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FINAL ASSESSMENT REPORT
(INQUIRY - SECTION 17)

APPLICATION A379

OIL AND LINTERS FROM BROMOXYNIL-TOLERANT COTTON TRANSFORMATION EVENTS 10211 AND 10222

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EXECUTIVE SUMMARY

Background

An application was received from Aventis CropScience Pty Ltd and the Stoneville Pedigreed Seed Company on 30 April 1999 for the approval of oil and linters from genetically modified (GM) cotton plants. The cotton has been genetically modified to be tolerant to the herbicide bromoxynil and is known commercially as OXY or BXN cotton. BXN cotton is not grown in either Australia or New Zealand and the only imported foods derived from the crop are refined oil and cellulose linters.

Issues addressed during assessment

(i) Safety evaluation

Food from bromoxynil tolerant cotton has been evaluated according to the safety assessment guidelines prepared by ANZFA. The assessment considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of antibiotic resistance genes to micro-organisms in the human digestive tract; (3) toxicological issues; and (4) nutritional issues.

On the basis of the available information, it is concluded that food from bromoxynil tolerant cotton is as safe and wholesome as food from other commercial cotton varieties. A detailed report on the safety of food from bromoxynil-tolerant cotton is provided at **Attachment 2** to this report.

(ii) Labelling information for consumers

Under the current Standard A18, which remains in effect until 7 December 2001, food derived from bromoxynil tolerant cotton transformation events 10211 and 10222 does not require labelling as it is regarded as substantially equivalent to food derived from non-genetically modified cotton varieties.

When the amended Standard comes into effect on 7 December 2001, food products containing oil or linters from bromoxynil-tolerant cotton will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

(iii) Public consultation

ANZFA undertook two rounds of public consultation in relation to this application and a total of 66 submissions were received overall – 45 submissions in the first round and 21 submissions in the second round. The majority of submissions were not supportive. Those opposing the application did so primarily on the basis that they perceive GM food to be unsafe. The food safety concerns raised in submissions have been addressed by the safety assessment carried out by ANZFA, the details of which are in **Attachment 2** to this report.

Conclusions

- There are no public health and safety concerns associated with the two genes introduced into bromoxynil-tolerant cotton transformation events 10211 and 10222.

- Oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222 are as safe and wholesome as that from other commercially available cotton varieties.

- On 7 December 2001, food products containing oil or linters from bromoxynil-tolerant cotton will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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

1. BACKGROUND TO THE APPLICATION

Cotton (*Gossypium hirsutum*) has been made tolerant to the herbicide bromoxynil through the transfer of the *oxy* gene from the soil bacterium *Klebsiella pneumoniae* subspecies *ozaenae*. This gene codes for an enzyme, nitrilase, which degrades bromoxynil into non-phytotoxic compounds.

Bromoxynil is primarily used on field corn, wheat and grain crops and is found to be effective against a variety of grasses and broadleaf weeds. Such weeds are also common in cotton crops, however low doses of bromoxynil will kill conventional varieties of cotton. The production of bromoxynil-tolerant (BXN) varieties of cotton will enable bromoxynil-containing herbicides to be used for the post-emergence control of broadleaf weeds in cotton crops.

The BXN cotton lines to which this application relates are the result of crossing the original transformation events – 10222 and 10211 – with elite cotton varieties. Current commercial lines are derived from event 10222, with future lines to be derived from event 10211.

BXN cotton is not grown in either Australia or New Zealand and is only imported as refined oil and cellulose linters for use in various processed foods. Cottonseed oil is premium quality oil that may be used in a variety of foods including frying oil, mayonnaise, salad dressing, shortening, margarine and packing oil. Linters are short fibres removed from the cottonseed during processing and are used in a number of food products including high fibre dietary products, thickeners in ice cream and salad dressings. The linters consist primarily of cellulose (>99%).

The main benefits of BXN cotton are agronomic in nature, and are therefore likely to accrue mainly to the primary producer – cottonweeds should be cheaper and easier to control, with lower expenditure on labour and herbicides. More general benefits may flow to the community as a result of reduced primary production costs.

2. PUBLIC CONSULTATION

Upon receipt of the application, ANZFA completed an information summary, which was released for public comment on 3 November 1999. A total of 45 submissions were received in response to the information summary.

ANZFA then conducted an assessment of the application, including a safety evaluation of the food, taking into account the comments received. A Full Assessment Report was released for public comment on 7 March 2001 resulting in a further 23 submissions being received. ANZFA then finalised its assessment of the application taking into account the public comments. Attachment 5 contains a summary of all submissions received.

3. 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 GM foods, the proposed amendments are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters and were therefore notified to the WTO.

4. ISSUES ADDRESSED DURING THE ASSESSMENT

4.1 Safety assessment

Food from bromoxynil tolerant cotton has been evaluated according to the safety assessment guidelines prepared by ANZFA[1]. The assessment considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues. On the basis of the available information, ANZFA concluded that food from bromoxynil tolerant cotton is as safe and wholesome as food from other commercial cotton varieties. The full safety assessment report can be found at Attachment 2 to this document.

4.2 Labelling of food derived from BXN cotton

On 28 July 2000, the Australia New Zealand Food Standards Council agreed to a revised standard which requires labelling of food where novel DNA and/or protein is present in the final food and also where the food has altered characteristics. The revised Standards (A18 in the Volume 1 of the *Food Standards Code*, 1.5.2 in Volume 2 of the *Food Standards Code*) were gazetted on 7 December 2000 and will come into effect 12 months from the date of gazettal.

Until the new labelling requirements take effect, the provisions in the original Standard A18 apply. Under these provisions, food derived from bromoxynil tolerant cotton transformation events 10211 and 10222 does not require labelling as it is regarded as substantially equivalent to food derived from non-genetically modified cotton varieties.

4.3 Issues arising from public submissions

General issues

Many of the submissions received in both the first and second rounds of public comment 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 more general issues raised can be found in Attachment 6.

[1] ANZFA (1999) Guidelines for the safety assessment of foods to be included in Standard A18 – food produced using gene technology.

