Gross necropsy of all animals at the end of the study revealed abnormalities only in those animals that died: in the test animal that died on day 1, there was perforation of the oesophagus, in the test animal that died on day 2, there were abnormalities of the lungs and liver and in the control animal that died on day 1, there were lung and stomach content abnormalities.

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 globule of solid material was sufficient to prevent passage of food or water into the stomach and is the likely cause of death of this animal. 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.

An additional study was submitted that supports the safety and nutritional adequacy of food derived from animals fed genetically modified stockfeed.

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.

Feeding study of Bt-176 and Bt-11 corn in the diet of laying hens

A 14 day study was conducted by Wildlife International Ltd using methods and species based on procedures specified in the Environmental Protection Agency's Registration Guidelines,

Pesticide Assessment Guidelines, FIFRA Subdivision O, Hazard Evaluation: Pesticide-Residue Chemistry Guidelines. Single comb, white laying hens (28-week old at start of treatment) were fed diets containing 64% corn meal from Bt-176 or Bt-11 derived genetically modified corn. The study birds were from the same lot and age and were acclimated to the test facility for 11 days prior to the start of the pre-treatment phase. From this lot, 40 hens were randomly assigned to the control and treatment groups (10 per group).

One group received a diet prepared with Bt-176 grain and a second group received a diet prepared with Bt-11 grain. A third group received a diet prepared with non-genetically modified control hybrid grain (Bt-176) and a fourth group received a diet prepared with non-genetically modified control hybrid grain (Bt-11). The birds were fed the diets *ad libitum* for 14 days and evaluated for survival, body weight and general health. The number and weight of eggs produced were measured daily. Data on feed consumption, egg production and egg weight for each pen was compared to the comparable values from the pre-treatment phase (final 7 days of acclimation). Eggs from the final two days of the study were collected for analysis for the transgenic proteins. Hens from the control and treatment groups were sacrificed at the end of the 14 day exposure period and selected tissues were taken for analysis of the transgenic proteins.

There were no mortalities observed in the control or treatment groups during the course of the study. No effect was observed on survivability, health, egg production or egg weight when compared to birds fed non-modified control corn meal. There were no differences from the pre-treatment phase in the parameters measured. Additionally, the Cry1A(b) and PAT proteins were not detected in any of the five tissue types analysed (egg white, egg yolk, liver, breast and thigh).

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 and no effects on egg weight or production in laying hens. No differences were observed in tissues from laying hens fed genetically modified corn or control corn.

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.

Acknowledgements

ANZFA gratefully acknowledges Professor Ken Reed, Director, Queensland Agricultural Biotechnology Centre, Queensland Department of Primary Industries, for expert comments on this safety assessment report.

References

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

Australia New Zealand Food Authority (ANZFA) 1999a Guidelines for the safety assessment of foods to be included in Standard A18 - food produced using gene technology.

Australia New Zealand Food Authority (ANZFA). *Food Standards Code* 1999b Standard A14 – Maximum Residue Limits.

Astwood, J. 1995 *Bacillus thuringiensis* susp. *kurstaki* HD-1 insecticidal protein (*B.t.k.* HD-1 protein) shares no significant sequence similarity with proteins associated with allergy or Coeliac disease. Monsanto Company, USA 63198. MSL-14172

Benfey, P.N. and Chua, N-H. 1990 The Cauliflower Mosaic Virus 35S promoter: combinatorial regulation of transcription in plants. ***Science*** **250**: 959-966

Beriault, J.N., Horsman, G.P. and Devine, M.D. 1999 Phloem transport of D,L-glufosinate and acetyl-L-glufosinate in glufosinate-resistant and –susceptible *Brassica napus.* ***Plant Physiol*** **121**: 619-627

Botterman J., V Gossele, C Thoen and M Lauwereys. 1991. Characterisation of phosphinothricin acetyltransferase and C-terminal enzymatically active fusion proteins. Gene 102:33-37

Brake, J. and Vlachos, D. 1998 Evaluation of transgenic event 176 "Bt" corn in broiler chickens. ***Poultry Sci*** **77**: 648-653

