DRAFT FINAL RISK ANALYSIS REPORT

APPLICATION A386

Food derived from insect-protected, herbicide-tolerant Bt-11 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

ANZFA received an application from Syngenta Seeds (formerly Novartis Seeds Pty Ltd) on 30 April 1999 for the approval of food from insect-protected, herbicide-tolerant corn lines containing the Bt-11 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 tolerant to applications of the herbicide glufosinate ammonium. The genetically modified corn is known commercially as Bt-11 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 insect-protected, herbicide-tolerant Bt-11 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, herbicide-tolerant Bt-11 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, herbicide-tolerant Bt-11 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-11 corn is protected against lepidopteran pest attack through the transfer of the *cry*1Ab gene from the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki.* It is also tolerant to applications of the herbicide glufosinate ammonium through the transfer of the *pat* gene from *Streptomyces viridochromogenes*. This gene, commonly used as a selection marker, codes for the enzyme phosphinothricin acetyl transferase (PAT), and enables plants to detoxify the broad-spectrum herbicide phosphinothricin. Tolerance to commercial levels of the herbicide is conferred to Bt-11 corn, enabling the crop to survive under conditions that would kill conventional corn. The modified corn is known commercially as Bt-11 corn and was developed for cultivation in the United States. The applicants have indicated that they will apply for approval for general release of Bt-11 corn in Australia.

Bt-11 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. In addition to these processed products, sweet corn varieties are also grown for human consumption. According to the applicant, grain harvested from Bt-11 field corn will enter the food chain only after processing. However, Bt-11 corn has also been bred with sweet corn varieties, and it is possible that these hybrid varieties may be consumed as fresh produce, as well as canned, frozen or dehydrated in powder form.

The main benefits of Bt-11 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. 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 Technical 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 recent changes to Standard A18 are considered to raise potential Technical Barrier to Trade or Sanitary/Phyto-sanitary 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-11 corn was generated by the transfer of two new genes, a truncated *cry*1A(b) gene (referred to as the *Btk* gene) and the *pat* gene. Both genes were derived from bacteria and were modified at the DNA sequence level to increase their 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 proteins. The corn transformation was carried out using a protoplast transformation/regeneration system.

The *cry*1A(b) gene is one of several isolated from the bacterium *B. thuringiensis*, which encode a group of toxins known as the *Bt* toxins. These toxins are selectively active against several groups of insects such as moths and butterflies, beetles, and flies and mosquitos. The *Bt* toxin produced by the *cry*1A(b) gene is known as Cry1A(b) and is selectively active against lepidopteran insects. The protein becomes active against the target insect through ingestion. In the insect gut, the protein binds to specific receptors on the insect midgut, inserts into the cell membrane and forms ion–specific pores. These events disrupt the digestive processes and cause the death of the insect.

The *pat* gene is derived from the microorganism *Streptomyces viridochromogenes* strain Tu494, and codes for the enzyme phosphinothricin acetyl transferase (PAT). This modifies and inactivates the herbicide glufosinate ammonium, and its presence thus confers tolerance to the plant. As discussed earlier, the gene was originally used only as a selection marker to distinguish genetically modified plant cells from unmodified cells. However, since the enzyme is expressed at a level high enough to confer tolerance to the plant, it has the added benefit that the herbicide can be used in the field.

In addition to the two genes transferred to the final plant, an antibiotic resistance gene, the *bla* gene was used as a selection marker when the plasmid was being generated in *E. coli*. However,

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

the gene was removed from the plasmid prior to transformation of the final plant, and is thus not present in Bt-11 Corn.

Both the *cry*1A(b) and the *pat* gene were found to be stably integrated as single copies, and 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 expression level of the protein was generally low, and varied in different plant parts. The level of expression was fairly low in the kernel, the part used for human consumption, with a maximum level of 3.17 μg/g fresh weight. Once processed (canned) the kernels were found to contain no detectable Bt-protein.

Phosphinothricin acetyl transferase (PAT) is specific for the herbicide phosphinothricin (as well as the natural substrate produced by *S. viridochromogenes*), neither of which are found in the human body. Although the level of expression of the enzyme in Bt-11 corn is sufficiently high for the corn to be regarded as herbicide-tolerant, these high levels were only found in the tassels and leaves. No enzyme was detectable in the kernels, pollen, silk, stalk or root.

Although the plasmids used in the transformation process of Bt-11 corn contain the antibiotic resistance gene *bla*, the gene was not transferred to the modified plant. The impact on human health from its potential transfer to gut micro-organisms was therefore not considered. The transfer of novel genetic material from Bt-11 corn to human cells via the digestive tract was assessed, but was considered to be extremely unlikely to occur, and unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Toxicological issues

The presence of naturally-occurring toxins and allergens in Bt-11 corn was investigated, as well as the potential toxicity and allergenicity of the *cry*1A(b) and PAT proteins.

Corn contains no naturally-occurring toxins or allergens, and as noted above 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. 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, herbicide-tolerant corn line Bt-11, 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 homology with known allergens, history of use and common physicochemical properties of allergens, including the sensitivity to digestion by digestive enzymes. As already discussed, Cry1A(b) has a long history of safe use, and shares no characteristics or 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 too was found to be rapidly digested in conditions that mimic human digestion. In addition, it is present at very low levels, if at all, in corn kernels, and shows no amino acid similarity to known allergens.

Nutritional issues

Detailed compositional analyses were carried out to establish the nutritional adequacy of Bt-11 corn, and to look for any unintended effects by comparing it to non-modified control lines. The composition of maize and sweet corn lines were assessed, including fresh and canned sweet corn lines. The effect of glufosinate ammonium use on the composition of corn kernels was also examined. Samples were taken from trials in both Europe and the USA. Composition in terms of key chemical components (total protein, oil, starch and fibre), including fatty acids, amino acids, vitamins and minerals was investigated.

Results revealed few significant differences between Bt-11 corn and control samples, confirming that insect-protected, herbicide-tolerant Bt-11 corn is compositionally equivalent to other commercial corn lines. Although small but significant differences were seen in protein content and the levels of two amino acids (cysteine and arginine) of some Bt-11 lines, these effects were not consistent between field trial sites or hybrid lines, and are likely to reflect natural variation rather than any effect of the modification. Levels of vitamins and minerals, chemical composition, and fatty acid content were all unaffected by the modification, in both canned and fresh produce. Glufosinate ammonium treated samples were also examined and there were no significant differences between genetically modified and control lines, except for the levels of the amino acids proline and alanine, which were lower in treated Bt-11 corn than in the control lines. These differences are not considered to raise safety or nutritional concerns and are considered likely to reflect normal variation in corn hybrids.

Corn does not contain natural toxins or anti-nutrients at a level that is considered biologically significant.

Animal feeding studies were not considered essential in this case because sufficient information had been provided about the genetic modification and the composition of the food. However, studies were submitted and no adverse effects were found in chickens or their products that consumed genetically modified corn. It can be concluded from the data provided that Bt-11 corn is nutritionally adequate.

Conclusion

On the basis of the data submitted in the present application, insect-protected, herbicide-tolerant Bt-11 corn is equivalent to other commercially available corn in terms of its safety and nutritional adequacy.

2. Labelling of food produced from insect-protected Bt-11 corn

Under the current Standard A18, which remains in effect until 7 December 2001, food derived from insect-protected Bt-11 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-11 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.

(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

(ii) Toxicity of glufosinate ammonium breakdown products

The South Australia Public and Environmental Health Service raised the point that the ANZFA safety assessment should address the issue of whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL. The Consumers' Association of South Australia Inc. & National Council of Women of Australia raised similar concerns.

Response

There is currently no MRL for either glufosinate ammonium or its metabolites in corn in Australia. Similarly, in New Zealand no MRL exists, although a level of 0.1 ppm is allowed under default clause 6b of the regulation 257 (2A). A Codex MRL of 0.1 ppm also exists. There is no evidence to suggest that the metabolites MPP and MPA are any more toxic than glufosinate, and sub-chronic and developmental studies in the US concluded that they were of similar or lower toxicity compared to the parent compound.[2] The chemical is permitted for use on Bt-11 corn in the USA and the US regulatory assessment concluded that a single tolerance limit of 0.2 ppm was suitable for field corn. The consumption of food produced from Bt-11 corn is therefore not considered to pose a risk to human health.

[2] US Federal Register, Volume 65 (98), May 19 2000.

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 in her submission 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 commented 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[3] 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

[3] 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.

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) 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.

(v) 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.

(vi) 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[4] of processed corn products per person is 3.48 g/day in Australia, and 3.23 g/day in New Zealand. If, however, the consumption figures are based only on those in the population who report consuming such corn products, then

[4] Calculated for all respondents

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 Cry1Ab protein concentrations (3.80 µg protein/g fresh weight) 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[5]). 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 two 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 corn and Bt-176. 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.

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

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

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, Syngenta Seeds submitted one study that assessed the animal products produced from animals fed genetically modified stockfeed. This study on laying hens fed the two Bt corn lines 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[6]. 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.

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

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

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 Table 1 to clause 2 of Standard A18 be amended to include food from insect-protected, herbicide-tolerant Bt-11 corn. The proposed amendment is provided in Attachment 1.

A public discussion paper on the safety assessment process for GM food[7] 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.

5. Regulatory impact assessment

The benefits and costs associated with the proposed amendment to Standard A18 to include food from insect-protected, herbicide-tolerant Bt-11 corn have been analysed in a draft Regulatory Impact Statement (Attachment 3). The benefits of the revised Standard A18 amendment,

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

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. Draft safety assessment report
3. Draft regulatory impact assessment
4. World Trade Organization Agreements
5. Summary of public comments
6. General issues raised in public comments

ATTACHMENT 1

DRAFT VARIATION TO THE FOOD STANDARDS CODE

A386 – FOOD DERIVED FROM INSECT-PROTECTED, GLUFOSINATE AMMONIUM TOLERANT Bt-11 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, glufosinate ammonium-tolerant Bt-11 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, glufosinate ammonium-tolerant Bt-11 corn.

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

A386 – FOOD DERIVED FROM INSECT-PROTECTED, HERBICIDE-TOLERANT CORN Bt-11

SUMMARY AND CONCLUSIONS

Nature of the genetic modification

A proprietary inbred corn line, H8540, was transformed with two genes — the *pat* and *cry*1A(b) genes to generate Bt-11 corn. Bt-11 corn contains a single copy of each gene at one chromosomal location in the corn genome. No other genes were transferred.

The *cry*1A(b) gene is one of several genes from the bacteria *Bacillus thuringiensis*, which encode toxins collectively known as the *Bt* toxins. These toxins are selectively active against 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 the Cry1A(b) protein and is selectively active against lepidopteran insects. This gene has been transferred to corn to protect it specifically against the European Corn Borer.

The *pat* gene is derived from the bacteria *Streptomyces viridochromogenes* and encodes for the enzyme phosphinothricin acetyl transferase (PAT), which enables plants to detoxify the broad-spectrum herbicide phosphinothricin (which is the active moiety of glufosinate ammonium). This protein enables the selection of genetically modified plant cells from unmodified cells and also confers herbicide tolerance to the genetically modified corn line.

The transformed corn was shown to be phenotypically and genotypically stable by segregation and mapping studies over 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 an enormous number and diverse range of foods, and have a long history of safe use. Products derived from Bt-11 corn may include highly processed corn products such as flour, breakfast cereals, high fructose corn syrup and other starch products as well as products derived from fresh sweet corn varieties (frozen, canned and powdered products).