However, in light of the rapid developments in this field, some general issues raised in the second round of public consultation have been addressed again taking into account more recent outcomes of intensive deliberations on gene technology issues, such as the publishing of the report of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on "New Biotechnology Food and Crops: Science, Safety and Society", and the deliberations of various Codex Alimentarius and OECD Task Forces and FAO/WHO Expert Consultations.

(i) ANZFA's processes

Several criticisms of ANZFA's general processes for the risk assessment of GM foods were raised by submitters including: the Public Health Association of Australia (PHAA), the GeneEthics Network, the National Council of Women of Australia (NCWA), Consumers' Institute of New Zealand, GE Free New Zealand, Paul Elwell-Sutton, Sandra Jacobs, Brian Lister and Lorraine Leader, Claire Bleakely, Julian Yates, Oraina Jones, Leila Huebner and Dr Kate Clinch-Jones.

Response

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

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

(ii) *Substantial equivalence*

Several submitters (PHAA, GeneEthics, Dr Kate Clinch-Jones, Consumer's Institute of New Zealand) raised concerns with the use of the concept of substantial equivalence

Response

On the issues of the appropriate use of the concept of substantial equivalence, ANZFA reiterates that it uses this tool as a starting point in the safety assessment process for GM foods as supported by international bodies such as Codex Alimentarius, OECD, FAO/WHO, other regulators such as the UK, the EU, Japan, Canada and the recent report of the Canadian Royal Society.

(iii) *Antibiotic resistance marker genes*

Several submitters (PHAA, GeneEthics Network, Dr Kate Clinch-Jones) raised some concerns about the use of antibiotic resistance marker genes (ARMGs) in the development of GM foods. In particular, the PHAA submission asserts that ANZFA is "remarkably out-of-step with scientific opinion…" and quotes the JETACAR Report as evidence of this.

Response

The JETACAR Report states (page 117 referring to a specific gene called *nptII*) that the use of antibiotic resistance genes in GM foods is unlikely to contribute in any significant way to the spread of antibiotic resistance in humans. The issue of the use of antibiotic resistance marker genes in GM foods was discussed at the recent Ministerial Council meeting held in Adelaide in late July 200. At that meeting, Professor John Turnidge, former Chair of JETACAR and now Chair of the NHMRC Expert Advisory Group on Antibiotic Resistance (EAGAR) appeared at the Council meeting to present his expert advice on the safety of the use of ARMGs in GM foods in support of ANZFA's views on this issue.

(iv) *Source of data*

Some submitters (PHAA, GeneEthics) raised concerns over the independence of the source of the data submitted to ANZFA

Response

It is a requirement of the ANZFA assessment process that raw data from experiments supporting the safety of a GM food are submitted to ANZFA for assessment. These data are assessed in detail by ANZFA scientists and then the assessment report undergoes a robust process of internal review by ANZFA's own scientific experts and external review by ANZFA's expert panel and senior health officials from State and Territory and New Zealand Health Departments. The quality and sources of the data supplied to ANZFA in support of applications for approval of GM foods was the subject of particularly intense scrutiny during ANZFA's evidence at the New Zealand Royal Commission on Genetic Modification. ANZFA submitted a full data package (15 volumes of raw data on Roundup Ready Soybeans) to the Commission for inspection. The Commission states that it looked closely at the quality of this data and came to the view that ANZFA did receive and assess raw data and that its processes were not wanting in this regard.

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

(v) *Imported GM foods versus GM crops*

Some submitters (GeneEthics Network, National Council of Women of Australia) have argued that approvals for GM foods for import is a tacit approval for the GM crop to be grown in Australia

Response

The regulatory framework for approval by ANZFA of safety of GM foods (imported foods and derived from GM crops grown in Australia) is separate from that of the Office of the Gene Technology Regulator (OGTR), which has responsibility for approving the environmental release of GM crops. ANZFA's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard A18 of the Food Standards Code (Standard 1.5.2 of the joint Australia New Zealand Food Standards Code) is not, and would never be, a tacit approval for the environmental release of the crop in Australia since the environmental issues are completely separate and entirely different to food safety issues.

Specific issues

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

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

(i) Toxicity of bromoxynil breakdown products

Both the New Zealand Ministry of Health and the Public and Environmental Health Service in Australia 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. This is of relevance to bromoxynil-tolerant cotton. The Consumers' Association of South Australia Inc. & National Council of Women of Australia raised similar concerns, suggesting that the US FDA had not adequately assessed the persistence and toxicity of bromoxynil, and that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself.

Response

This issue has been fully addressed in the safety assessment report (**Attachment 2**).

Briefly, nitrilase hydrolyses bromoxynil (3,5-dibromo-4-hydorxybenzonitrile) into 3.5-dibromo-4-hydroxybenzoic acid (DBHA) and ammonia. As DBHA is a by-product specifically resulting from the activity of the introduced nitrilase it was necessary to include a consideration of its potential toxicity in the safety assessment. Moreover, it is reported that significant residues of DBHA can be present on BXN cotton; although it is not clear to what extent these residues persist in refined oil and linters.

The potential toxicity of DBHA was recently considered by the United States Environment Protection Agency (US EPA) in its re-evaluation of bromoxynil. The US EPA examined the chemical structures of bromoxynil and DBHA and, based on this examination, concluded "there was no concern that DBHA would exhibit significant toxicity over that of the parent bromoxynil", which they consider poses a negligible human health risk. The chemical structure of DBHA is such that it is less fat soluble than bromoxynil, and this is expected to reduce the amount of residue present in the oil.

There is no maximum residue limit (MRL) set for bromoxynil in cottonseed in either Australia or New Zealand, and nor is there a Codex MRL for bromoxynil. The absence of an MRL in both Australia and New Zealand, as well as a Codex MRL, means that in Australia residues of either bromoxynil or its metabolites are not permitted in food products derived from cotton, and in New Zealand residues are not permitted above 0.1ppm.