Callis, J., Fromm, M. and Walbot, V. 1987 Introns increase gene expression in cultured maize cells. ***Genes Dev*** **1**: 1183-2000

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

Canadian Food Inspection Agency 1995 Novel Food Information – Food Biotechnology: Insect resistant corn, 176, 19 December 1995 Office of Food Biotechnology. http://www.agbios.com/decdocs/ofb-095-353-a.pdf

Canadian Food Inspection Agency 1996 Decision Document DD96-09: Determination of environmental safety of event 176 Bt corn (*Zea mays* L.) developed by Ciba Seeds and Mycogen Corporation. 16 April 1996 Plant Biotechnology Office.
http://www.cfia-acia.agr.ca/english/plaveg/pbo/dd9609e.shtml

Cao, J., Duan, X.L., McElroy, D. and Wu, R. 1992 Regeneration of herbicide resistant transgenic rice plants following microprojectile-mediated transformation of suspension culture cells. ***Plant Cell Reports*** **11**: 586-591

Cooper, D. 1991 *Bacillus thuringiensis* toxins and mode of action. *in* The Proceedings from the Workshop on *Bacillus thuringiensis.* Editors R. Milner and C. Chandler. CSIRO, Canberra.
Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. and Dean, D.H. 1998 Revision of nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. ***Microbiol Mol Biol Rev*** **62**: 807-813

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

Drummond, J. and D. Pinnock. 1991. Host spectrum of *Bacillus thuringiensis.* In *The Proceedings from the Workshop on* Bacillus thuringiensis*.* Editors R. Milner and C. Chandler. CSIRO, Canberra.

Ebert, E., Leist, K.H. and Mayer, D. 1990 Summary of safety evaluation toxicity studies on glufosinate ammonium. ***Food Chem Toxicol*** **28**: 339-349

Estruch, J.J., Kadwell, S., Merlin, E. and Crossland, L. 1994 Cloning and characterization of a maize pollen-specific calcium dependent calmodulin-independent protein kinase. ***Proc Natl Acad Sci USA*** **91**: 8837-8841

Fearing, P.L., Brown, D., Vlachos, D., Meghji, M. and Privalle, L. 1997 Quantitative analysis of Cry1A(b) expression in *Bt* maize plants, tissues, and silage and stability of expression over successive generations. ***Mol Breed*** **3**: 169-176

Frick, O.L. 1995 The potential for allergenicity in transgenic foods. *in Genetically Modified Foods: Safety Aspects*. K.-H. Engel, G.R. Takeoka and R. Teranishi. (eds) American Chemical Society, Washington DC.

Gallo-Meagher, M. and Irvine, J.E. 1996 Herbicide resistant sugarcane plants containing the *bar* gene. ***Crop Sci*** **36**: 1367-1374

Geiser, M., Scweitzer, S. and Grimm, C. 1986 The hypervariable region in the genes encoding entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the kurhd1 gene of subsp. *kustaki* HD1. ***Gene*** **48**: 109-118

Gill, S.S. 1995 Mechanism of action of *Bacillus thuringiensis* toxins. ***Mem Inst Oswaldo Cruz*** **90**: 69-74

Hack, R., Ebert, E., Ehling, G. and Leist, K.H. 1994 Glufosinate ammonium – some aspects of its mode of action in mammals. ***Food Chem Toxicol*** **32**: 461-470

Hadley, W.M., Burchiel, S.W., McDowell, T.D., Thilsted, J.P., Hibbs, C.M., Whorton, J.A., Day, P.W., Friedman, M.B. and Stoll, R.E. 1987 Five-month oral (diet) toxicity/infectivity study of *Bacillus thuringiensis* insecticides in sheep. ***Fundam Appl Toxicol*** **8**: 236-242

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

Hudspeth, R.L. and Grula, W. 1989 Structure and expression of the maize gene encoding the phosphoenolpyruvate carboxylase isozyme involved in C_4 photosynthesis. ***Plant Mol Biol*** **12**: 579-589

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

Klein, R.M., Wolf, E.D., Wu, R. and Sandford, J.C. 1992 High-velocity microprojectiles for delivering nucleic acids into living cells. ***Biotechology*** **24**: 384-386