The transformed corn produces two new proteins: Cry1A(b) and phosphinothricin acetyltransferase (PAT). The expression of both proteins in the corn kernels is low – Cry1A(b) was expressed to a maximum level of 3.17 μg/g fresh weight of sweet corn and a maximum of 1.6 μg/g fresh weight of maize varieties and the PAT protein was below the limit of detection in all lines tested. Cry1A(b) was below the level of detection in canned sweet corn.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells in the human digestive tract. This concern primarily refers to the presence of antibiotic resistance genes in genetically modified foods. Bt-11 corn does not contain any antibiotic resistance genes and therefore it was not necessary to address this issue in this assessment. Transfer of the *cry*1A(b) and *pat* genes from Bt-11 corn to human cells via the digestive tract was considered to be unlikely. As the amount of novel genetic material in Bt-11 corn is minute compared to the total amount of DNA present, it is unlikely to pose any additional risks compared with the large amount of DNA naturally present in all foods.

Toxicological issues

Corn does not have any naturally-occurring toxins or allergens and has a long history of safe use.

The Cry1A(b) and PAT proteins are present at low levels in kernels of Bt-11 corn lines tested. The potential toxicity and allergenicity of the Cry1A(b) and PAT proteins were investigated.

In acute toxicity studies of the Cry1A(b) and PAT proteins in mice, there were no signs of toxicity at a dose of approximately 3.5 g/kg and 2.6 g/kg bodyweight respectively. The newly expressed proteins were readily degradable in simulated gastric conditions and neither protein has similarity with known toxins or allergens. The Cry1A(b) protein is present in low levels in kernels of both maize and sweet corn varieties, and could not be detected after processing (canning) of sweet corn. The PAT protein was below the level of detection in kernels of all varieties tested. These results suggest that dietary exposure to Cry1A(b) and PAT from consumption of Bt-11 corn kernels would be very low.

Nutritional issues

Detailed compositional analyses were assessed to establish the nutritional adequacy of Bt-11 corn and to compare it to non-modified control lines of a similar genetic background. No consistent differences in major components or nutrients were observed in Bt-11 corn varieties compared to their respective control lines, or in plants treated with herbicide compared to untreated controls.

Although some statistically significant differences were observed, these were small and random and are not considered to have any biological significance or raise any safety or nutritional concerns. All values reported in the study are consistent with ranges cited in the published literature. The results support the conclusion that Bt-11 corn is nutritionally and compositionally comparable to non-modified corn hybrids and that no health risks are associated with consumption of food derived from the genetically modified corn.

Conclusion

No potential public health and safety concerns have been identified in the assessment of insect protected, herbicide tolerant Bt-11 corn. On the basis of the data considered in the present application, genetically modified Bt-11 corn is equivalent to other commercially available corn in terms of its safety and nutritional adequacy.

1. BACKGROUND

Syngenta Seeds have made an application to ANZFA to amend Standard A18 of the Australian *Food Standard Code*, to include food derived from corn that has been genetically modified for protection against insects, specifically the European corn borer (ECB) and tolerant to the herbicide glufosinate ammonium. The corn is referred to as 'Bt-ll corn'.
Protection against the European corn borer is achieved through the expression in the plant of a modified, truncated version of the *cry*1A(b) gene which produces a nature identical insecticidal protein, CryIA(b). Cry1A(b) is produced naturally by the spore-forming soil bacterium *Bacillus thuringiensis kurstaki* strain HD-1 (*B.t.k.*).

Tolerance to the herbicide glufosinate ammonium is achieved through the expression of the *pat* gene, which produces the enzyme, phosphinothricin acetyl transferase (PAT) that chemically modifies the herbicide, thus rendering it inactive.

Bt-11 corn has been crossed into both maize and sweet corn varieties. Maize varieties are generally classified into flint, pop, dent and flour lines based on the hardness of the kernel. Flint varieties are preferred by dry millers for flour, grits and meal based products such as cereals and dent varieties are preferred by wet millers for starch and starch based products such as high fructose corn syrup. Corn oil may be produced from the germ of all varieties. Fermentation of cereal grains is also used for beverage and alcohol production.

A wide variety of food products are derived from the genetically modified corn including highly processed corn-based food ingredients such as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Maize starch is also used by the food industry for the manufacture of dessert mixes and canned foods. Corn-based ingredients can also be processed into breakfast cereals, baking products, extruded confectionary and corn chips.

As well as these highly processed foods, foods produced from sweet corn varieties may be consumed as fresh, canned or frozen corn or dehydrated in powder form.

2 DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modification

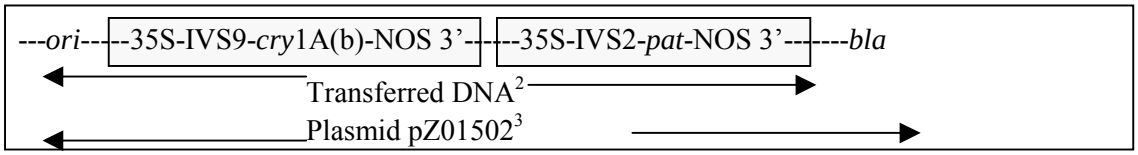
A proprietary inbred corn line, H8540, was transformed with the vector pZ01502 to transfer two new genes, a truncated *cry*1A(b) gene (referred to as the *cry*1A(b) gene) and the *pat* gene. The line was transformed using a protoplast transformation/ regeneration system similar to that described by Negrutiu *et al* (1987). The vector is derived from the plasmid pUC18 and contains the following additional sequences:

- the *bla* (or *amp*) gene under the control of a bacterial promoter, encoding a β-lactamase, which confers resistance to ampicillin;
- a nonfunctional *lac Z* gene, encoding a portion of a β-galactosidase; and
- the pUC origin of replication derived from the plasmid pBR322.

This plasmid does not contain the *tra* (transfer) and *nic/bom* (nick/basis of mobility) genes required for conjugation. The *bla* gene was used as a selection marker when the plasmid was being generated in *Escherichia coli*, but was removed before transformation of plant cells. Thus,

the transformation of corn resulted in the transfer of only one *cry*1A(b) gene and one *pat* gene. The insect-protected, herbicide-tolerant corn varieties designated 'Bt-11 corn', are the subject of this application and were derived from the original transformant.

Figure 1: Schematic diagram of pZ01502[1]



[1]See text or Table 1 for an explanation of the abbreviations.
[2]The transferred DNA is denoted by the arrows. The two boxed regions denote the novel genes introduced into Bt-11 corn.
[3]The genes in the entire plasmid including the antibiotic resistance gene, *bla*.

2.2 Function and regulation of the novel genes

The genes transferred to the corn genome and their regulatory sequences are outlined in Table 1.

Table 1: Description of Genes transferred to Corn

Genetic Element	Origin	Role
***cry*1A(b)**	Bt gene from *Bacillus thuringiensis*	A crystal protein toxic to Lepidopterans
35S Promoter	cauliflower mosaic virus 35S gene	Promoter of high level constitutive gene expression in plant tissues
IVS9 Enhancer	intron from corn alcohol dehydrogenase 1S gene	A regulatory sequence that enhances gene expression in the plant
NOS 3' Untranslated region	*A. tumefaciens* nopaline synthase gene	Contains the signal for the termination of transcription and directs polyadenylation
Pat	Phosphinothricin acetyl transferase from *Streptomyces viridochromogenes*	Confers tolerance to glufosinate ammonium
35S Promoter	modified figwort mosaic virus 35S promoter	Promoter of high level constitutive gene expression in plant tissues
IVS2Enhancer	intron from corn alcohol dehydrogenase 1S gene	A regulatory sequence that enhances gene expression in the plant
NOS 3' Untranslated region	*Agrobacterium tumefaciens* nopaline synthase gene	Contains the signal for the termination of transcription and directs polyadenylation

The cry1A(b) gene

The *cry*1A(b) gene derived from the soil bacterium *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*) strain HD1 confers protection against attack from certain species of lepidoptera, including the European corn borer (ECB) (Geiser *et al*, 1986). The DNA sequence of the gene has been truncated at the 3' end and modified to increase the level of expression in corn, but the amino acid sequence of the protein has not been altered (Perlak *et al*, 1991). The *cry*1A(b) gene in Bt-11 corn codes for the Cry1A(b) protein, a truncated version of the δ-endotoxin produced by *B. thuringiensis*.

Plasmid pZ01502 contains one copy of the *cry*1A(b) gene, controlled by the untranslated 35S promoter from cauliflower mosaic virus (CaMV) and the NOS 3' untranslated region from the nopaline synthetase gene of *Agrobacterium tumefaciens* (NOS 3'). The *cry*1A(b) gene is fused to an intron from the corn alcohol dehydrogenase 1S gene (IVS9) to enhance gene expression in the plant (Mascarenhas *et al*, 1990).

The pat gene

The *pat* gene is derived from the soil microorganism *Streptomyces viridochromogenes* strain Tu494. It codes for the enzyme phosphinothricin acetyl transferase (PAT) which modifies and inactivates the herbicide glufosinate ammonium (Strauch *et al*, 1988).

Plasmid pZ01502 contains one copy of the *pat* gene, which uses the same promoter and 3' untranslated sequence to direct initiation and termination of transcription of the mRNA as the *cry*1A(b) gene (the CaMV 35S promoter and NOS 3' termination signal). The *pat* gene is also fused to an intron from the corn alcohol dehydrogenase 1S gene (IVS2) to enhance gene expression in the plant (Mascarenhas *et al*, 1990). The native DNA sequence of the gene has been altered to optimise expression in plants (Wohlleben *et al*, 1988) but the amino acid sequence of the PAT protein is unaltered. The changes to the DNA sequence alter codon usage to lower the GC content.

The bla gene

A *bla* gene was used as a selectable marker to distinguish transformed bacterial cells from non-transformed cells. It codes for a β-lactamase enzyme that confers resistance to some β-lactam antibiotics, including the moderate-spectrum penicillin and ampicillin. Bacterial cells that contained the pZ01502 plasmid were selected through their resistance to ampicillin. The *bla* gene was excised from the gene construct before transformation of corn embryos and is therefore not present in Bt-11 corn. This has been demonstrated by Southern blot and specific-primer PCR analyses.

2.3 Characterisation of the genes in the plant

Syngenta Seeds submitted the following study regarding characterisation of the novel genes in Bt-11 and stability of genetic changes.

Hilleshög NK (1996). Molecular characterisation of the genetically modified (Bt-11) maize.

Southern blot experiments confirmed the presence of the *cry*1A(b) and *pat* genes in bt-11 corn lines and the absence of the *bla* gene. Prior to transformation, the plasmid DNA was digested with restriction enzymes to produce the DNA fragment containing only the *cry*1A(b) and *pat* genes. The *bla* gene was specifically removed by this digest therefore producing a DNA fragment without any antibiotic resistance genes (illustrated in figure 1).

Southern blot and polymerase chain reaction (PCR) analyses of the Bt-11 corn line was used to support the absence of the *bla* gene. No positive signal was obtained when using a *bla* probe in Southern blots. PCR analysis of the genetically modified corn line, Bt-11, also indicated that it did not contain the *bla* gene. Both Southern blotting and PCR are sensitive enough to detect a single copy of the *bla* gene.

The PCR-walking technique was used to determine that a 1.4 Kb DNA fragment of the vector sequence, upstream from the *cry*1A(b) gene including the origin of replication is transferred to the Bt-11 corn genome. The DNA fragment transferred to Bt-11 corn includes the two novel genes and the bacterial origin of replication (*ori*) from the pUC18 plasmid.

2.4 Stability of the genetic changes

The stability of the inserted DNA in Bt-11 corn was demonstrated by a Mendelian inheritance pattern. The segregation of the *cry*1A(b) and *pat* genes and their phenotypic traits was followed over multiple generations. F1 plants (first generation hybrids) were identified as containing the *cry*1A(b) and *pat* genes. These plants were self-fertilised to produce the S1 population. This S1 population was screened for protection against the European corn borer and for tolerance to glufosinate ammonium. The S1 plants were again self-fertilised. The insect protection and herbicide tolerance traits were then backcrossed into two genetic backgrounds (H8540 and 977), and in some cases, followed by further self-fertilisation.