(ii) Allergenic effects of novel genes

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

Response

The safety assessment carried out by ANZFA has addressed the issue of the potential allergenicity of nitrilase in some depth. Data was evaluated on a comparison of the amino acid sequence of nitrilase to that of known allergens, its resistance to acid and protease digestion, and its presence in the food as consumed. Nitrilase does not come from a source that is known to be allergenic and has none of the characteristics that are common to food allergens, nor does it have any significant amino acid sequence similarity to known allergens. This, combined with the fact that refined oil and linters are essentially devoid of protein, means that in the case of BXN cotton, nitrilase has very limited potential to become a food allergen.

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

(i) New data

Aventis CropScience submitted additional data from a study done on April 12, 2001 on the levels of nitrilase and NPTII in leaf and seed material derived from bromoxynil-tolerant cotton event 10211.

Response

Analyses of nitrilase and NPTII levels in leaf and seed material of event 10211 had not been submitted as part of the original application. Such data however was not considered essential for safety assessment purposes, as data had been provided on novel protein expression levels in the main food product – the oil. The new data that has now been provided by Aventis CropScience for novel protein expression levels in leaf and seed material from event 10211 are consistent with those levels determined for event 10222. The safety assessment report has been amended to incorporate the additional data provided. The assessment of the new data has not resulted in any changes to the conclusions of the safety assessment.

(ii) Use of cottonseed flour for human consumption

Aventis CropScience submitted that the statement made by ANZFA in the draft risk analysis report that "whole cottonseed, cottonseed meal, and cottonseed flour are not used for human consumption" is not completely correct.

Aventis has advised that cottonseed flour is approved for human consumption in the United States. It was approved with the specification that free gossypol (a natural toxicant of cotton) not exceed 450 ppm. At present however no cottonseed flour products are produced for human consumption in the US. This is apparently due to both economic and technical factors preventing any significant use of cottonseed in this form.

Aventis further advised in their submission that food grade cottonseed flour made from finely ground cottonseed meal, specifically processed to minimise the toxicological properties of gossypol, has been marketed internationally where it is used as a low cost, high quality protein ingredient in special products including "incaparina" developed by the Institute of Nutrition of Central America and Panama (INCAP) to help ease malnutrition in developing countries where cottonseed meal is inexpensive and readily available. Incaparina or similar high protein mixtures have been marketed in Nicaragua, El Salvador, Columbia and India.

Response

The safety assessment report has been updated to reflect the latest information about the use of cottonseed flour for human consumption. In addition, ANZFA has made enquiries with a number of manufacturers to ascertain whether cottonseed flour is being used in any products available for sale in Australia and New Zealand, in particular in any infant products. All manufacturers contacted to date have confirmed that they do not use cottonseed flour.

In relation to the GM varieties of cotton that have been assessed, including BXN cotton, ANZFA has thus far only recommended approval for the oil and linters. This is because of the potential for meal and flour derived from cottonseed to contain toxic substances such as gossypol. This is not a concern for oil and linters.

In considering whether approval should be extended to cottonseed products, other than oil and linters, ANZFA would first need to determine if such products were novel foods according to the definition in Standard 1.5.1 Novel Foods. This standard prohibits the sale of novel foods unless they have first been assessed as safe and added to the table in the standard. Whether or not cottonseed flour would be classed as a novel food would depend on the extent to which there is a history of significant human consumption by the broad community in Australia and New Zealand, and also whether there is sufficient knowledge in the broad community to enable safe use in the form or context in which it is presented. These are questions that would apply to cotton broadly, not just GM varieties. In the interim, ANZFA will continue to only recommend approval for oil and linters from cottonseed as these foods have been routinely used in foods and have an established history of safe use.

(iii) Compositional analyses

The Public Health Association of Australia Inc (PHAA) and the National Council of Women of Australia Inc. Ltd. commented that some of the components of the genetically modified cotton lines were statistically different to the control line and therefore that the GM lines cannot be considered as comparable or 'substantially equivalent' to the control cotton line.

Response

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

The use of published ranges and historical control data in safety assessment studies is standard procedure in the interpretation of biological and analytical components of variation. Although the most appropriate control group for interpretative purposes is always the concurrent control, there are instances in which the use of historical control information can aid an investigator in the overall evaluation of safety data. Studies suggest that statistically significant findings that are not biologically or toxicologically important will be present in many safety assessment studies with a standard design. Over reliance on the result of standard prepackaged statistical analyses for determining the presence of toxicologically or biologically significant findings can lead to misinterpretation of data. It is well recognized that sound judgment must be applied to study findings using appropriate statistical analyses as a tool for pattern recognition.

(iv) Acute oral toxicity study of the nitrilase protein

The PHAA expressed concern that measurements other than gross pathology (e.g. microscopy or biochemistry) had not been done on animals subjected to acute oral toxicity testing of the nitrilase protein and also questioned the manner in which the study had been reported by ANZFA (i.e. ANZFA did not report individual body weight data for the animals in the report and no description was provided as to what would constitute "clinical signs").

Response

ANZFA requires, as with all methods of analysis, that acute oral toxicity studies are conducted according to international guidelines. For example, the OECD Guidelines for Testing of Chemicals ("Acute Oral Toxicity"). These are based on a number of different documents, including: the *Principles and Methods for Evaluating the Toxicology of Chemicals*, Environmental Health Criteria 6, World Health Organisation; the *Principles and Procedures for Evaluating the Toxicity of Household Substances*, National Academy of Sciences; and *A European Community Study on an Intercomparison Exercise on the Determination of Single Dose Oral LD50 in Rats*, Commission of the European Communities.

The OECD guidelines require that animals should be carefully examined at least once a day for 'clinical signs'. Clinical signs include changes to the skin and fur, eyes and mucous membranes of the animal as well as respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behaviour patterns. The guidelines advise that particular attention should be directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Individual body weights of animals should be determined shortly before the test substance is administered, and usually weekly thereafter and at death. Any animals that die during the test are subject to necropsy as are those animals that survived and have been sacrificed at the end of the test.

In terms of examination after death, the guidelines recommend that necropsy of all animals should be carried out and all "gross pathological" change be recorded. Gross pathology is the first step in examination of organs and refers to clear and obvious changes, abnormalities or lesions visible upon inspection. Microscopic examination of organs as well as measurement of biochemical parameters is usually only undertaken when clinical signs are evident and/or there is evidence of gross pathology. Animals not showing any clinical signs or gross pathology are generally not required to be examined in greater detail.