Komari, T., Hiei, Y., Ishida, Y., Kumashiro, T. and Kubo, T. 1998 Advances in cereal gene transfer. ***Curr Opin Plant Biol*** **1**: 161-165

Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R., Warren, G.W., Wright, M. and Evola, S.V. 1993 Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. ***Bio/Technology*** **11**: 194-200

Koziel, M.G., Carozzi, N.B. and Desai, N. 1996 Optimizing expression of transgenes with an emphasis on post-transcriptional events. ***Plant Mol Biol*** **32**: 393-405

Kues, U. and Stahl, U. 1989 Replication of plasmids in gram-negative bacteria. ***Microbiol Rev*** **53**: 491-516.

Kumada, Y., Anzai, H., Takano, E., Murakami, T., Hara, O., Itoh, R., Imai, S., Satoh, A. and Nagaoka, K. 1988 The bialaphos resistance gene (*bar*) plays a role in both self-defense and bialaphos biosynthesis in *Streptomyces hygroscopicus*. ***J Antibiot (Tokyo)*** **41**: 1838-1835

Lehrer, S.B. and Reese, G. 1998. Food allergens: implications for biotechnology. In: Thomas JA (ed.) Biotechnology and safety assessment. Taylor and Francis, Philadelphia.

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

Merriman, T.N. 1996 An Acute Oral Toxicity Study in Mice with Phosphinothricin Acetyltransferase (PAT) Protein. DEKALB Genetics Corporation, 62 Maritime Drive, Mystic, CT 06355-1958. DEKALB Study No. DGC-95-A18.

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

Noteborn H.P., Bienenmann-Ploum M.E., van den Berg J.H., Alink G.M., Zolla L., Reynaerts A., Pensa M. and Kuiper H.A. 1995. Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein Cry1A(b) expressed in transgenic tomatoes. In: Genetically modified foods. American Chemical Society Symposium Series 605. Engal K-H, Takeoka GR and Teranishi R (eds) American Chemical Society, Washington, DC.

Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L., Fischhoff, D.A. 1991 Modification of the coding sequence enhances plant expression of insect control protein genes. ***Proc Natl Acad Sci USA* 88**: 3324-3328

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

Rogers, J. 1990 What food is that? and how healthy is it? Weldon Publishing, Sydney p 326-327

Sanders, P.R., Lee, T.C., Groth, M.E., Astwood, J.D. and Fuchs, R.L. 1998 Safety assessment of insect-protected corn. *in* Thomas, J.A. (ed.) Biotechnology and safety assessment. Taylor and Francis, Philadelphia.

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

Taylor S.L. and S.B. Lehrer. 1996. Principles and characteristics of food allergens. ***Crit Rev Food Sci Nutr* 36** Suppl: S91-S118.

Thompson, C.K., Rao Movva, N., Tizard, R., Crameri, R., Davies, J.E., Lauwereys, M. and Botterman, J. 1987 Characterization of the herbicide resistance gene *bar* from *Streptomyces hygroscopicus.* ***EMBO J* 6**: 2519-2623

United States Department of Agriculture, Animal and Plant Health Inspection Service. 1995 USDA/APHIS Petition 94-319-01 for determination of nonregulated status for Event 176 corn. Environmental assessment and finding of no significant impact. http://www.agbios.com/decdocs/9431901p.htm

United States Environmental Protection Agency 1997 Phosphinothricin Acetyltransferase and the genetic material necessary for its production in all plants; exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register Volume 62 Number 70 pp17717-17720. http://www.epa.gov/fedrgstr/EPA-PEST/1997/April/Day-11/p9373.htm

United States Environmental Protection Agency 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision O, Hazard Evaluation: Pesticide-Residue Chemistry Guidelines,* subsection 171-4, Environmental Protection Agency, Office of Pesticide Programs. Washington , D.C.