Seed was collected from corn plants exhibiting both new traits representing different backcross stages and planted in the field for analysis in 1994 and 1995. Plants were tested for protection against the European corn borer and tolerance to glufosinate ammonium. All plants were either both tolerant to the herbicide and protected against insect attack or susceptible to both with segregation patterns consistent with the expected ratio for a single dominant locus, for that particular generation.

The stability of the insert and specifically the *pat* and *cry*1A(b) genes was also demonstrated from R_3 and R_6 generations using Southern blot analysis. Segregation analyses for Bt 11 corn for the six generations of backcrosses and also for crosses with two inbred corn lines are consistent with a stable, single dominant gene segregating according to Mendelian genetics.

Plants screened for protection against insect attack (bioassays with the European corn borer) and for tolerance to the herbicide glufosinate ammonium demonstrated these phenotypes and inheritance patterns consistently over multiple generations. These studies also demonstrated that the *cry*1A(b) and *pat* genes are closely linked, as they always segregated together.

Restriction fragment length polymorphism (RFLP) mapping was used to determine the location of the novel genes in Bt-11. The progeny of Bt-11 plants crossed with the two inbred corn lines were screened with RFLP probes, corresponding to different regions of the corn genome. Comparison of the genotypes of the progeny with isogenic controls demonstrated that the site of integration for the genetic material in Bt-11 corn is located on the long arm of chromosome 8.

2.5 Conclusion regarding the nature of the genetic modification

A single copy of the *cry*1A(b) and *pat* genes are transferred to corn resulting in the development of an insect protected (lepidopteran), herbicide tolerant (glufosinate ammonium) Bt-11 corn. Segregation analyses indicate that the transferred DNA is integrated into the corn genome as a single and stable insert. Further molecular studies indicated that the insertion site is on the long arm of maize chromosome 8.

3. GENERAL SAFETY ISSUES

The Bt-11 corn has been assessed according to the safety assessment guidelines developed by ANZFA, relating to Group D foods - food ingredients, ie plants or animals that contain new or altered genetic material (ANZFA 1999).

3.1 History of use

Corn has been cultivated for centuries and is used as a basic food item by people throughout the world (Wright, 1987). Most corn production is used for human consumption, and a wide variety of food products are derived from corn kernels. Sweet corn varieties are grown largely for human consumption. Corn grain is also widely used as an animal feedstuff.

Two milling procedures are used in corn processing – dry 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). Food products derived from dry milling include flakes and grits. Corn flakes are produced by a process that involves high temperature and pressure and grits are prepared by boiling.

The wet milling process 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 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. These treatments would be expected to degrade and remove proteins (May, 1987). Oil is produced from wet-milled corn by solvent extraction and heat (ie 120ºC) and corn oil is considered free of protein (Rogers, 1990).

Bt-11 has been crossed with elite maize and sweet corn hybrid varieties. Grain harvested from Bt-11 maize corn (ie. predominantly dent corn varieties) will be consumed only after processing as either starch based products like high fructose corn syrup or dry milling corn-based products such as breakfast cereals and flour. Bt-11 sweet corn may also be consumed fresh, canned, frozen or dehydrated in powder.

A summary of the Bt-11 lines analysed is given in Table 2. These have been divided into the elite dent and sweet corn hybrid lines. Additionally, compositional data for genetically modified plants that have been treated with herbicide during growing have been analysed.

Table 2. Summary of lines evaluated in the application[1].

Lines	Protein Expression	Proximate[2]	Fatty Acids	Amino Acids[3]	Vitamins & Minerals
Initial transformant (greenhouse data)					
H8540 Bt$^+$/Bt$^+$		+	+	+	
Control H8540		+	+	+	
H8540 Bt$^+$/Bt$^-$ hybrid		+	+	+	
Control hybrid		+	+	+	
Dent Corn					
N4640-CBR					+
X4734-CBR	+	+	+	+	
X4334-CBR	+	+			
N4242-CBR					+
N4640		+	+	+	+
NK4242	+	+			+
X6534-CBR	+	+	+	+	
X6514		+			
N6800			+	+	
X7634-CBR	+	+			
X7514	+	+			
Sweet Corn Varieties					
0943	+	+			+
Jubilee	+	+			+
0937	+	+			+
Bonus	+	+			+
0941	+	+			+
Empire	+	+			+
Herbicide treated plants					
Madera-Bt		+			+
Madera		+			+
Manuel-Bt		+			+
Manuel		+			+
Magister-Bt		+			+
Magister		+			+

[1]A "+" indicates the data that was provided for that line. Control lines are in italics and genetically modified corn lines are in bold and are denoted as CBR – corn borer resistant or Bt. Control lines are either corresponding isogenic non-GM lines or are of a similar genetic background.

[2]Proximate components analysed were: *Initial transformants*: Total nitrogen, moisture, ash, starch, cellulose, xanthophyll; *Dent corn*: protein, oil, starch and fibre; *Sweetcorn*: moisture, protein, fat, ash, carbohydrates (total), calories, calories from fat, sugars, other carbohydrates, total dietary fibre; *Treated*: energy, carbohydrate, protein, fat, fibre.

[3]Some analyses did not assess all amino acids.

Corn-based food products are derived from many different corn varieties, particularly dent corn lines and sweet corn lines. The applicant has provided data on the original transformant (H8540 and hybrids), and has extended their analysis to those Bt-11 corn lines that are widely used in food production. This includes several dent corn and sweet corn lines that have been developed from conventional breeding with the original transformed line. This information on additional lines enables a comprehensive analysis of the potential impact of the novel genes in different corn genotypes.

3.2 Nature of novel proteins

Two new proteins are expressed in Bt-11 corn: a truncated form of the insecticidal protein CryIA(b), and phosphinothricin acetyl transferase (PAT). The protein products of the novel genes in the transgenic corn have been characterised and the extent of expression determined.

Cry1A(b)

The *cry*1A(b) gene transferred to Bt-11 corn codes for the Cry1A(b) protein, which is an identical but truncated version of the δ-endotoxin produced by *B. thuringiensis*. In the gut of a susceptible insect, the δ-endotoxin is broken down to yield a smaller protein that binds to specific receptors and lyses cells in the gut, preventing feeding and thus causing death.

During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 μm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxin-containing crystals. The protoxin is then activated by trypsin-like gut proteases which cleave off domains from the carboxy- and amino- termini, leaving a protease resistant core which is the active toxin. The active toxin binds to a highly specific glycoprotein receptor on the surface of midgut epithelial cells in the insect. Aggregation of the core toxins results in the formation of a pore through the cell membrane. These cells eventually swell and burst` causing loss of gut integrity and resulting in larval death within 1 to 2 days (Hofte and Whitely, 1989; Schnepf et al, 1998)

The Cry1A(b) protein produced by *B. thuringiensis* subsp. *kurstaki* is a 130 kDa protoxin, which is cleaved in the gut of a susceptible insect to give an insecticidally active 65 kDa fragment. This fragment can be generated *in vitro* by digestion of the protoxin with trypsin. The modified truncated *cry*1A(b) gene product in Bt-11 corn is a protein of 615 amino acids identical to the first 615 amino acids of the native protein, with a molecular weight of approximately 65 kDa.

PAT

S. viridochromogenes produces the tripeptide antibiotic, bialaphos, which consists of phosphinothricin, an analogue of L-glutamic acid bearing two alanine residues. Peptidases hydrolyse bialophos releasing free phosphinothricin. The *pat* gene encodes phosphinothricin acetyl transferase (PAT) which breaks down bialaphos thus allowing the microorganism to protect itself against the toxic compound it produces. When transferred to plants, the *pat* gene product enables the plant to detoxify the broad-spectrum herbicide phosphinothricin (the active moiety of glufosinate ammonium herbicides).

In plants, the enzyme glutamine synthetase, plays a central role in the uptake of nitrogen by catalysing the incorporation of ammonia into glutamine. The herbicide glufosinate ammonium inhibits this enzyme in plants, leading to an accumulation of ammonia in the tissues, which kills the plant. The PAT protein catalyses the acetylation of phosphinothricin, eliminating its herbicidal activity. Acetylation of phosphinothricin produces N-acetyl-glufosinate (NAG) and two further metabolites, 3-methylphosphinicopropionic acid (MPP) and 3-methylphosphinicoacetic acid (MPA).

Although Bt-11 corn is marketed only as an insect-protected plant, the presence and expression of the *pat* gene enables tolerance to commercial applications of the herbicide glufosinate ammonium and is therefore also regarded as a herbicide tolerant plant. The expression level of the PAT protein is discussed in detail in section 3.3. Bialophos, an antibiotic produced by *S. viridochromogenes* is the natural substrate for PAT. No additional substrates, apart from phosphinothricin, have been reported.

3.3 Expression of novel protein in the plant

Syngenta Seeds submitted two studies related to this area:

Schramm S. and Warnick D. (1998). Quantification of Cry1A(b)protein in Attribute insect-protected sweet corn tissues, whole plants and processed products. Performing laboratory: Novartis Seeds Inc, Gilroy, CA, USA. Determination of phosphinothricin N-acetyl-transferase levels in Bt11 maize. Performing laboratory: Xenos Laboratories Inc.

The expression of the PAT and Cry1A(b) proteins in Bt-11 plants has been determined for several maize lines grown both in field trials and in greenhouses and also for three sweet corn lines (refer to Table 2). Expression levels of the introduced proteins were measured using enzyme linked immuno-sorbent assay (ELISA), which is a highly sensitive technique that can detect the presence of a protein generally to a sensitivity of 10 - 100 pg.

In a greenhouse experiment, various plant tissues at several stages of development were analysed for the novel proteins. A second experiment determined the expression levels for four Bt-11 maize hybrids grown in two locations (ie 2 hybrid lines per location) and a third study determined the level of the novel proteins in three sweet corn hybrids. ELISA analysis was used in the analysis of leaf tissue, kernel and canned kernels from the Bt-11 corn.

ELISA analysis of the Cry1A(b) protein levels in Bt-11 corn plants grown in the greenhouse determined that the highest levels were found in the leaf tissue (Table 3) with the highest level at day 25 on the fifth leaf (data not shown).

Table 3: Specific concentration of the Cry1A(b) protein in Bt-11 dent corn tissues during the life cycle of plants grown in the greenhouse[1].

	ng Cry1A(b)/mg plant protein – days post planting (± SE)				
Tissue	**10**	**25**	**59**	**84**	**119**
Roots	11.7 ± 1.7	-	12 ± 3.4	18.2 ± 4	2.2 ± 1.2
2nd Leaf	106 ± 4.7	125 ± 5	-	-	-
15th Leaf	-	-	37.9 ± 2.2	10.2 ± 1.1	-
Pollen	-	-	1.25 ± 0.8	-	-
Kernel	-	-	-	8.2 ± 2.5	0.4 ± 0.4

[1]Values are means of samples from 5 replicate plants (n=5). Data points that are not available at a certain developmental stage are denoted as '-'.

The Cry1A(b) protein was detected in all plant tissue samples. A summary of the results from the greenhouse tissues is given in Table 3. Generally higher levels were detected at the younger stages of tissue development. The level of Cry1A(b) protein decreased as the plant reached full maturity and the tissues became senescent.