In this respect, the studies supplied by the applicant have been consistent with such guidelines and have been reported appropriately.

All the raw data for the acute oral toxicity study with nitrilase is held on file at ANZFA and is a matter of public record – the data being available both for inspection as well as copying by any member of the public. ANZFA has fully assessed all the raw data for the acute toxicity study and provided a summary of the findings, plus ANZFA's conclusions, in the assessment report. Given the large volume of data generally provided with toxicity studies it would be impractical to detail all this information in the safety assessment report.

4.4 Risk management

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

On the basis of the conclusions of the safety assessment, together with a consideration of the public submissions, it is recommended that Table 1 to Clause 2 of Standard A18/Standard 1.5.2 be amended to include oil and linters from BXN cotton events 10211 and 10222. The recommended variation is provided in Attachment 1.

In relation to the concerns raised in the public submissions with regard to gene technology and GM food, ANZFA has prepared a public discussion paper on the safety assessment process for GM food[2]. This is widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

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

4.5 Regulatory impact assessment

The benefits and costs associated with the proposed amendment to the *Food Standards Code* have been analysed in a Regulatory Impact Assessment (see **Attachment 3**). The benefits of the proposed amendment to approve food from BXN cotton primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

5. CONCLUSIONS

- There are no public health and safety concerns associated with the two genes introduced into bromoxynil-tolerant cotton transformation events 10211 and 10222.

- Oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222 are equivalent to that from other commercially available cotton in terms of their safety and nutritional adequacy.

- On 7 December 2001, food products containing oil or linters from bromoxynil-tolerant cotton will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety assessment report
3. Regulatory impact assessment
4. World Trade Organization agreements
5. Summary of public submissions
6. General issues raised in public submission
7. Statement of Reasons

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A379 – OIL AND LINTERS DERIVED FROM BROMOXYNIL-TOLERANT COTTON TRANSFORMATION EVENTS 10211 AND 10222

To commence: on gazettal

The Food Standards Code is varied by:

[1] *Standard A18 of Volume 1 and Standard 1.5.2 of Volume 2 are varied by inserting in Column 1 of the Table to clause 2 -*

Oil and linters derived from bromoxynil-tolerant cotton transformation events 10211 and 10222.

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

A379 - OIL AND LINTERS DERIVED FROM BROMOXYNIL-TOLERANT COTTON TRANSFORMATION EVENTS 10211 AND 10222

SUMMARY AND CONCLUSIONS

Oil and linters from bromoxynil-tolerant cotton has been assessed by ANZFA to evaluate its safety for human consumption. A number of criteria are used in this assessment including a characterisation of the transferred genes, the modifications at the DNA, protein and whole food levels, compositional analyses, and the potential allergenicity and toxicity of the newly expressed proteins. This enables the intended as well as any significant unintended changes to be identified, characterised and evaluated for their safety.

Nature of the genetic modification

Cotton transformation events 10211 and 10222 were made tolerant to the herbicide bromoxynil through the *Agrobacterium*-mediated transfer of a single copy of the *oxy* gene from the soil bacterium *Klebsiella pneumoniae* subspecies *ozaenae.* The bromoxynil-tolerant cotton lines derived from these transformation events are known commercially as either BXN or OXY cotton.

The *oxy* gene is responsible for the production of the enzyme nitrilase that hydrolyses bromoxynil to an inactive, non-phytotoxic compound. Low concentrations of bromoxynil kill conventional cotton varieties therefore the purpose of the genetic modification is to enable bromoxynil-containing herbicides to be used for weed control in cotton crops.

Both cotton transformation events also each contain a single copy of the *nptII* gene that was used as a marker for selection of transformed plant lines during the cotton transformation procedure. The *nptII* gene codes for the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the antibiotics neomycin, kanamycin, and geneticin (G418).

Both genes are stably integrated into the cotton genome and the bromoxynil-tolerant trait is stably maintained from one generation to the next in a variety of different genetic backgrounds.

General safety issues

Cotton (*Gossypium hirsutum*) is grown primarily for the value of its fibre; cottonseed (and its processed products) is very much a by-product of the crop. Cottonseed itself is not used as a food for human consumption because it contains naturally occurring toxic substances. These toxic substances can however be removed or reduced by the processing of the cottonseed into various fractions of which it is really only the oil and linters that are used for human consumption. Both the oil and linters have been routinely used in foods and have an established history of safe use. The types of food products likely to contain cottonseed oil are frying oils, mayonnaise, salad dressing, shortening, and margarine. After processing, linters, which are >99% cellulose, may be used as high fibre dietary products, and as thickeners in ice cream and salad dressings.

Transformation events 10211 and 10222 express two novel proteins — nitrilase and NPTII. While both proteins can be readily detected in leaf tissue as well as in cottonseed and meal, neither could be detected in crude cottonseed oil at a detection limit of 0.1 ppm.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells in the human digestive tract. Much of the concern in this regard is with antibiotic resistance genes. In the case of transformation events 10211 and 10222, it was concluded that the *nptII* gene would be extremely unlikely to transfer to bacteria in the human digestive tract because refined oil and linters are essentially devoid of DNA. Even were DNA to be present in refined oil and linters, horizontal DNA transfer would be extremely unlikely because the number and complexity of steps that would be required to take place consecutively. Regardless of the above, the human health impacts of such a transfer would be negligible anyway because kanamycin resistant bacteria are already commonly found in the human digestive tract and in the environment.

Toxicological issues

The levels of naturally occurring toxins in transformation events 10211 and 10222 were assessed as well as the potential toxicity and allergenicity of the two novel proteins — nitrilase and NPTII. The potential toxicity of 3,5-dibromo-4-hydroxybenzoic acid (DBHA), a by-product of the detoxification of bromoxynil by nitrilase, was also considered in the assessment.