United States Food and Drug Administration 1999 Foods derived from new plant varieties derived through recombinant DNA technology: final consultations under FDA's 1992 policy. Office of Premarket Approval, Center for Food Safety & Applied Nutrition, US FDA http://vm.cgscan.fda.gov/~lrd/biocon.html

Watanabe, T. and Sano, T. 1998 Neurological effects of glufosinate poisoning with a brief review. ***Hum Exp Toxicol* 17**: 35-39

Watson S.A. 1987. Structure and composition. *in* Corn: Chemistry and Technology. ed. Watson, S.A. and Ramstead, P.E. American Association of Cereal Chemists Inc, St Paul, Minnesota.

Weber, E.J. 1987 Lipids of the kernel. *in* Corn: Chemistry and Technology. ed. Watson, S.A. and Ramstead, P.E. American Association of Cereal Chemists Inc, St Paul, Minnesota. pp 311-349

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

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

Wohlleben, W., Broer, I., Hillemann, D., Strauch, E. and Puhler, A. 1988 Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from *Strepromyces viridochromogenes* Tu494 and its expression in *Nicotiana tabacum*. ***Gene*** **70**: 25-37

Wright, K.N. 1987 Nutritional properties and feeding value of corn and its by-products. *in* Corn: Chemistry and Technology. ed. Watson, S.A. and Ramstead, P.E. American Association of Cereal Chemists Inc, St Paul, Minnesota. pp 447-478

ATTACHMENT 3

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[8]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[8] 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

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

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

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

4. **Australian GeneEthics Network**
   - Believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
     - Direct health effects of pesticide residues
     - Possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
     - The possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
     - Insertion of viral DNA could create new and virulent viruses
     - The possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
     - The threat to GE-free status export markets

- Believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

5. Public and Environmental Health Service (Australia)

- Believes that the data provided should cover both the intentional and unintentional effects of the genetic modification. The unintended consequences of random insertion of new genetic material into the host genome could include loss or change of function of gene or controlling element, disregulation or amended regulation of the gene or controlling element, or production of a novel hybrid protein which could occur in an unregulated manner. They should also cover any compositional changes e.g. nutrients, antinutritional factors, natural toxicants, and define when a change would be considered 'significant'
- Potential effect of introduced proteins on metabolic pathways should be addressed e.g. over-expression or inhibition of enzymes
- Data should include details of whether introduced proteins are detectable in whole commodities, processed products and highly processed derivatives
- Data should include details of toxicity and allergenicity tests to prove that food is safe, as well as address issues of specificity and potency of proteins. It should also address the ability to support typical growth and well-being
- Data for herbicide-tolerant plants should be derived from studies performed on plants treated with herbicide. They should address the human toxicity of the herbicide and whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.

6. David Grundy (Australia)

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

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

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

8. Australian Food and Grocery Council

- Fully endorses the policy of minimum affective regulation, supports these applications, and considers that food manufacturers should make their own choice with regard to use of GM crops or products derived from them

- Believes that since the growth of GM crops has been approved overseas, they would support their growth in Australia if approved through the GTAC/GMAC/OGTR process
- Considers it unfortunate that ANZFA has not negotiated "equivalence" agreements for products already approved overseas to enable approval without having to carry out its own safety assessment. In the absence of such an agreement it supports the ANZFA safety assessment process.
- Believes that an appropriate information and labelling scheme would enable consumers to make an informed choice.

9. New Zealand Ministry of Health

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

10. 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.

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

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

A379, A388

- State concern over the persistence and toxicity of bromoxynil, and consider that these have not been adequately assessed by the US FDA. They understand that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil

itself, and believe that a safety assessment needs to be done on this too. This is apparently the main residue, and they believe that this may appear in cotton oil and linters.

A372, A375, A380, A381, A386

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

A380, A382, A383, A384, A385, A386

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

A387

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

12. Health Department of Western Australia

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

13. Meat New Zealand

A379

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

14. BRI Australia

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

15. Food Technology Association of Victoria Inc.

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

16. Diane Davie (Australia)

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

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

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

18. Richard and Sharon Moreham (see also above)

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

19. Vicky Solah (Australia)

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

20. Dr Rosemary Keighley (Australia)

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

21. Nicola Roil (Australia)

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

22. Ian and Fran Fergusson (Australia)

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

23. Lyndal Vincent (Australia)

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

24. Fay Andary (Australia)

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

25. John and Francesca Irving (Australia)

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

26. Diana Killen (Australia)

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

27. Sheila Annesley (Australia)

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

28. David and Edwina Ross (Australia)

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

29. Beth Schurr (Australia)

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

30. Beth Eager (Australia)

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

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

- Believe that safety has not been, and cannot be satisfactorily determined, and that any party associated with GM foods could be legally liable should adverse health effects be seen. Thalidomide, smoking, 'Agent Orange' and asbestos all show that such things can affect subsequent generations