A second analysis was done on leaf, husk, stalk and kernels for four Bt-11 corn hybrids grown in field trials and respective control corn lines that have similar background genetics. All tissues were physiologically mature, green and healthy when sampled: leaf - distal half of the ear and next leaf up; stalk: 20 cm section from the stalk above ear; husk: the upper third of the outer husk leaf. The kernels from one location were picked at the early dent stage and at the late dent stage at the second location. The Cry1A(b) protein is expressed at very low levels in these tissues (Table 4). This is equivalent to less than 0.02% of the total protein in the seed. The highest level of the Cry1A(b) protein was found in leaf tissue, with the other plant tissues having significantly lower levels of the protein. The four hybrids produced similar levels of the Cry1A(b) protein.

The PAT protein was analysed in two Bt-11 hybrid corn lines. The protein level is below the limit of detection (ie 1ng/ml extract) in the kernel, husk and stalk and is expressed in trace amounts in the leaf (Table 4). The level of the PAT protein in the leaf represents less than 0.0005% of the total protein.

Table 4: Mean levels of the Cry1A(b) and PAT proteins in corn tissues[1].

		Mean levels in leaf and kernel (µg/g fresh weight)			
		leaf	**kernel**	**husk**	**stalk**
X4334-CBR	Cry1A(b)	4.3 ± 0.66	1.5 ± 0.21	1.1 ± 0.26	0.71 ± 0.11
	PAT	0.0386 ± 0.0029	lod[2]	lod	lod
X4734-CBR	Cry1A(b)	5.05 ± 0.35	1.30 ± 0.28	0.84 ± 0.18	0.55 ± 0.06
	PAT	0.0494 ± 0.005	lod	nd	nd
Control NK4242	PAT	lod	lod	lod	lod
X6534-CBR	Cry1A(b)	5.30 ± 0.90	1.50 ± 0.04	0.79 ± 0.03	0.64 ± 0.04
X7634-CBR	Cry1A(b)	5.24 ± 0.78	1.60 ± 0.13	1.04 ± 0.23	0.53 ± 0.06
Control NK7514	Cry1A(b)	0	0	0	0

[1]n=4 for all Cry1A(b) means and n=3 for all PAT means.
[2]lod (limit of detection) for the procedure is 1ng PAT/ml extract. These values are considered not above background. nd: no data

A third analysis determined the level of the Cry1A(b) protein in tissues from three Bt-11 sweet corn hybrid varieties and control lines with a similar genetic backgrounds (Jubilee, Bonus and Empire). The Cry1A(b) protein levels in kernels tested at prime harvest stage were also assessed in these sweet corn hybrids that had been canned.

The level of the Cry1A(b) protein was present at low levels (Table 5) in Bt-11 sweet corn hybrids. Cry1A(b) protein was not detectable in any of the canned corn samples tested.

Given the low levels of the Cry1A(b) protein determined in kernels for all Bt-11 corn varieties (field and sweet corn) and that it was not detected in canned corn, dietary exposure to the novel protein is expected to be very low.

Table 5: Cry1A(b) protein levels in tissues from Bt-11 sweet corn hybrids[1].

	Cry1A(b) levels in Bt-11 tissues (µg/g fresh weight)					
	Leaves		Kernel		Canned[3]	
	mean	range	mean	range	mean	range
Control[2]	0	-	0	-	nd	-
Hybrid 0943	4.53	3.87-5.18	3.17	2.54-3.80	nd	-
Hybrid 0937	3.10	2.60-3.86	1.59	1.41-1.80	nd	-
Hybrid 0941	3.31	2.66-3.92	0.78	0.51-1.08	nd	-

[1]Values are µg/g fresh weight. n=3 for all means except in leaves and kernels from 0943 where n=2.
[2]Control plants varieties are Jubilee, Bonus and Empire. Control plants had ELISA values corresponding to 0ng Cry1A(b)/g fw.
[3]The absorbance generated for canned samples did not exceed background (nd = not detectable). The lower limit of quantification was 2ng/g fw

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

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[8]/WHO Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). That consultation concluded that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

The major concern in relation to the transfer of novel genetic material to 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.

No antibiotic resistance genes were transferred to Bt-11 corn as indicated by Southern blot and PCR analysis.

In relation to transfer of novel genetic material from genetically modified food to human cells via the digestive tract, this is also equally 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 additional risks compared with the large amount of DNA naturally present in all foods.

[8] Food and Agriculture Organization.

3.5 Conclusions regarding general safety issues

The *cry*1A(b) and *pat* genes are expressed at low levels in Bt-11 corn. Both proteins are expressed highest in the leaf tissue. The expression level of the Cry1A(b) protein is much lower in the kernel representing less than 0.02% total protein in the seed and the PAT protein is below the limit of detection in the kernel. The level of DNA and protein in highly processed corn based products is expected to be very low and in some cases, negligible. It is also likely that the proteins will be degraded and/or removed during processing steps.

No antibiotic resistance genes were transferred to Bt-11 corn during the transformation process. The novel genetic material in Bt-11 corn comprises only a minute fraction of the total DNA present in the corn and is therefore unlikely to pose any additional risks.

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally-occurring toxins

Corn contains no naturally-occurring toxins that occur at biologically significant levels (Wright, 1987).

4.2 Potential toxicity of the novel proteins

The potential for toxicity of the newly expressed proteins, Cry1A(b) and PAT, were evaluated based on:

. the amino acid sequence similarity with known toxins
. acute toxicity testing in mice.
. the resistance to digestion by proteases and acids in the model digestive/gastric system
. their presence as a major protein component in a specified food.

The potential for acute toxicity of the Cry1A(b) and PAT proteins was assessed by evaluating physical and chemical characteristics of the proteins and also by acute oral toxicity in mice. The scientific basis for using an acute test is that known protein toxins generally act via acute mechanisms (Jones and Maryanski 1991). Another study was submitted that demonstrated equivalence of the corn-expressed Cry1A(b) protein to the microbially produced Cry1A(b) protein in terms of molecular weight, immunological reactivity, trypsin resistance, amino acid sequence, glycosylation and bioactivity.

Reports submitted by Syngenta Seeds:

Kuhn JO (1994a). Cry1A(b) B.t.k. delta-endotoxin. Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC, USA.

Kuhn JO (1995). Phosphinothricin acetyltransferase (Sample PAT-0195) Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC

Meeusen R. and Mettler I. (1994), revised by Goy P.A. (1998). Equivalence of plant and microbially produced Bacillus thuringiensis subsp. kurstaki HD-1 protein. Performing laboratories: Novartis Seeds/Northrup King Co, University of Wisconsin, Kendrick Laboratories and Washington University School of Medicine, USA.

4.2.1 Cry1A(b)

(i) History of consumption

Cry1A(b) has a long history of safe use as an insecticide and has been repeatedly shown to be non-toxic to humans and other vertebrates. There is no evidence from the history of long use that there is any associated toxicity to humans. The toxicity of this protein is very specific to Lepidopteran insects. The 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 Cry1A(b)-specific gut receptors in other organisms (Frick, 1995). Additionally, there is evidence that demonstrates that the mammalian gut contains receptors that are not comparable to those found in the gut of susceptible insects. *In vivo* studies with rats given Cry1A(b) orally, and *in vitro* binding studies with gut tissue isolated from rats, mice, rhesus monkeys and humans did not reveal receptors for the protein (Noteborn *et al* 1995).

(ii) Similarity with known toxins

An amino acid sequence comparison of the Cry1A(b) protein to a database of 2632 sequences detected significant similarities only to other *B. thuringiensis* insecticidal crystal proteins. The sequences were obtained from the GenBank, EMBL, Swissprot and PIR databases.

(iii) Equivalence of the plant Cry1A(b)protein to the bacterially produced protein.

The test protein used in acute toxicity tests and characterisation studies was produced in *E. coli* because the genetically modified corn plants did not express enough protein for purification of large quantities. Data was presented to indicate that the bacterially produced Cry1A(b) protein is equivalent to the plant produced Cry1A(b) protein in terms of its molecular mass, N-terminal amino acid sequence, lack of glycosylation, and biological activity. The *E. coli* produced Cry1A(b) is considered a suitable substitute for plant produced Cry1A(b) in toxicity testing.

In this study, the trypsin resistant fragment of Cry1A(b) expressed by Bt-11 corn was purified by extraction of leaf tissue, trypsin digestion and immunoaffinity purification. Analysis by SDS-polyacrylamide gel electrophoresis and Western blotting demonstrated that two related proteins are present in Bt-11 corn; one of 69 kDa (the full 615 residues coded for by the *cry*1A(b) gene) and one of 65 kDa (the expected size if the first 28 amino acids have been removed by proteolysis). Both proteins are reactive with antibodies to microbially-produced CryIA(b). Trypsin treatment resulted in a single band of 65 kDa, which is equivalent to the trypsin resistant fragment of the native protein. Some lower molecular weight immunoreactive material (42 and 15 kDa) was also present, probably representingpartially digested Cry1A(b) protein. Similar results were obtained with the microbially-produced Cry1A(b) protein.

N-terminal amino acid sequencing confirmed that the Bt-11 65 kDa protein had the expected sequence of a fragment extending from residue 29 of the native protein, consistent with the fragment having been cleaved at the trypsin sensitive site at residue 28. There was no evidence of glycosylation of either the Bt-11 or the microbially-produced CryIA(b). The plant and microbially-produced Cry1A(b) had similar bioactivity against ECB, with LD_{50}s (µg/mL) of 0.47 and 0.50 respectively.

(iv) Acute oral toxicity in mice – native CryIA(b)

Hsd:S-D ICR albino mice (source: Harlan Sprague Dawley Inc, Texas) were acclimatised for at least 5 days before dosing (5/sex). They were housed individually in controlled conditions with free access to food and water, except for the 16 hours before dosing when food was withheld. *Bacillus thuringiensis* Cry1A(b) δ-endotoxin (lot BFL0194, purity 70%, source SIGMA Chemical Co, produced in *E. coli*) in carboxymethylcellulose was administered to the mice (5/sex) at 5050 mg/kg bw by single oral gavage. A 20% w/v concentration in 2% w/v aqueous carboxymethylcellulose was used, as this was the highest concentration able to be administered through the gavage tube.

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. There did not appear to be any ill effects from the dosing volume. 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. Any abnormalities were recorded and the gastrointestinal tracts were preserved in formalin for later histopathological examination if required.

There were no deaths during the study. The only abnormal clinical sign observed in the test group was piloerection (hair standing on end), which occurred only on day 1. During the second week after dosing, one female in the test group lost weight; all other mice showed normal bodyweight gains for their age and sex. No abnormalities were detected on necropsy. The acute oral LD_{50} for Cry1A(b) δ-endotoxin in mice is therefore 70% of 5050 mg/kg bw (ie 3535 mg/kg bw given that the protein was 70% pure). 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) and do not demonstrate any potential mammalian toxicity from Cry1A(b) protein.

4.2.2 PAT

(i) History of consumption

The *pat* gene encodes the phosphinothricin-N-acetyl transferase enzyme which has a very narrow substrate specificity for phosphinothricin and demethyl-phosphinothricin, both of which are not found in humans. Acetyl transferases are a class of enzymes common to all bacterial, plant and animal cells and play a major role in both the synthesis and oxidation of fats. Since proteins from this family are naturally present in virtually all cells, they can be considered a component of the human diet.

(ii) Similarity with known toxins

A comparison of the amino acid sequence of the PAT protein to a database of known toxins demonstrated that it does not share any significant similarity with any known protein toxins. The sequences were obtained from the GenBank, EMBL, Swissprot and PIR databases. Additionally, no reports were found of toxicity associated with acetyl transferases as a class and that the donor organism has no known pathogenic potential.

(iii) Equivalence of the plant PAT protein to the bacterially produced protein.

PAT expression was at the limit of detection in Bt-11 corn plants and it was not possible to extract it in sufficient quantities to be used in model digestion system or oral toxicity studies or

to be compared to the bacterially produced protein. The PAT protein was therefore derived from expression of the recombinant protein in *E. coli*. However, the modified *pat* gene transferred to corn plants produces a protein of 183 amino acids, the sequence of which is identical to that of the PAT protein encoded by the native *pat* gene.