Cotton contains two naturally occurring toxins that are of interest – gossypol and cyclopropenoid fatty acids. Refined cottonseed oil is generally free of gossypol but generally contains small amounts (typically <1.0%) of cyclopropenoid fatty acids. Compositional data from several field trials conducted with plants derived from transformation events 10211 and 10222, both sprayed with herbicide and unsprayed, demonstrates that the gossypol and cyclopropenoid fatty acid levels in BXN cotton are equivalent to those of conventional cotton varieties and that these levels are unaffected by herbicide spraying.

In relation to the potential toxicity and allergenicity of nitrilase and NPTII, it was concluded from the protein expression data that humans are highly unlikely to be exposed to either protein through the consumption of refined cottonseed oil and cellulose products from BXN cotton. Moreover, the absence of toxicity of nitrilase and NPTII has been confirmed through acute toxicity testing in mice, and neither protein also demonstrates any potential to become a food allergen.

In relation to DBHA, the evidence indicates that this compound is likely to be no more toxic than its parent compound, bromoxynil, which is considered to pose negligible risk to human health at expected exposure levels.

Nutritional issues

Detailed compositional analyses were done to establish the nutritional adequacy of the food products derived from BXN cotton and also to demonstrate that unintended changes to the composition of the cotton plants had not occurred as a result of the genetic modification.

Analyses done were: fibre, moisture, fat/oil, ash and protein content of cottonseed; nitrogen, protein and amino acid content of cottonseed meal; and fatty acid and tocopherol content of crude cottonseed oil. Analyses were done of both herbicide-sprayed and unsprayed plants. The most important analyses, in terms of nutritional adequacy, were those of the oil which is the principal human food product.

On the basis of the data provided, cotton transformation events 10211 and 10222 were found to be compositionally no different to other commercially available cotton varieties.

Conclusion

Based on the data submitted in the present application, refined oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222 are as safe and wholesome as refined oil and linters from other commercially available cotton varieties.

1. BACKGROUND

Rhone Poulenc Rural Australia Pty Ltd (now trading as Aventis CropScience Pty Ltd after its merger with AgrEvo) and the Stoneville Pedigreed Seed Company (formerly owned by Monsanto Co.) have made a joint application to ANZFA to amend Standard A18 of the Australian *Food Standards Code* to include food derived from cotton which has been genetically modified to be tolerant to the oxynil family of herbicides comprising bromoxynil and ioxynil. The genetically modified cotton is known commercially either as OXY cotton or BXN cotton.

The oxynil family of herbicides act by inhibiting electron transport in photosystem II in plants. Inhibition of electron transport causes superoxide production resulting in the destruction of cell membranes and an inhibition of chlorophyll formation, leading to plant death (Comai and Stalker 1986). Tolerance to either bromoxynil (3,5-dibromo-4-hydorxybenzonitrile) or ioxynil (3,5-di-iodo-4-hydroxybenzonitrile) is achieved through expression in the plant of a bacterial nitrilase enzyme that hydrolyses the herbicide to an inactive, non-phytotoxic compound. The nitrilase is derived from the bacterium *Klebsiella pneumoniae* subspecies *ozaenae* which is responsible for rapidly degrading bromoxynil in soil. The nitrilase enables the bacterium to utilise bromoxynil as a sole source of nitrogen (McBride *et al* 1986).

The oxynil herbicides are primarily used on field corn, wheat and grain crops to control a variety of grasses and broadleaf weeds. Low concentrations of bromoxynil-containing herbicides kill conventional cotton varieties. Therefore, current weed control practices in cotton involve either prophylactic pre-plant, pre-emergence herbicide application or post-directed herbicide sprays to avoid crop injury. The rationale for engineering cotton to be bromoxynil-tolerant is to enable bromoxynil-containing herbicides to be used for the post-emergence control of dicotyledonous weeds in cotton crops.

The major human food products obtained from cotton are refined oil and linters. Cottonseed oil is a premium quality oil that may be used in a variety of foods including frying oil, mayonnaise, salad dressing, shortening, margarine and packing oil. Linters are short fibres removed from the cottonseed during processing (delinting).

After extensive processing at alkaline pH and high temperatures, the linters may be used as high fibre dietary products, and thickeners in ice cream and salad dressings. The linters consist primarily of cellulose (>99%).

The BXN cotton lines currently in commercial production, or planned for future commercial release, are derived from transformation events 10222 (current lines) and 10211 (future lines). The currently available BXN cotton lines include BXN 47 and BXN 16. The first of these, BXN 47 cotton, was commercialised in 1997. Therefore, cottonseed oil derived from BXN cotton or processed products containing cottonseed oil or linters derived from BXN cotton may have been imported into Australia and New Zealand since that time.

2. DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modification

Cotton (*Gossypium hirsutum*) line Coker 315 was transformed with plasmid pBrx75 (see Figure 1 below), using the method of *Agrobacterium tumefaciens*-mediated transformation as described by Fillatti *et al* (1990) and Radke *et al* (1990). The transformation resulted in the selection of nine independent transformant events, two of which, 10211 and 10222, are the subject of this application and have been, or will be, used to derive the BXN cotton lines for commercial production.

2.2 Function and regulation of the novel genes

The transformation of cotton with plasmid pBrx75 resulted in the transfer of two gene expression cassettes denoted *oxy* and *nptII*. These gene expression cassettes are described in Table 1 below.

Table 1: Description of the gene expression cassettes in pBrx75

Cassette	Genetic element	Source	Function
oxy	35S promoter	The cauliflower mosaic virus (CaMV) 35S promoter region (Gardner *et al* 1981).	A promoter for high level constitutive (occurring in all parts of the plant and at all stages of development) gene expression in plant tissues
	oxy	Gene isolated from *Klebsiella pneumoniae* subspecies *ozaenae* encoding the enzyme nitrilase (Stalker *et al* 1988).	Inactivates the herbicide bromoxynil and confers bromoxynil tolerance when expressed in plants.
	tml 3'	The 3' non-translated region of the *tml* gene from *Agrobacterium tumefaciens* plasmid pTiA6 (Barker *et al* 1983).	Contains signals for termination of transcription and directs polyadenylation.
nptII	35S promoter	as above	as above
	nptII	The gene coding for neomycin phosphotransferase II from Tn5 in *Escherichia coli* (Beck *et al* 1982).	Confers resistance to the antibiotics kanamycin and neomycin. Used as a selectable marker for plant transformation (Horsch *et al* 1984, DeBlock *et al* 1984).
	tml 3'	as above	as above

The oxy gene

The *oxy* gene was isolated from the soil bacterium *Klebsiella pneumoniae* subsp. *ozaenae* and encodes an enzyme that metabolises the herbicide bromoxynil (Stalker and McBride 1987). The *oxy* gene has been fully sequenced and its encoded enzyme, nitrilase, has been fully characterised (Stalker *et al* 1988). When transferred into plants, the gene, through its encoded protein, confers tolerance to the oxynil family of herbicides including bromoxynil and ioxynil. The mechanism of tolerance involves the detoxification of the herbicide by the nitrilase enzyme. This degradation effectively inactivates the herbicide and enables the normally bromoxynil-sensitive plant to survive and grow when treated with applications of the herbicide.