- Believe that an increase in use of pesticides will result from pesticide-tolerant crops, and that the emphasis should be on organic and/or safe agriculture
- Believe that GM-food is a retrograde step, contrary to nature and has the potential to destroy the human race.

32. Chitta Mylvaganum (Australia)

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

33. John Stevens (Australia)

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

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

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

35. Jan Kingsbury (Australia)

- Believes that GM-foods could result in loss of economic advantage for Australia and New Zealand since they are known internationally for pure and safe products
- Believes that foods are being complicated and pushed by big internationals, and organic farmers are being contaminated by cross-pollination.

36. Teresa Sackett (Australia)

- Believes that:
  - The KPMG report on labelling was prepared in a ridiculously short time and provided limited analysis
  - The proposal of ‘no label’ for foods which ‘may contain’ or in which there is ‘no evidence’ of GM material is inadequate
  - Inadequate testing procedures should not be used to declare a product is GM-free just because material can’t be detected. In fact testing methods have been developed that can be used to work out the GM content
  - Government and industry seem to be favouring the introduction of GM foods. This will result in the increased use of chemicals and the destruction of soil life
  - Organic farming pay high costs for producing healthy plants, while conventional farmers have little restriction on pollution of air, soil and water.

Salinity problems, the death of the Great Barrier Reef, rivers and streams has resulted from ignorance in farming and broader community. Such problems will increase with GM foods.
  - The implication that the public will not understand the issues is wrong. Everyone needs to be fully informed.
- Asks the question of whether workers in the food industry are to be better informed, and also why no 'verification documents' are to be required by retailers? Believes that certification schemes should be on a par with those for Kosher foods and organics.

37. John and Sandy Price (Australia)

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

38. John Scott (New Zealand)

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

39. R A Randell (New Zealand)

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

40. National Council of Women of New Zealand

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

41. Safe Food Campaign (New Zealand)

- Believes that approval should be rejected, and a moratorium be put in place until after the Royal Commission of Inquiry, for various reasons:
  - Possible effects on non-target insects
  - Spread of GM pollen may cause contamination of non-GM (especially organic) crops, and may result in the spread of herbicide-tolerance genes and an increase in resistance development. Cross-pollination is considered a particular risk for canola (A372 & A388). Bt resistance development is noted as being a particular risk for A382, A383 & A384
  - Lack of long-term testing means health risks are not known

- Use of broad-spectrum pesticides affects wild flowers and non-target insects.

42. Jocelyn Logan, Caroline Phillips (New Zealand)

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

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

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

44. Stephen Blackheath (New Zealand)

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

45. Claire Bleakley (New Zealand)

- Believes that approval should be rejected for various reasons:
  - They may be against Maori views
  - Further long-term trials are needed and should be carried out by ANZFA themselves - certain trials have apparently shown effects on immune system, allergies and rare syndromes
  - Health concerns of pesticide overuse

- The possibility of horizontal gene transfer with respect to antibiotic resistance transfer
- Lack of labelling and the use of the unsatisfactory 'substantial equivalence' concept, which makes hazard difficult to assess
- There is no substantial gain to consumers

SUMMARY OF SECOND ROUND PUBLIC SUBMISSIONS

The draft Risk Analysis Reports (formally referred to as the Full Assessment Report) for A385 and A386 were released for a 6 week period of public comment on 4 October 2000. At the end of the public comment period (15 November 2000) a total of 10 submissions had been received. They are summarized below.