Based on the *pat* gene construct, there is no reason to expect that the plant produced PAT protein would be different in any way to the bacterially produced PAT protein.

(iv) Equivalence of the PAT protein produced by the bar *gene.*

Phosphinothricin acetyl transferase is also produced by *Streptomycin hygroscopicus* (Thompson *et al*, 1987) which is encoded for by the *bar* gene. A functional and structural comparison of both protein products has concluded that both proteins have comparable molecular weights and show similar immuno-cross-reactivity to their respective polyclonal antisera (Wehrmann *et al*, 1996). Both enzymes have a similar substrate affinity (for L-phosphinothricin) and do not acetylate any of the other L-amino acids tested. Both proteins were rapidly broken down in model digestion system studies and had decreased enzymatic activity (Wehrmann *et al*, 1996). These studies are discussed in the next part and also under Section 4.4.

(v) Acute oral toxicity in mice – bacterially produced PAT

Hsd:S-D ICR albino mice (source: Harlan Sprague Dawley Inc, Texas) were housed individually in controlled conditions with free access to food and water, except for the 16 hours before dosing when food was withheld. Groups (5/sex) of mice were given a single oral dose (gavage) of PAT protein (PAT-0195, purity 51% phosphinothricin acetyltransferase, expressed by the *bar* gene in *E. coli*) in carboxymethyl cellulose; heat inactivated PAT (PAT-0195C, 52% purity) in carboxymethyl cellulose; or carboxymethyl cellulose to a total dose of protein of approximately 2600 mg/kg bw (ie 51-52% of 5050 mg/kg bw, given that this was the purity of the protein).

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. The only notable clinical signs were decreased activity, piloerection and ptosis (drooping eyelid) on days 6–8 in the male that died. One male receiving the reference substance showed slight piloerection on the day of dosing. However, as no other clinical signs were observed in animals of any group, these signs are not considered to be treatment related. 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 results do not indicate any potential toxicity from the PAT protein.

4.3 Levels of naturally-occurring allergenic proteins

Corn does not contain any known naturally-occurring allergens (Wright 1987).

4.4 Potential allergenicity of novel proteins

Although there are no simple predictive assays available to assess the allergic potential of proteins, a number of characteristics are common among many of the allergens that have been

characterised. For instance, 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). In terms of the chemical and physical nature of proteins, known allergens tend to be glycosylated proteins with a molecular weight of 10–70 KDa (Lehrer *et al*, 1996). 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). The Cry1A(b) and PAT proteins are evaluated for potential allergenicity against these criteria: molecular size, amino acid sequence similarity to known allergens, and how easily the protein is degraded by heat, acid and gastric enzymes (Lehrer and Reese 1998, Jones and Maryanski 1991).

Syngenta Seeds submitted three studies relevant to the possible allergenicity of the novel proteins which are listed below. The *in vitro* digestibility of the proteins was investigated to consider the potential allergenicity of the novel protein products which can be related to the presence of large undigested protein molecules.

Studies submitted by Syngenta Seeds:

Privalle L (1994). In vitro digestibility of CryIA (b) protein from Bt maize (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.

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.

Meeusen R. and Mettler I. (1994), revised by Goy P.A. (1998). Equivalence of plant and microbially produced Bacillus thuringiensis subsp. kurstaki HD-1 protein. Performing laboratories: Novartis Seeds/Northrup King Co, University of Wisconsin, Kendrick Laboratories and Washington University School of Medicine, USA.

4.4.1 Cry1A(b) protein

As described in Section 3, the Cry1A(b) protein produced by Bt-11 corn was demonstrated to be equivalent to the microbially-produced protein in terms of the N-terminal sequence, immunoreactivity and post-translational modification. The microbially-produced protein is considered to be a suitable substitute for plant-expressed Cry1A(b) for allergenicity studies.

(i) Physical properties of the protein

The Cry1A(b) core protein has a molecular weight of 63 kDa, which is in the size range of known allergens.

The amino acid sequence of the Cry1A(b) protein was compared to the amino acid sequences of 219 known allergens present in public domain databases (eg GenBank, EMBL, Swissprot, PIR). No biologically similarity was found with any of these known allergens.

(ii) Model digestive system studies

Native Cry1A(b) protein obtained from *Bacillus thuringiensis* subsp *kurstaki* was digested under simulated gastric conditions. The protein was extracted from a cell paste of *Btk* strain HDI-9.

Simulated gastric fluid (SGF) was prepared containing NaCl, HCl and pepsin. The pepsin content (X) was initially 3.2 mg/mL, with a pH of 1 to 1.2. Solutions of SGF containing dilutions of pepsin (0.1, 0.01 and 0.001 times the standard dilution) were also prepared to investigate the degradation of the protein over time. Gastric fluid without pepsin was also prepared.

In an initial trial, 10 µL of protein sample (100 µg of protein) was added to 90 µL of SGF. A 50 µL aliquot was immediately removed, neutralised and heated to 75^{o}C for 10 minutes. The remainder was incubated at 37^{o}C for 2 minutes before neutralising and heating. Following the initial trial, a trial to investigate the time course of degradation was performed using 0.01X pepsin solution. 40 µL of protein sample was added to 360 µL of SGF. 50 µL was removed at 0, 1, 2, 5, 10 and 30 minutes and neutralised and heated as above. The protein content of each sample (in the initial and time course trial) was analysed by western blot.

Following incubation with a solution containing a standard quantity of pepsin, the native Cry1A(b) protein was almost all degraded after 2 minutes. In the time course trial, the protein was undetectable after 5 minutes with 0.01 times the standard dilution of pepsin.

This trial using simulated gastric conditions indicates that Cry1A(b) protein obtained directly from *Bacillus thuringiensis* subsp *kurstaki* is digested as normal dietary protein, being rapidly degraded under simulated gastric conditions. This result is consistent with published studies (Noteborn *et al* 1995, Sanders *et al* 1998). As Cry1A(b) produced by Bt-11 was found to be identical to the microbially produced protein (as discussed in Section 3), it can be concluded that the Bt-11 Cry1A(b) would rapidly degrade in the digestive tract. As Cry1A(b) is present at low levels in the kernel, is easily digested and does not show any amino acid sequence similarity with known allergens, it is not considered to be allergenic.

4.4.2 PAT protein

(i) Physical properties of the protein

A comparison of the amino acid sequence of the PAT protein to a database of known allergens demonstrated that it does not share any significant similarity with any known protein allergens. Additionally, acetyltransferases in general have no similarity to any reported mammalian allergens.

(ii) Model digestive system studies

The PAT protein used in this trial was obtained from an *E. coli* expression system and was purified following fermentation. SGF contained NaCl (2 mg/mL), HCl and pepsin (3.2 mg/mL), the pH was 1.0 to 1.2, and the activity of the fluid was determined before use. Solutions were prepared containing successive dilutions of pepsin (0.1, 0.01 and 0.001 times the standard dilution). The reactions were started by adding 10 µL to PAT sample (26 µg total protein) to 90 µL of the appropriate gastric solution. After mixing, 50 µL was removed, neutralised and heated. This sample was designated the time zero sample. The remainder was incubated for 2 minutes before neutralisation and heating. The presence of PAT in the fluid following incubation was determined by SDS-PAGE analysis. The enzymic activity of the solution was also determined at the pH optimum for the enzyme, at gastric pH and following serial incubation with a gastric solution containing 0.0032 mg/mL pepsin.

In the presence of SGF containing a standard concentration of pepsin, the PAT protein was completely degraded at time zero. After 2 minutes of incubation with 0.1 or 0.01 times the standard pepsin concentration, PAT degradation appeared complete. When 0.001 times the standard concentration was used, a significant amount of PAT remained after a 2-minute incubation period. This concentration was thus selected for the enzyme inactivation studies.

The enzyme activity of PAT decreased to 56% of initial values after a 10-minute incubation at 37^{o}C. This reflects the thermal sensitivity of the enzyme above 35^{o}C, and would represent the maximum activity were gastric pH or pepsin to have no effect on PAT activity. Immediately after addition to SGF without pepsin, PAT activity decreased to 2.6% of the initial activity, and reached zero by 1 minute. When pepsin was included in the SGF, the initial activity was even lower. Activity was not restored by neutralisation, indicating that inactivation of the PAT enzyme was irreversible. The half-life of the PAT protein in SGF containing 0.0032 mg/mL was between 1 and 2 minutes.

This study demonstrates that PAT loses enzymatic activity immediately upon exposure to gastric pH, and that the protein is readily digested in the stomach. As the PAT protein is present at low levels in the kernel, is easily digested and does not show amino acid sequence similarity with known allergens, it is considered highly unlikely to be allergenic.

4.5 Conclusions regarding toxicological issues

Analysis of the physical and chemical properties of the Cry1A(b) and PAT proteins have not revealed any similarities to known toxins and allergens. No adverse reactions were observed in mice that were administered either protein in acute toxicity tests. No evidence suggests that either protein has been derived from a potentially toxic or allergenic source and the Cry1A(b) protein has a long history of safe use. Both proteins are present in corn kernels at low levels and are shown to degrade in conditions that mimic the human digestive system. Therefore it is highly unlikely that either the Cry1A(b) or PAT protein would be toxic or allergenic to humans.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

The safety assessment includes an analysis of the composition of the food in comparison with other commercial varieties of the crop. Given that food is produced from many corn varieties, the applicant has provided data on several different dent and sweet corn varieties. Refer to Table 2 for a complete summary of the lines analysed.

Four major studies have been conducted on Bt-11 kernels that assess the major components in inbred and hybrid lines at different stages of maturity and a comparison with their respective near-isogenic controls. The first study is an analysis of the glasshouse grown original transformant. The second suite of studies have been conducted on six dent corn lines developed from conventional breeding with the original transformant. The third set of data has been provided on sweet corn lines also derived from conventional breeding with the original transformant. A final study assesses the potential effect of Bt-11 corn treated with the herbicide glufosinate ammonium during growing.

Studies submitted by Syngenta Seeds:

Compositional analysis of Bt11 maize: determination of the substantial equivalence — chemical composition analysis done with Bt-11 maize with a European background. Performing laboratory: Association Generale des Producteurs de Mais).

Compositional analysis of Bt11 maize: determination of the substantial equivalence — chemical composition analysis done with Bt-11 maize with a US background. Part 1: Properties of grain produced from ECB protected maize hybrids; Part 2: Characterization of grain attributes of normal, wild-type maize hybrids and the Bt11 converted iso-hybrid counterparts; Part 3: Analyses of fatty acid and amino acid profiles of grain from Bt-11 maize. Report No. NSB-004-97.Performing laboratory: Novartis Seeds/Northrup King Co.

Comparison of nutritional composition of fresh and canned grain prepared from Attribute insect protected and control sweet corn hybrids. Report No. NSV-002-98. Novartis Seeds Inc.

Comparison of vitamin and mineral composition of Bt11 maize and non-modified maize hybrids. Report No. NSB-004-97. Novartis Seeds.

Goy P.A. (1999). Novartis Seed's genetically modified Bt11 maize: biochemical composition of kernels from plants treated with a glufosinate ammonium herbicide.

5.1.1 Study 1: Analysis of Bt-11 corn grown in greenhouses in Europe

The following greenhouse grown plants were analysed: an inbred line (H8540-Bt), a hybrid line (hybrid Bt^+/Bt^-) and their respective controls (isogenic non-modified H8540 and control hybrid). Between 45 and 56 ears were taken from each plant. Ears were harvested and dried four months after sowing and 500 g samples were analysed for moisture, total nitrogen, ash, starch, cellulose, xanthophyll, fat composition and amino acid composition. Statistical comparison with STAT-ITCF software was made on the values of two replicate analyses, except in the case of xanthophyll, fatty acids and amino acids, where data points are the result of a single analysis.