The nptII gene

The *nptII* gene is widely used as a selectable marker in the transformation of plants (Kärenlampi 1996). The gene functions as a dominant selectable marker in the initial, laboratory stages of plant cell selection following transformation. It codes for the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the aminoglycoside antibiotics, neomycin, kanamycin, and geneticin (G418). The *nptII* gene is transferred along with the *oxy* gene, enabling those plant cells successfully transformed with the *oxy* gene to grow in the presence of kanamycin. Those cells that lack the *nptII* gene, and hence the *oxy* gene, will not grow and divide in the presence of kanamycin.

Other genetic elements

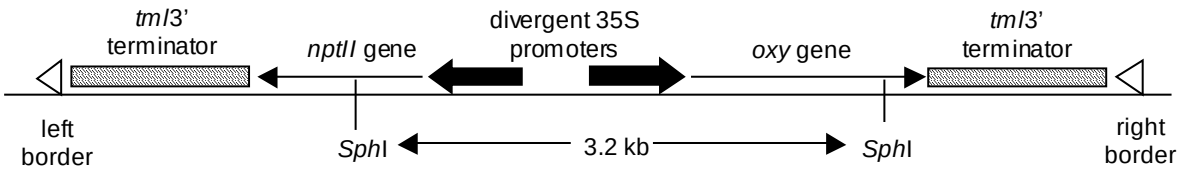
The plasmid pBrx75 is a 16.1 kb double border binary plant transformation vector derived from the *Agrobacterium* binary vector pCGN1559 (McBride and Summerfelt 1990). The plasmid contains well characterised DNA segments required for its selection and replication in bacteria as well as the right and left borders delineating the region of DNA (T-DNA) which is transferred into the plant genomic DNA. This is the region into which the gene of interest, and the plant cell selectable marker, is inserted. DNA residing outside the T-DNA region does not normally get transferred into plant genomic DNA (Zambryski 1992). The additional genetic elements contained within pBrx75 are described in Table 2 below and a map of the T-DNA region is provided in Figure 1. The host for all DNA cloning and vector construction was *E. coli* strain MM-294, a derivative of the common laboratory *E. coli* K-12 strain.

Table 2: Description of other genetic elements contained within pBrx75

Genetic element	Source	Function
aac (resides outside the T-DNA)	Gene derived from *Escherichia coli* coding for gentamicin-3-N-acetyltransferase (Hayford *et al* 1988, Carrer *et al* 1991).	Confers resistance to the antibiotic gentamicin. Used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells.
LB	A DNA fragment of the pTiA6 plasmid containing the 24 bp nopaline-type T-DNA left border (LB) region from *A. tumefaciens* (Barker *et al* 1983).	Terminates the transfer of the T-DNA from *A. tumefaciens* to the plant genome.

pRi *ori* (resides outside the T-DNA region)	Origin of replication region derived from the *Agrobacterium rhizogenes* plasmid pRiHRI (Jouanin *et al* 1985).	Allows the binary vectors to be stably maintained in *A. tumefaciens* without antibiotic selection.
***ori-322/rop* region** (resides outside the T-DNA region)	A 1.8 kb segment of the plasmid pBR322 which contains the origin of replication region and the *bom* site for the conjugational transfer.	Allows for autonomous replication of plasmids in *E. coli* as well as their conjugal transfer into *A. tumefaciens* cells (Bolivar *et al* 1977, Sutcliffe 1978).
RB	A DNA fragment from the pTiA6 plasmid containing the 24 bp nopaline-type T-DNA right border (RB) region from *A. tumefaciens*. (Barker *et al* 1983).	The RB region is used to initiate T-DNA transfer from *A. tumefaciens* to the plant genome.

Figure 1: Map of the T-DNA region of pBrx75



2.3 Characterisation of the genes in the plant

Selection of the plant lines

Initial screen of T_1 plants

The plants resulting from the *Agrobacterium*-mediated transformation (the T_1 generation) were tested for the presence of a functional T-DNA insert by a bromoxynil dab assay. Observations of plant morphology were made, including (but not limited to) leaf size, internode distance, plant stature, flower morphology, fertility of flowers, relative flower and boll abortion rates, boll size, seed per boll and total seed per plant. This information was then used to select individuals for field-testing and for comparison with field observations on subsequent generations. Infertility, due to flower structure, pollen inviability, premature flower abortion or boll abortion, are morphological criteria used to drop non-commercial lines from the product development program before contained field testing of the T_2 generation.

Production and analysis of T_2 material

A total of nine transformation events passed the initial T_1 screen (events 10103, 10109, 10206,10208, 10209, 10211, 10215, 10222, and 10224) and were self-fertilised to produce T_2 seed. T_2 progeny were then planted in the field and sprayed with Buctril® (a proprietary herbicide containing bromoxynil as the active ingredient) when the plants were between a two leaf stage and 12 inches tall.

In early spray experiments, four rows of T_2 progeny from each event were planted and each row was sprayed at different herbicide rates up to 3 times the recommended field application rate. Only plants that exhibited tolerance to Buctril® at 3 times the recommended field application rate were selected for further development.