1. Robert Anderson (Physicians and Scientists for Responsible Genetics)

- Supports the article by Joe Cummins that the genetically modified foods have been inadequately tested and that Bt toxin may have adverse effects (attached letter: *Bacillus thuringiensis* and its toxins as biopesticides).
- Considers that the applications should be refused on scientific grounds because the Bt toxin arising from the modified Bt gene is not identical to the Bt toxin used in organic sprays which are regarded as safe. He is concerned that
  - The Bt corn toxin does not have a history of safe use in human food supply.
  - The modified Bt toxin may have altered properties due to the truncation of sequences before and after the gene.
  - The Bt toxin produced in corn is more powerful than the natural toxin and therefore riskier than the natural toxin.
  - The concentration of secondary plant chemicals in the total plant might change causing increased levels of toxic chemicals that would normally be present at low levels
  -
- Is concerned that dried Bt spores are harmful to the human immune system due to the presence of toxins other than the Bt toxin.
- Is concerned that pleiotropic effects could create unexpected proteins, toxins or allergens within the plant.
- Considers that the foods are not safe because the genetically modified foods have not been subjected to long term testing.
- Is concerned that the antibiotic resistance marker gene could transfer to bacteria and generate antibiotic resistance in bacteria.

2. Australian Fruit and Grocery Council (AFGC)

- Supports the approval of the two corn applications: A385 insect protected corn Bt-176 and A386- insect protected, herbicide tolerant corn line Bt-11.
- Submits that as ANZFA has concluded that food derived from the two Bt corn applications do not raise any public health and safety concerns, that there should be no reason for retaining the generic prohibition on their use merely because they are GMOs.
- Commented that they support the application of the more extensive labelling requirements of Standard A18 to the GM corn lines and their products.

3. Kate Clinch-Jones

- Comments that ANZFA states they address the majority of submissions in the body of the report, but it does not refute these claims with any scientific evidence.
- No scientific references are provided to support ANZFA's surmise that horizontal gene transfer is unlikely to occur. She states that horizontal gene transfer is very real and is a potential hazard that is ignored by ANZFA.
- Cites a number of scientific articles as evidence that ingested viral DNA survives digestion and can be incorporated into the cells of hosts, including their foetuses and that transgenic DNA can transfer into soil bacteria and fungi. She also refers to unpublished work showing that transgenic DNA from pollen ends up in the bacteria in the gut of bees.
- Cites evidence that the cauliflower mosaic virus 35S promoter has a recombination hot spot and is able to function in a number of different organisms such as yeast, *E. coli*, algae, higher plants and humans. She refers to concerns expressed by Professor Ho that this can cause inappropriate gene expression and may lead to cancer. Urges that until such time as Professor Ho's hypothesis has been scientifically invalidated extreme caution is needed with GM foods containing 35S or related promoters.
- Comments that the Bt toxin used is not identical to the conventional form and has been shown to accumulate in soil and is not biodegradable. Submits that despite this knowledge, ANZFA has continued to extrapolate toxicity data from the conventional form, with no confirmatory testing.
- Is concerned that the use of a gene for resistance to the antibiotic ampicillin (beta lactamase – *bla* gene in Application A385) is considered acceptable. Because this penicillin based antibiotic are commonly used in human and veterinary care, she recommends ANZFA seek advice from microbiology and infectious disease specialists.
- In relation to allergy testing submits that ANZFA's approach of comparing the structure of novel proteins to a list of known allergens is inadequate to exclude unexpected allergens and cannot substitute for proper in vivo testing.
- Comments that full proteome analysis could and should be done on any transgenic food.
- Comments that no studies submitted by the applicant have been published and therefore have not been peer reviewed and therefore submits that these studies are therefore not scientifically credible.
- Expresses concern about the animal feeding studies and submits that they were conducted using very poor scientific methodology and would not stand up to peer review.
- Comments that there were adverse in the acute toxicity of the Bt protein (both native and Bt-176 Cry1Ab proteins) i.e. weight loss in some individual mice. She comments that no reasons were given for the death of mice in test or control groups. She raises the possibility that all mice may have inadvertently been given the test substance.
- Comments that in the acute oral toxicity of the PAT protein, the mouse that died due to an obstruction in the oesophagus may not be related to the treatment procedure given that the mouse died 8 days later and that the tests need to be repeated.
- Comments that toxicity testing on the Cry1Ab and PAT proteins should be repeated with larger numbers of animals and that given some animals died, that the foods should not be regarded as safe.
- In the nutritional analyses, several significant differences were noted (protein, fatty acid, and moisture content) and were dismissed in an unscientific manner. She says

that they have not been regarded as indicators of unexpected effects that could be toxic. Even if the differences are not likely to be allergenic or toxic, the varieties are substantially equivalent to their traditional counterparts.