(i) Compositional analyses

All values for chemical composition were within the normal range for data obtained by the Association Générale des Producteurs de Mais (AGPM), except for total nitrogen, which was higher than normal for both the control and genetically modified corn plants (Table 6).

Table 6: Summary of compositional analysis for Bt-11 and control corn plants[1].

	Inbred line H8540-Bt	***Isogenic control H8540***	**Hybrid Bt+/Bt-**	***Control hybrid***	**Normal range[2]**
Total nitrogen[3]	13.18 ± 0.07	12.35 ± 0.06	12.28 ± 0.03	12.30 ± 0.07	7.7–10[4]
Moisture	12.3	12.6	12.6	13.3	7–23
Ash	1.47 ± 0.04	1.79 ± 0.007	1.70 ± 0.02	1.6 ± 0.02	1.1–3.9
Starch	68.02 ± 0.4	67.57 ± 0.4	70.83 ± 0.81	70.25 ± 0.48	61–78
Cellulose	2.99 ± 0.007	2.9 ± 0.05	2.67 ± 0.28	2.92 ± 0.05	3.3–4.3 1.93–2.5[4]
Xanthophyll	24.2	21.0	21.6	19.1	19.2–33.1[4]

[1]Samples are 500g of kernels from: Bt^+/Bt^+ H8540 ears n=54, Control H8540 n=56, Bt^+/Bt^- hybrid n=50, Control hybrid ears n= 45. Each data point represents the mean of two replicate analyses made with the 500g sample. Data from AGPM. All data except moisture (% H_2O) and xanthophyll (mg/kg dry weight basis) are presented on a % dry weight basis.

[2]Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA.

[3]All values from control and genetically modified lines are significantly different to range.

[4]Data from AGPM

Protein levels were higher than the normal range for all plants assessed. As protein content is affected by soil nitrogen, it is possible that the fertiliser used in culturing the plants caused the high level of nitrogen for all plants in the study.

(ii) Amino acid analysis

A summary of amino acid values for plants homozygous for the *cry*1A(b) gene is shown in Table 7. A single analysis was done on 500g samples of kernels from Bt^+/Bt^+ H8540 (number of ears n=54), isogenic control H8540 (number of ears n=56), Bt^+/Bt^- hybrid (number of ears n=50), control hybrid (number of ears n= 45). Values for amino acid composition (once corrected for the high total nitrogen) had minor variations to control values but were within the normal range according to APGM and the published literature. There were no differences in these values greater than 10% (which allows for experimental error) between the modified corn and isogenic controls. The levels of glutamine, asparagine, tryptophan were not determined. No spectrum or literature ranges were available for some of the amino acids, as some of these analyses are not routinely carried out by the laboratory assaying these samples.

Table 7: Summary of amino acid composition data for Bt-11 corn plants[1].

Amino acid composition	**Bt^+/Bt^+ line H8540**	***Control H8540***	**Bt^+/Bt^- hybrid line**	***Control hybrid line***	**Range[2]**
Aspartic acid	9.8	9	8.7	8.4	
Threonine	5.2	5	4.9	5	3.2–3.4
Serine	6.6	6.4	6.1	6.1	
Glutamic acid	28.1	25.7	26.2	25.1	
Proline	12	12.5	12	11.4	
Glycine	4.1	4.2	4	4	
Alanine	11.5	10.8	10.8	10.1	
Cysteine	2.4	2.4	2.5	2.7	
Valine	6.5	6.1	5.9	6.2	4.2–4.6
Methionine	2.5	2.5	2.7	2.9	1.8–1.9
Isoleucine	5.2	4.8	4.6	4.6	3.4–3.7
Leucine	19.4	17.5	17.7	17.3	10–11.3
Tyrosine	5.4	4.9	5	4.7	
Phenylalanine	7.2	6.5	6.4	6.3	4.4–4.5
Lysine	3.2	3.3	3.1	3	2.45–2.6
Histidine	3.5	3.4	3.4	3.5	
Arginine	4.4	4.8	4.9	4.8	4.1–5.2

[1]Values are expressed as g/Kg dry matter.
[2]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

(iii) Fatty acid analysis

A summary of fatty acid values for plants homozygous for the *cry*1A(b) gene is shown in Table 8. A single analysis was done on 500g samples of kernels from Bt^+/Bt^+ H8540 (number of ears n=54), isogenic control H8540 (number of ears n=56), Bt^+/Bt^- hybrid (number of ears n=50), control hybrid (number of ears n= 45). Values for fatty acid composition had minor variations to control values but were within the normal range according to APGM and the published literature. There were no differences in these values greater than 10% (which allows for experimental error) between the modified corn and controls. Literature ranges were available for most of the common fatty acids and not the minor ones as analyses of these fatty acids are not routinely carried out.

Table 8: Summary of fatty acid composition data for Bt-11 corn plants[1].

Fatty acid composition	**Bt^+/Bt^+ line H8540**	***Control H8540***	**Bt^+/Bt^- hybrid line**	**C*ontrol* hybrid line**	**Range**
C16 palmitic acid	15.1	14.5	15.3	14.6	6–22[2]
C18 stearic acid	1.7	1.6	1.6	1.5	1–15[2]
C18:1 oleic acid	20.6	21.9	21.8	21.8	14–64[2]
C18:2 linoleic acid	58.9	58.2	58.1	60	19–71[2]; 56–65[3]
C18:3 linolenic acid	1.7	1.7	1.2	1.1	0.5–2[2]
C20 arachidic acid	0.5	0.4	0.4	0.4	
C20:1 gadoleic acid	0.2	0.2	0.2	0.2	
C22: behenic acid	0.2	0.2	0.1	0.1	

[1]Samples are 500g of kernels from: Bt^+/Bt^+ H8540 ears n=54, Control H8540 n=56, Bt^+/Bt^- hybrid n=50, Control hybrid ears n= 45. Values are expressed as % of the analysed fatty acid relative to the total amount of fatty acids.
[2]From Weber, "Lipids of the kernel", Chapter 10 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. data
[3]AGPM

5.1.2 Study 2: Analysis of Bt-11 dent corn grown in USA

5.1.2.1 Data set 1

An analysis of the major components and nutritional qualities of elite Bt-11 dent corn lines has also been assessed. These lines are derived from the original transformant. Two genetically modified Bt-11 hybrid corn lines and their near-isogenic controls were grown in three field locations in the USA in 1995. Kernels were analysed for percentage of starch, protein, oil and fibre. These components were estimated by near infrared reflectance (NIR) spectroscopy by the Illinois Crop Improvement Association Inc. NIR analyses are methods used by the American Association of Cereal Chemists.

The kernels from insect-protected corn hybrids were comparable to control hybrids for percentage starch, protein, oil and fibre (Table 9) and fell within the normal ranges expected for these components.

[2]From Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA.
[3]Average value

Table 9: Summary of compositional analysis for Bt-11 and control corn plants[1].

	X6534CBR	**Isogenic control X6514**	**X7634CBR**	**Isogenic control X7514**	**Normal range[2]**
Protein	9.89 (9.40-10.60)	9.96 (9.10-11.40)	10.55 (10.24-11.00)	9.68 (8.90-10.94)	6-12
Oil	4.09 (4.00-4.16)	4.11 (4.10-4.13)	4.02 (4.00-4.02)	4.07 (3.80-4.31)	3.1-5.7
Starch	70.09 (68.80-71.07)	70.19 (67.80-71.50)	69.32 (68.60-70.36)	70.36 (69.07-71.40)	61-78
Fibre	2.95 (2.86-3.00)	2.97 (2.92-3.00)	2.93 (2.89-3.0)	2.91 (2.90-2.92))	2.5[3]

[1]Values presented as % dry weight. Values are means of 3 samples taken from 3 locations (ie 1 sample/location), ranges are given in brackets. Genetically modified corn lines are denoted CBR and are isogenic to their controls except for the presence of the novel genes.

5.1.2.2 Data set 2

A second nutritional study on Bt-11 dent corn that included additional hybrids was done. Three to five ears were picked from the centre two rows of a four row strip plot for each hybrid per two sites within three geographical regions to give a total of six locations per hybrid. Two of the hybrids are 'northern' (early-season) hybrids and two were 'southern' (mid-late-season) hybrids and were grown with their respective isogenic controls. The grain was analysed by the Illinois Crop Improvement Association using Near Infrared Reflectance Spectroscopy (NIRS) according to methods of the American Association of Cereal Chemists.

(i) Compositional analyses

The compositional data for the Bt-11 corn (denoted as corn borer resistant – CBR) and control corn plants were analysed for significant differences by Analysis of Variance (SAS GLM procedure). The components measured were % protein, oil, starch and fibre (Table 10). Kernels

from the early season (northern hybrids) genetically modified corn hybrids (X4334CBR and X4734CBR) have a significantly lower protein content than kernels from the control corn lines (P=5 and P=1 respectively). All other components were comparable between the Bt-11 corn hybrids and their respective control corn lines.

Table 10: Summary of compositional analysis for Bt-11 and control corn plants from a second field trial[1].

Northern / Early	**X4334CBR**	**Control N4242**	**X4734CBR**	**Control N4640**	**Normal range[2]**
Protein	8.65[3] (8.03-9.11)	9.25 (8.63-9.63)	8.19[4] (7.74-9.16)	8.96 (8.28-9.53)	6-12
Oil	3.17 (2.81-3.73)	3.23 (3.04-3.50)	3.34 (3.36-3.48)	3.30 (3.12-3.68)	3.1-5.7
Starch	72.93 (71.8-73.2)	72.57 (71.7-73.4)	72.73 (71.5-73.7)	72.62 (71.3-73.2)	61-78
Fibre	2.69 (2.66-2.83)	2.75 (2.67-2.93)	2.77 (2.68-2.83)	2.77 (2.69-2.83)	2.5[5]
Southern / Mid-late	**X6534CBR**	**X6514**	**X7634CBR**	**X7514**	
Protein	9.52 (8.35-10.60)	9.93 (9.10-11.40)	9.85 (8.63-11.00)	9.87 (8.67-10.94)	6-12
Oil	3.80 (3.63-4.16)	3.93 (3.27-4.13)	3.37 (2.59-4.00)	3.48 (2.70-4.31)	3.1-5.7
Starch	70.77 (68.8-72.5)	71.07 (67.8-72.7)	71.33 (68.6-74.3)	71.12 (69.1-73.9)	61-78
Fibre	2.78 (2.55-3.00)	2.80 (2.61-3.0)	2.74 (2.53-3.00)	2.72 (2.46-2.92)	2.5[5]

[1]Values presented as % dry weight. Values are means of a total of 6 samples taken from 2 sites in 3 locations (ie 2 distinct samples from each of the 3 locations), ranges are given in brackets.
[2]From Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA.
[3]Values are significantly different to that of control value at 5% level of probability.
[4]Values are significantly different to that of control value at 1% level of probability.
[5]Average value

The "northern" and "southern" hybrids were derived from separate backcross conversion processes using the same original transformation event (plant). Although the protein was lower in the northern hybrids, there is a lack of consistent differences between the non-modified hybrids and their genetically modified equivalents. This may indicate that the effects observed, are not likely to be a result of the genetic modification itself but more likely from differences arising out of an incomplete backcross conversion in the normal breeding process. Values for all the parameters measured fell within the ranges cited in the literature (refer to Table 10).