Counts were made of tolerant (alive, no symptoms) and susceptible (dead) individuals to Buctril® to determine the segregation ratio of the trait. Individual tolerant T_2 plants were selected from agronomically promising events that segregated in a 3:1 or 15:1 ratio of tolerant to susceptible to bromoxynil (consistent with one or two independently segregating loci, respectively). Seed from each individual plant was then harvested and maintained separately. Ideally, a single genetic locus is preferred because, while not essential for the performance of the cotton or the *oxy* gene, it simplifies the breeding of the trait into other elite commercial cultivars.

Production of T_3 material

Progeny rows from each T_2 selection were grown in the next field generation and were again sprayed with Buctril®. Events which segregated 3:1 in the T_2 generation are expected to produced progeny rows, one third of which are 100% tolerant to Buctril® (indicating the individual parent was homozygous for the *oxy* gene) and two thirds segregating 3:1 (indicating that the individual parent was heterozygous for the *oxy* gene).

Events that segregated 15:1 in the T_2 generation are expected to produce progeny rows that segregate 3:1, 15:1 or that are 100% tolerant. In this case, the rows segregating 3:1 were the ones of most interest because they have inherited only one of the two loci originally in the T_1 plant. Selections from these 3:1 progeny rows were harvested to identify T_4 homozygous lines, as was the case with the events segregating 3:1 in the T_2 generation.

Homozygous T_3 progeny rows from 3:1 segregating T_2 events were evaluated for potential agronomic acceptability. Individual plant selections were made and these were advanced to the next generation. Advanced progeny rows were then grown from these selections. A bulk harvest of the remaining plants from promising progeny rows were also made for initial yield and quality testing. In all, five events were selected for further testing. The best homozygous rows were selected from each of these events and individual plant selections were made from within each selected row.

Subsequent generations

In subsequent generations, more stringent selection based on yield, fibre quality and good agronomic performance (including earliness, height, and pest and disease resistance) were used to further select and reduce the candidates for commercial release.

Thus, although nine independent transformation events were originally selected, this application only relates to events 10211 and 10222. These events have been, or are being used to derive the BXN lines for commercial release.

Characterisation of the inserted T-DNA

Progeny of the nine independently derived transformation events were analysed, using a combination of genetic analyses and Southern blot analysis, to characterise the genes from the T-DNA region of pBrx75 that had been inserted into the plant genome. The data for events 10211 and 10222 are presented below.

Genetic analysis

As described above, the total number of functional (bromoxynil-tolerant) loci that have been integrated into an individual transformed plant can be determined by spraying seedlings with the herbicide Buctril® and determining the Mendelian segregation ratios of the bromoxynil tolerant trait. Progeny of single plants are grown and sprayed with the herbicide and plants whose progeny segregate with a ratio of 3 tolerant to 1 susceptible are assumed to contain one functional locus or insertion site. This method cannot however determine the number of copies of the *oxy* gene that have been inserted into the single site, nor can it be used to determine if there has been an insertion of non-functional copies of the *oxy* gene because this method detects functional expression of the trait only. The results of the spray analyses of the T_2 generation of events 10222 and 10211 are provided in Table 3 below.

Table 3: Segregation ratios[1] for events 10211 and 10222 sprayed with Buctril®

	Event 10211	**Event 10222**
1.5 lb/acre:		
No. of tolerant plants	61	53
No. of susceptible plants	18	17
Chi-Square value 3:1	0.21	0.02
Chi-Square value 15:1	36.86	38.86
3.0 lb/acre:		
No. of tolerant plants	62	66
No. of susceptible plants	13	18
Chi-Square value 3:1	2.35	0.57
Chi-Square value 15:1	15.72	33.03
4.5 lb/acre:		
No. of tolerant plants	65	69
No. of susceptible plants	22	17
Chi-Square value 3:1	0.00	1.26
Chi-Square value 15:1	53.81	26.82
All spray rates:		
No. of tolerant plants	188	188
No. of susceptible plants	53	52
Chi-Square value 3:1	1.16	1.42
Chi-Square value 15:1	101.92	97.35

[1] Chi-Square values of 3.84 or less fit the expected ratios with a 95% level of confidence

The results of these analyses show that the bromoxynil tolerant trait in events 10222 and 10211 segregates as a single functional locus. Further analysis of the transferred T-DNA was done using Southern blot analysis (Southern 1975).

Southern blot analysis

Southern blotting is a sensitive technique that enables the detection and characterisation of specific sequences among DNA fragments separated using gel electrophoresis.

For events 10222 and 10211 the Southern analyses were used to characterise the inserted T-DNA in terms of insert number (number of integration events), copy number (number of T-DNA copies at a particular genetic locus), insert integrity (gene size, composition and linkage), and sequences outside the T-DNA borders (including the gentamicin resistance gene). Genomic DNA was isolated from leaf tissue of non-transformed control *G. hirsutum* (var. Coker 315) plants and from the homozygous T_3 progeny of BXN cotton events 10222 and 10211 transformed with pBrx75.

To determine the copy number of each of the genetic elements genomic DNA was digested with the restriction enzyme *Sph*I and probed with DNA corresponding to each of the regions of interest (see Table 4). Because the *Sph*I restriction sites in the T-DNA were known (see Figure 1), the size of the hybridising fragments that would be expected to result from a single copy inserted at a single genomic location could be predicted. The expected fragment sizes are detailed in Table 4 below.

Table 4: Expected fragment sizes for a single copy of T-DNA inserted at a single genomic location

Probe	Expected fragment size
oxy	3.2 kb + 1 larger right border fragment
nptII	3.2 kb + 1 larger left border fragment
tml 3'	right and left border fragments
35S	3.2 kb fragment

Hybridising DNA fragments of the expected size (as indicated above), without any additional fragments, were detected using Southern analysis for both 10222 and 10211 indicating that a single copy of each genetic element is present at a single insertion site in the genome. These results confirm the findings of the genetic analysis above. This experiment also demonstrates physical linkage between the *oxy* and *nptII* genes (both genes inserted at the same site within the genome) because of the common 3.2 kb fragment identified when either the *oxy* or the *nptII* probe is used.