- States that if the reason for the differences in carotenoids is a difference in storage time of the grain, then the experiment had flimsy scientific design.
- States that the evidence supporting the claims made in the regulatory impact assessment on the economic, industry and consumer benefits should be provided.
- Suggests that an expert team of advisors be established to design scientifically sound feeding studies that also consider the ethics of such studies.
- Would be interested in receiving substantiating documentation on all the points she has raised and that until such time as the evidence is made freely available it is impossible to conclude that the corn, or any other GM food, is fit for human consumption.
- Submits that she rejects ANZFA's risk analysis and the foods on the basis that there are far too many potential hazards from transgenic foods.

4. IP Hancox

- Concerned that once GM crops are grown and in the food supply, it will be difficult to turn the clock back if they are found to have any adverse effect.
- Is against genetically modifying foods.

5. Susie Lees

- The main issue of concern is that ANZFA should not rely on US FDA approval process as some individuals in US regulatory agencies may have been formerly employed in biotechnology companies.
- Submits that just because the organic Bt toxin used as a biopesticide is regarded as safe that does not follow that the corn produced Bt toxin is safe.
- All genetically modified food products should be labelled so that consumers have the choice.

6. National Genetic Awareness Alliance

- There has been no independent scientific research conducted by ANZFA in their risk assessment process, unlike irradiated food which has included multigenerational animal studies and studies using volunteers who ate only irradiated food.
- Comment that peanut allergies have increased dramatically and it is generally understood but rarely publicised that such allergies may be due to residual proteins in peanut oil in infant formula. Despite earlier assurances that oils cannot sensitise because they are protein free, peanut oil is now banned from infant formula.
- In terms of the new GM labelling laws, what guarantee can there be that refined oils do not contain any residual DNA or protein? Will ANZFA set in place sophisticated independent testing to ensure that claims for GM crop derived oils to be totally free of protein or DNA are truthful? Will all such oils be labelled?
- Enclosed a number of documents discussing the hazards associated with the use of the CaMV promoter, a document on the potential problems associated with "Golden Rice", and a copy of an open letter from world scientists to all governments concerning GMOs which was submitted to the State of the World Forum in September 2000.

7. Eva Naylor

- The main issue of concern is that the safety assessments are flawed and that genetically modified food is unsafe.
- Submits that scientists' warnings have been ignored.

8. New Zealand Ministry of Health

- The Ministry of Health submitted that they agree with the conclusions reached in the assessments, i.e. that the foods are safe for human consumption.
- They raised the following comments that they believe would enhance the safety assessments:
  - the safety assessment of A385 should be based on the corn kernels and not any downstream processing products (although processed products are more likely to be prevalent at this time;
  - a No Observable Adverse Effect level in A385 should have been calculated based on the observed piloerection in the mouse studies;
  - further experimentation should be done in A385 to resolve the equivocal result of a mouse death in both the control and test animal groups in the acute oral toxicity studies, which was dismissed as not being associated with the Bt toxin.
  - further experimentation in A385 should also have been conducted to address the unacceptable number of experimental adverse effects explained by mis-dosing.
  - analysis of appropriate vitamins (particularly vitamin E), sugars and minerals in A385 would improve the comparative analysis.
  - the stability of the Cry1Ab protein in simulated intestinal fluids should have been calculated for A385.
  - the dietary intake estimate for Cry1Ab protein should be calculated for A386
  - ANZFA should have standard application formats, data requirements and analytical methodologies to facilitate comparisons across applications.
  - state that comparisons to literature values in the comparative analyses are of little value because the literature ranges quoted are quire large, allowing large differences between GM and parent line to be accommodated within the literature range.
  - chronic toxicity studies on the Cry1Ab and PAT proteins should have been included in order to rule out the possibility of a chronic mode of action.
  - histopathological examinations should be done as part of the toxicity studies.
  - ANZFA should give consideration to whether toxicity studies of the whole corn would provide more meaningful information that studies of only the purified proteins.