(ii) Amino acid analyses

Amino acid analyses were performed on kernels obtained from two Bt-11 hybrid corn lines: X6534CBR (a mid-late maturity variety) and X4734CBR (an early maturity variety) and their genetically equivalent controls (N6800 and N4640 respectively). The kernels were sampled from two locations, three samples per line. For further comparison, kernels from another seven non-modified reference hybrids were grown in one of the field trial locations (N4242, N5220, N5866, N6223, N6822, N7070 and N790). Two separate statistical analyses were performed — the first to analyse the variation between hybrids to determine whether there were significant differences between hybrids. The second study analysed differences specifically between genetically modified hybrids and their near-isogenic controls (Table 11).

The first statistical analysis determined the variation between hybrids. Since not all hybrids were replicated, the analysis used the variation observed in hybrids with multiple replicates as an indication of "error" for the other hybrids. The rationale for this is that other hybrids would have been equally variable. There were significant differences between the hybrids for all values except that for tyrosine (P=5).

Small but significant differences at the 5% level were found between the genetically modified corn hybrid X4734CBR and its control line N4640 for arginine and cysteine. This difference is not consistent for all genetically modified corn hybrids and is consistent with the variability that is observed between lines. Some variability may arise as a result of incomplete backcrossing. This variation is not considered to be a result of the genetic modification nor is it biologically significant.

(iii) Analysis of fatty acid profiles

Fatty acid analyses were also done on the kernels sampled as described above. The kernels were sampled from two locations, three samples per line from two Bt-11 hybrid corn lines X6534CBR and X4734CBR and their genetically equivalent controls (N6800 and N4640 respectively). Additionally, grain from another seven non-modified reference hybrids were also analysed. As outlined above for the amino acid analysis, two separate statistical analyses were performed — the first to analyse the variation between hybrids to determine whether there were significant differences between hybrids. The second study analysed differences specifically between genetically modified hybrids and their isogenic controls. The results are shown in Table 12.

A statistical analysis to determine the variation between hybrids, as described above for the amino acid analysis, found no significant differences between the hybrids for fatty acid values (P=5).

Table 11: Amino acid profile for Bt-11 hybrids and control corn plants[1].

	X6534-CBR	N6800	X4734-CBR	N4640	N4242[4]	N5220[5]
Tryptophan	0.05-0.06	0.05-0.06	0.05[2]	0.05-0.06	0.05[2]	0.07
Aspartic Acid	0.61-0.67	0.60-0.66	0.54[2]	0.55-0.57	0.55[2]	0.64
Threonine	0.35-0.38	0.35-0.38	0.29-0.30	0.30-0.31	0.30-0.32	0.36
Serine	0.50-0.55	0.50-0.55	0.42-0.43	0.43-0.44	0.43-0.44	0.52
Glutamic Acid	1.54-1.72	1.55-1.79	1.17-1.25	1.22-1.30	1.30-1.32	1.63
Proline	0.77-0.88	0.83-0.91	0.68-0.70	0.63-0.68	0.61-0.66	0.84
Glycine	0.34-0.37	0.35[2]	0.29-0.30	0.31-0.33	0.32-0.34	0.36
Alanine	0.75-0.82	0.75-0.87	0.60-0.62	0.58-0.63	0.61-0.63	0.74
Cysteine[3]	0.21-0.22	0.22-0.23	0.17[2]	0.20-0.21	0.18-0.21	0.22
Valine	0.41-0.43	0.40-0.45	0.32-0.33	0.32-0.34	0.32-0.36	0.43
Methionine	0.19-0.21	0.19-0.22	0.17-0.20	0.20-0.23	0.19-0.21	0.24
Isoleucine	0.28-0.32	0.28-0.33	0.23-0.25	0.24-0.26	0.23-0.27	0.32
Leucine	1.23-1.37	1.23-1.45	0.93-0.98	0.96-0.98	0.92-1.01	1.32
Tyrosine	0.14-0.18	0.14-0.16	0.13[2]	0.13-0.14	0.14[2]	0.17
Phenylalanine	0.44-0.49	0.44-0.51	0.37-0.39	0.36-0.40	0.35-0.38	0.50
Histidine	0.32-0.35	0.34-0.37	0.26-0.27	0.28-0.29	0.27-0.28	0.31
Lysine	0.25-0.26	0.24-0.26	0.23-0.24	0.24-0.25	0.23-0.25	0.27
Arginine[3]	0.36-0.37	0.37-0.38	0.31-0.32	0.32-0.34	0.33[2]	0.39

Table 11: continued.

	N5866[5]	N6223[5]	N6822[5]	N7070[5]	N7590[5]	Range[6]
Tryptophan	0.06	0.07	0.06	0.06	0.08	
Aspartic Acid	0.58	0.68	0.59	0.71	0.67	
Threonine	0.34	0.38	0.34	0.40	0.39	0.32-0.34
Serine	0.47	0.55	0.45	0.53	0.56	
Glutamic Acid	1.54	1.83	1.53	1.61	1.83	
Proline	0.77	0.93	0.79	0.75	1.03	
Glycine	0.34	0.36	0.33	0.40	0.36	
Alanine	0.73	0.85	0.70	0.90	0.83	
Cysteine	0.22	0.22	0.20	0.21	0.23	
Valine	0.40	0.45	0.39	0.48	0.47	0.42-0.46
Methionine	0.23	0.26	0.24	0.27	0.34	0.18-0.19
Isoleucine	0.31	0.35	0.30	0.33	0.34	0.34-0.37
Leucine	1.24	1.46	1.20	1.28	1.47	0.10-0.11
Tyrosine	0.15	0.17	0.16	0.15	0.16	
Phenylalanine	0.47	0.54	0.46	0.46	0.54	0.44-0.45
Histidine	0.31	0.35	0.30	0.37	0.32	
Lysine	0.26	0.26	0.25	0.32	0.25	0.25-0.26
Arginine	0.36	0.40	0.36	0.36	0.38	0.41-0.52

[1]Values are ranges for three samples taken from 3 field sites (ie 1 sample/site) and are expressed as g/100g dry weight.
[2]The same value was obtained for all three samples.
[3]Values for genetically modified corn plants are significantly different to those of control corn plants.
[4]Range is obtained from two values
[5]Single value only.
[6]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

Table 12: Fatty acid profile for Bt-11 hybrids and control corn plants[1].

	Palmitic	Stearic	Oleic	Linoleic	Linolenic
X6534CBR	10.99-11.14	1.99-2.16	27.15-27.36	56.88-57.31	1.16-1.25
N6800	10.78-11.11	2.11-2.24	26.85-26.90	56.81-57.07	1.29-1.43
X4734CBR	10.76-10.97	2.38-2.41	25.93-26.04	57.62-57.86	1.61-1.67
N4640	10.61-10.65	2.45-2.52	26.31-27.06	56.69-57.59	1.56-1.59
N4242[2]	10.76-11.27	2.15-2.31	25.51-25.89	57.32-57.85	1.59-1.66
N5220[3]	13.14	1.89	26.55	55.13	1.40
N5866[3]	9.17	2.18	21.05	64.53	1.28
N6223[3]	11.53	2.01	26.58	57.04	1.24
N6822[3]	12.05	2.27	18.79	64.30	1.18
N7070[3]	10.11	1.77	25.49	59.77	1.19
N7590[3]	9.86	2.17	20.59	64.68	1.18
Range[4]	6–22	1–15	14–64	19–71 56–65[5]	0.5–2

[1]Values are ranges for three samples taken from 3 field sites (ie 1 sample/site) unless otherwise indicated and are expressed as % of fatty acid as a proportion of total fatty acid.
[2]Values are the range for two samples.
[3]Single values given only.
[4]From Weber, "Lipids of the kernel", Chapter 10 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. data
[5]Data from AGPM.

A second statistical analysis of the fatty acid values investigated specifically differences between the genetically modified corn hybrid plants versus the non-modified control hybrids. Small but significant differences at the 5% level were observed for palmitic acid (higher in the genetically modified corn line) and stearic acid (lower in the genetically modified corn line). Using the information from the first analysis on the variation that exists between hybrids, the values determined for the Bt-11 hybrids fall within the range determined for the control hybrids. Additionally, all values are within the range reported in the literature (see Table 7).

(iv) Vitamins and minerals

One-pound (2.24 kg) samples of grain were taken from each of three locations from two Bt-11 corn hybrids N4242-Bt and N4640-Bt and their corresponding near-isogenic non-modified hybrids and analysed for their vitamin and mineral content. The grain was analysed for the minerals copper, magnesium, manganese and zinc as well as the vitamins folic acid, niacin, vitamin B_1 and vitamin B_2 (Table 13). No significant differences (p=0.05) between Bt-11 corn hybrids and their corresponding control hybrids were observed for any of the selected components.

Table 13: Vitamin and mineral profile for Bt-11 and control corn plants[1].

	N4242Bt	Control N4242	N4640Bt	Control N4640
Copper	0.17 ± 0.06	0.17 ± 0.06	0.20 ± 0.0	0.20 ± 0.0
Magnesium	95.7 ± 1.15	91.7 ± 5.51	90.0 ± 1.73	86.3 ± 4.73
Manganese	0.47 ± 0.06	0.43 ± 0.06	0.40 ± 0.0	0.33 ± 0.06
Zinc	1.93 ± 0.06	2.03 ± 0.29	1.77 ± 0.12	1.70 ± 0.10
Folic acid	0.051 ± 0.010	0.045 ± 0.002	0.57 ± 0.03	0.57 ± 0.03
Niacin	8.62 ± 1.32	8.03 ± 0.14	8.96 ± 0.21	9.49 ± 0.41
B_1	1.44 ± 0.10	1.37 ± 0.21	1.26 ± 0.23	1.48 ± 0.15
B_2	0.71 ± 0.04	0.70 ± 0.09	0.72 ± 0.04	0.71 ± 0.02

[1]Values are means of 3 samples, one from each of 3 locations. Minerals are expressed as % and vitamins are expressed in mg/lb.

5.1.3 Study 3: Comparison of nutritional composition of fresh and canned Bt-11 sweet corn

A fourth analysis of Bt-11 corn lines was done, specifically to assess the nutritional value of three Bt-11 sweet corn varieties. Corn was harvested from the Bt-11 sweet corn hybrids, Bt 98-0943, Bt 95-0937 and Bt 95-0941, and from their corresponding near-isogenic non-modified hybrids, grown in 1996 at one location in the United States. Ten ears of each of the hybrids were harvested at prime harvest and analysed as fresh corn on the cob. Corn from each hybrid was canned and also analysed (processed corn analysis).

Fresh and canned sweet corn was analysed for moisture, protein, fat, ash, carbohydrates, fibre, vitamins and minerals (Table 14) according to methods from the Association of Official Analytical Chemists. Given that there was only duplicate analysis of the one sample taken for each line, no statistical analysis was performed.

Comparable nutritional composition was observed between the three Bt-11 sweet corn hybrids and their corresponding isogenic hybrids for both the fresh corn and canned corn.

Table 14: Compositional profile for fresh and canned sweet corn Bt-11 hybrids[1].

Fresh	**Bt 95-0943**	**Jubilee**	**Bt 95-0937**	**Bonus**	**Bt 95-0941**	**Empire**
Moisture (g)	69.88 – 69.78	69.67-69.70	73.65	72.20-72.24	71.15-71.28	70.34-70.56
Protein (g)	3.7-4.09	3.20-4.35	3.75-3.37	3.89-4.06	3.75-3.83	4.17-4.26
Fat (g)	0.76-1.34	1.10-0.97	0.75-0.91	0.81-0.88	0.85-1.18	0.91-1.13
Ash (g)	0.90-0.93	0.91	0.99-1.05	1.00-1.03	1.01-1.02	0.91-0.95
Carbohydrates - total[2] (g)	24.28	24.63	20.94	22.06	22.89	23.36
Calories[2]	111	112	93	100	105	110
Calories[2] from fat	10	9	7	7	10	9
Sugars[2] (g)	6.8	6.31	4.14	4.38	5.21	4.86
Other Carbohydrates[2] (g)	14.71	15.59	13.77	15.01	14.81	16.04
Total Dietary Fibre (g)	2.83-2.71	2.93-2.54	2.61-3.44	2.64-2.70	2.36-3.38	2.38-2.54
Vitamin A[2] (IU)	230	137	280	211	95.8	160
Vitamin C[2] (mg)	0.869	1.63	7.35	6.53	7.25	7.69
Sodium (mg)	9.9-14.2	5.9-7.2	10.0-13.0	3.9-5.3	5.8-7.2	4.9-8.6
Potassium (mg)	293.5-286.2	326.0-322.6	287.6-307.4	292.6-306.7	372.7-391.8	255.6-322.9
Calcium (mg)	3.4-8.6	1.6	0.7-7.1	0.0-0.4	7.1-8.0	0.7-7.1
Iron (mg)	0.49-0.85	0.49-0.56	0.57-0.61	0.6-0.90	0.54-0.63	0.71-0.74
Canned	**Bt 95-0943**	**Jubilee**	**Bt 95-0937**	**Bonus**	**Bt 95-0941**	**Empire**
Moisture (g)	77.81 – 77.83	76.81-76.85	77.66-77.76	77.77-77.80	76.44-76.52	77.80-77.96
Protein (g)	2.95-2.99	2.62-2.97	2.95-3.00	3.09-3.18	2.85-2.94	2.93-3.02
Fat (g)	0.85-1.77	1.02-1.90	1.01-1.09	0.68-0.75	0.83-0.96	0.62-0.85
Ash (g)	0.97-1.01	1.01	0.84-0.85	0.85-0.87	0.85-0.87	0.83-0.83
Carbohydrates - total[2] (g)	16.91	17.92	17.42	17.5	18.87	17.59
Calories[2]	83	87	81	79	86	79
Calories[2] from fat	12	13	9	6	8	7
Sugars[2] (g)	1.8	1.92	1.54	1.3	1.89	1.53
Other Carbohydrates[2] (g)	12.99	13.85	13.38	13.72	14.65	13.56
Total Dietary Fibre (g)	1.99-2.23	2.01-2.29	2.47-2.55	2.41-2.54	2.19-2.48	2.18-2.82
Vitamin A[2] (IU)	175	209	192	185	175	206
Vitamin C[2] (mg)	2.07	2.32	2.25	2.31	2.15	1.99
Sodium (mg)	262.8-285.0	266.1-304.1	245.9-248.0	212.5-230.2	225.7-239.6	191.9-235.6
Potassium (mg)	199.9-202.8	212.2-262.4	210.3-228.4	191.4-202.6	181.1-205.3	176.3-200.2
Calcium (mg)	3.1-8.8	2.4-4.2	0.0-1.8	5.1-8.2	3.7-10.2	5.2-8.2
Iron (mg)	0.29-0.55	0.289-0.614	0.31-0.25	0.23-0.34	0.348-0.387	0.31-0.37

[1]Values are expressed per 100 g serving basis.
[2]Only one sample determined.

5.1.4 Study 4: Analysis of Bt-11 dent corn lines treated with herbicide

An additional study was done to assess the potential effects of herbicide treatment on the major components of the corn kernels. Three Bt-11 hybrids representing different maturity types (Madera, Manuel and Magister) and their isogenic controls were grown in open fields at two locations in France in 1998. Proximate analysis (carbohydrate, protein, fat and fibre), fatty acids and amino acid composition were compared between transgenic crops treated with a glufosinate ammonium herbicide (Liberty®) at a rate of 2.25 L/ha active ingredient at the 3 and 6–7 leaf stages and untreated transgenic and isogenic controls (Table 15). Values presented in this experiment are not directly comparable to values for other experiments because they have been performed by a different laboratory using slightly different methods.

(i) Compositional analyses

No significant differences in composition were found between the treated Bt-11 corn plants and untreated Bt-11 corn plants nor between the untreated Bt-11 corn plants and the unmodified control corn plants (P=5).

Table 15: Compositional analyses for Bt-11 hybrids and control corn plants[1].

	Treated	Untreated	Control
Energy	1441 ± 37	1430 ± 35	1433 ± 29
Carbohydrate	70.0 ± 2.0	69.5 ± 1.5	68.8 ± 1.5
Protein	7.6 ± 0.9	8.2 ± 0.8	8.4 ± 0.8
Fat	3.3 ± 0.6	3.0 ± 0.6	3.3 ± 0.8
Fibre	8.0 ± 1.0	8.0 ± 0.8	7.7 ± 0.2

[1]Values are means of 3 samples, one from each of the hybrids Madera, Manuel and Magister. Values are all expressed as a % except for energy (KJ/100g). *(ii) Amino acid analysis*

Amino acid levels were also analysed (Table 16a). The values for cysteine and tryptophan were not determined. Using the F test, significantly different values were obtained for glutamic acid, proline, alanine, isoleucine and phenylalanine when comparing all three treatments (treated GM, untreated GM and control) (at the P=5 level). In a comparison of the values for treated Bt11 hybrids to the non-modified control hybrids, only the values for proline and alanine were significantly different (lower in treated Bt-11 hybrids than in the control lines).

A breakdown of the values for proline and alanine for each of the three hybrids is shown in Table 16b. The difference between the treated modified and non-modified line was not consistent for all lines and may be a result of variability between the lines. This difference is not considered to raise safety or nutritional concerns.

Table 16a: Amino acid analyses for Bt-11 hybrids and control corn plants[1].

	Treated	Untreated	Control
Aspartic Acid	4690 ± 406	5033 ± 439	4703 ± 142
Threonine	2690 ± 423	2850 ± 165	2690 ± 423
Serine	3537 ± 353	3750 ± 260	3537 ± 353
Glutamic Acid	14533 ± 1595	16233 ± 1626	15700 ± 625
Proline	6967 ± 1154	8367 ± 234	8590 ± 769
Glycine	3047 ± 238	3187 ± 111	2920 ± 26
Alanine	5057 ± 415	5760 ± 606	5500 ± 207
Valine	2963 ± 552	3327 ± 654	3210 ± 183
Methionine	1030 ± 183	1270 ± 122	1170 ± 30
Isoleucine	1717 ± 315	2320 ± 368	2013 ± 42
Leucine	8153 ± 918	9320 ± 1105	8787 ± 420
Tyrosine	3800 ± 573	4240 ± 455	3957 ± 172
Phenylalanine	3163 ± 440	3540 ± 243	3363 ± 280
Histidine	1867 ± 376	2147 ± 170	1853 ± 169
Lysine	1967 ± 228	2223 ± 228	1967 ± 163
Arginine	3257 ± 319	3443 ± 119	3160 ± 236

[1]Values are means of 3 samples, one from each of a different maturity type. Values are all expressed as mg/kg.
[2]Data from L'alimentation des animaux monogastriques: porc, lapin, volailles. INRA 1989, Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data.

Table 16b: Significant differences in amino acid profiles between treated genetically modified hybrids and non-genetically modified hybrids.
[1]

Hybrid	Proline (mg/kg)		Alanine (mg/kg)	
	Bt11 hybrid[2]	Control hybrid	Bt11 hybrid[2]	Control hybrid
Madera	5640	7730	4720	5330
Manuel	7520	9210	5520	5730
Magister	7740	8830	4930	5440

[1]Values are all expressed as mg/kg.

(iii) Fatty acid analysis

Fatty acid levels were also analysed. No significant differences were found between fatty acid values for treated and untreated genetically modified corn plants and also between the untreated modified plant and control lines (P=5%) (Table 17).

Table 17: Fatty acid analyses for treated Bt-11 plants and control corn plants[1].

	Treated	Untreated	Control
Palmitic	12.4 ± 1.9	12.3 ± 1.2	11.2 ± 1.2
Stearic	2.3 ± 0.2	2.4 ± 0.3	2.2 ± 0.2
Oleic	28.0 ± 1.9	27.4 ± 2.0	27.2 ± 1.3
Linoleic	55.1 ± 2.7	55.8 ± 3.0	57.0 ± 2.3
Linolenic	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2

[1]Values expressed as a % of total fatty acids. Values are means of 3 samples, one from each of the hybrids Madera, Manuel and Magister.

5.2 Levels of anti-nutrients

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

5.3 Ability to support typical growth and well-being

In assessing the safety of a genetically modified food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well-being. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food. Where, on the basis of available data, there is still concern or doubt in this regard, carefully designed feeding studies in animals may provide further reassurance that the food is nutritionally adequate. Such studies may be considered necessary where the compositional analysis indicates significant differences in a number of important components or nutrients or where there is concern that the bioavailability of key nutrients may be compromised by the nature of the genetic changes to the food.

The compositional and other data presented in the application are considered adequate for establishing the nutritional adequacy of Bt-11 corn. Additional studies, including animal feeding studies are therefore not required.

5.4 Conclusions regarding nutritional issues

The nutritional qualities of insect-protected Bt-11 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.

There is a long history of safe use of corn. Based on the data submitted in the present application, grain derived from Bt-11 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 Dr Brian Jordan, Associate Professor, Director, Nutrition and Health, Institute of Food Nutrition and Human Health, Massey University, New Zealand, 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

ANZFA 1999. Guidelines for the safety assessment of foods to be included in Standard A18 — Food Produced Using Gene Technology.

Calva JJ, 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.

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.

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

Hammond BG and Fuchs RL 1998. Safety evaluation for new varieties of food crops developed through biotechnology. In: Thomas JA (ed.) Biotechnology and safety assessment. Taylor and Francis, Philadelphia.

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

INRA. 1989. L'alimentation des animaux monogastriques: porc, lapin, volailles. Feedstuffs ingredient analysis table, edition 1996, AEC Table and 1995 UCAAB data

Jones DD and Maryanski JH 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.

Kues U and Stahl U 1989. Replication of plasmids in gram-negative bacteria. Microbiological Reviews 53:491-516.

Lehrer, S.B., W.E. Horner and Reese, G. 1996. Why are some proteins allergenic? Implications for biotechnology. *Crit Rev Food Sci Nutr 36*: 553-564.

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

Mascarenhas D, IJ Mettler, DA Pierce and HW Lowe. 1990. Intron-mediated enhancement of heterolgous gene expression in maize. Plant Mol. Biol. 15:913-920

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

Negrutiu I, R Shillito, I Potryjus, G Biasini and G Sala. 1987. Hybid genes in the analysis of transformation conditions. Plant Molec. Biol. 8:363-373.

Noteborn HP, Bienenmann-Ploum ME, van den Berg JH, Alink GM, Zolla L, Reynaerts A, Pensa M and Kuiper HA 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

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

Sanders PR, Lee TC, Groth ME, Astwood JD and Fuchs RL 1998. Safety assessment of insect-protected corn. In: Thomas JA (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

Stewart GJ and Carlson CA 1986. The biology of natural transformation. Annual Review of Microbiology, 40:211-235.

Strauch E., Wohlleben, W. and A. Puhler. 1988. Cloning of a phosphinothricin N-acetyl transferase gene from *Streptomyces viridochromogenes* Tü4994 and its expression in *Streptomyces lividans* and *Escherichia coli*. Gene 63:65-77

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

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

Wehrmann, A., A. Van Vliet, C. Opsomer, J. Botterman and A. Schulz. 1996. The similarities of bar and pat gene products make them equally applicable for plant engineers. Nature Biotechnology 14:1274-1278

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

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

Wohlleben, W., Arnold, I. Broer, D. Hilleman, E. Strauch and. 1988. Nucleotide sequence of the phosphinothricin-N-acetyltransferase gene from a *Streptomyces viridochromogenes* T494 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[9]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[9] 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 ORGANISATION 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[10], 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*

[10] 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.