9. FE Peters (Canberra Consumer)

- Is concerned that ANZFA has not taken into account the possible pleiotropic effects – ANZFA has looked at the potential toxicity and dietary intake of novel proteins and not the possible overall pleiotropic changes that may occur.
- Believes there is a difference between the use of Bt as a biopesticide and the Bt toxin that is produced by the plants.
- Is concerned that there are no rat feeding studies
- Is concerned about the use of *bla* antibiotic resistance marker gene.
- Believes that the precautionary principle should be used.

10. S.P.C. Limited (Gillian Lawless and David Sutton)

- The main issue of concern for S.P.C. is that any genetically modified food entering the food supply incurs a significant cost for S.P.C. Ltd because it costs them to claim and substantiate GM free status of their products. This has other impacts that effect the costs to S.P.C. limited that then effects shareholders, employees and suppliers
- Is concerned that there has not been sufficient studies on the long term effect on flora and fauna.
- Is concerned about the escape of the novel genes to other crops and potential weediness.

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 and asserted that food produced using this technology is unsafe for human consumption. A number of general issues were raised in these submissions that are addressed 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 laboratory animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

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

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

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

3. *Substantial equivalence*

A number of submitters expressed concern regarding the use of the concept of substantial equivalence as part of the assessment process. 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 between the new food and traditionally-produced food. This can include physical characteristics and compositional factors, as well as an examination of the levels of naturally occurring allergens, toxins and anti-nutrients.

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food.

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

The concept of substantial equivalence was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*.'

The concept has been internationally recognised and embraced as a valuable tool in the safety assessment of foods produced using gene technology. 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 analytical data on any intentional or unintentional compositional changes to the food. This assessment encompasses the major constituents of the food (fat, protein, carbohydrate, fibre, ash and moisture) as well as the key nutrients (amino acids, vitamins, fatty acids). There is no evidence to suggest that genetic modification *per se* reduces the nutritional value of food.

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

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

- *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 comprehensive labelling of 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*

As early as August 1999, the Health Ministers comprising ANZFSC decided in-principle to require labelling of all genetically modified foods. However, due to the complexity of this issue, it was agreed that there was a need for a whole of government approach requiring input from all sectors of the community. To achieve this, the respective Cabinets of the Commonwealth, States, Territories and New Zealand established a Task Force to review the requirements for genetically modified food labelling.

On 28 July 2000, the ANZFSC met again to consider the outcomes of reports from the Task Force and other consultants, and agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard will be incorporated in to the new Joint Australia New Zealand Food Standards Code. To allow adequate time for compliance to the new provisions of the Standard, it will come into effect on 7 December 2001, twelve months after the date of gazettal. Guidelines, to assist with compliance with the amended labelling provisions of the Standard, were released for public consultation on 7 December in conjunction with gazettal of the Standard. The period for public comment closes on 26 February 2001.

The new Standard will require the labelling of food and food ingredients where novel DNA and/or protein is present in the final food and where the food has altered characteristics.

Exempt from these requirements are:

- highly refined food, where the effect of the refining process is to remove novel genetic material and/or protein;
- processing aids and food additives, except where novel genetic material and/or protein is present in the final food;
- flavours which are present in a concentration less than or equal to 0.1 per cent in the final food; and
- food prepared at point of sale (e.g. restaurants, takeaway food outlets).

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

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 has prepared a public discussion paper on the safety assessment process for GM foods[9], available at no charge on request. Since completion, this document has been widely distributed 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*

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

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. In relation to genetically modified crops actually cultivated in Australia or New Zealand, ANZFA would not recommend the approval of a food derived from such a crop unless the appropriate clearance for general release from either GMAC or ERMA had been obtained, following environmental assessment.

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

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

In addition, the Office of the Gene Technology Regulator (OGTR) has been established to 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 will continue to be 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). However, 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 A18).

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

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

14. Maximum residue levels of agriculture/veterinary chemicals

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

- *Response*

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

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