To further confirm the number of insertion sites as well as the T-DNA copy number, analyses were done to determine the number of border fragments that represent the junctions of the inserted genes with plant DNA. A plant would be suspected of having multiple copies of T-DNA at an insertion site if the number of right border fragments was not equal to the number of left border fragment, and/or if the intensity of the hybridisation signal was much stronger for some DNA fragments than for others. As indicated by Table 4 above, the *oxy* and *nptII* probes can be used to identify the right and left borders, respectively. This approach is valid because physical linkage between the *oxy* and *nptII* genes has been demonstrated. In addition, plants transformed with pBrx75 have copies of the *tml* 3' polyadenylation signal at each T-DNA border. Hybridisation with the *tml* 3' probe was used to further confirm the number of right and left border fragments in each event. The Southern analyses demonstrated that there is one left border and one right border only in both 10222 and 10211 thus confirming that one copy of each gene had been integrated at a single site in the genome.

The two events were also analysed for the transfer of DNA sequences from outside the T-DNA region. Three hybridisation probes were used. The first was the entire binary plasmid pCGN1532 (a precursor to pBrx75) that consists of the *A. rhizogenes* replicon region, the pBR322 origin of replication and the gentamicin resistance gene (*aac*; see Table 2). The second probe was the *aac* gene itself and the third probe was to the *nptII* region (the positive control).

If the pCGN1532 probe hybridises to any of the genomic DNA then transfer beyond the T-DNA region has occurred. Southern analysis showed that neither 10222 nor 10211 contains any sequences that hybridise to pCGN1532 indicating that transfer of DNA beyond the T-DNA borders has not occurred. To confirm this finding specifically in relation to the gentamicin resistance gene, the same Southern blot was re-probed with the *aac* probe. Once again, no hybridising sequences were detected in either 10222 or 10211.

Conclusion

A single copy of T-DNA, containing the *oxy* and *nptII* gene cassettes, has been integrated at a single site in transformation events 10222 and 10211. All transferred genes appear to be intact and no re-arrangements of the T-DNA were detected. An analysis of segregating plant populations using bromoxynil treatment indicated that the *oxy* gene is functional in both events and that the bromoxynil-tolerant trait is segregating according to standard Mendelian genetics. No sequences residing outside the T-DNA region had been transferred during the transformation.

2.4 Stability of the genetic changes

Analysis of integrated sequences

Southern analysis was done on later generations of events 10211 and 10222 to confirm that the DNA banding pattern observed in the homozygous T_3 plants (as described in Section 2.3 above) was maintained in subsequent generations. Two plant lines, derived from transformation events 10211 and 10222, were analysed at the T_5 generation. As plants from these two events had been previously analysed in the T_3 generation it enabled a direct comparison. In addition, events 10211 and 10222 had also been used in a backcrossing program to integrate the *oxy* gene into elite commercial cotton varieties, therefore the stability of the T-DNA in different genetic backgrounds could also be determined.

The pattern of hybridising DNA fragments from plants of the T_5 generation for lines 10211-20 and 10222-1 was shown by Southern analysis to be identical to that observed in DNA from T_3 generation plants. Southern analysis of late generations of these crosses between events 10211 and 10222 with elite cotton varieties also showed no difference compared to the analysis of the T_3 generation.

Inheritance of the bromoxynil tolerance trait by BXN cotton

The genetic stability and segregation of the bromoxynil tolerance trait was monitored using data obtained from field sprayed plants.

BXN cotton lines were screened for bromoxynil tolerance by spraying plants of each generation with the herbicide and selecting lines for commercialisation. As part of the normal screening process in the breeding program of BXN cotton, events with consistent segregation patterns and desirable characteristics are advanced, and those with unusual segregation patterns (not fitting classic Mendelian inheritance patterns) are not developed further.

The applicant reports that the *oxy* gene has been maintained for at least six seed generations (self-pollinated plants) and at least 5 generations of backcrossing with commercial varieties in the breeding program. Inheritance of the BXN tolerance trait was found to be consistent, not only with progeny produced by self-pollination but also in a backcross program involving introgression of the *oxy* gene into a variety of genetic backgrounds.

T_3 seed was collected from individual T_2 plants and processed separately. The seed from each plant was planted in an individual row and sprayed with a bromoxynil containing herbicide. The plant numbers obtained from the experiment should fit either a 3:1 tolerant to susceptible ratio or be 100% tolerant. The 3:1 ratio rows come from T_2 plants that were heterozygous for the insertion and the 100% rows come from T_2 plants that were homozygous tolerant.

Table 5 gives the fit to Mendelian inheritance in the T_2 generation for transformation events 10211 and 10222. Both events were found to fit an expected 3:1 ratio for one gene insertion site (as described above for the molecular characterisation).

Table 5: Segregation ratios for BXN cotton events

Event No.	Total:susceptible T_2 plants	Chi-Square fit for 3:1 ratio[a]	Chi-Square fit for 15:1 ratio[a]
10211	241:53	1.163	101.922
10222	240:52	1.422	97.351

[a] Chi-Square of < 3.84 has a 95% probability of a 3:1 or 15:1 segregation ratio

The vast majority of T_3 rows were found to fit reasonably closely to the expected ratios, the few rows that did not fit had too few plants to verify the fit statistically or were suspected to contain contaminant seed from processing.

The second statistic to verify expected segregation is the number of individual rows falling into each class. In the T_2 generation, a single insertion event is expected to segregate 3 tolerant: 1 susceptible. This is the observed phenotype, but genetically, the genotypes are 1 homozygous tolerant: 2 heterozygous tolerant: 1 homozygous susceptible. By examining the next generation from each surviving plant, it is possible to determine how many of the 3 tolerant T_2 plants were heterozygous and how many were homozygous. It was found that the ratio of genotypes was as expected, that is 1:2:1.

Overall, the T_2 and T_3 data presented support normal gene segregation for transgenes inserted into cotton plants. After the T_3 or T_4 generation, homozygous lines are selected, meaning these lines will no longer display segregation of the BXN trait. Screening with bromoxynil is then only done to monitor seed purity. The consistency of the tolerance trait in these lines is a good measure of the level of genetic stability (providing there is no contamination from bromoxynil-susceptible lines).

Table 6 shows the percentages of bromoxynil-sensitive plants found in the field of T_6 plants derived from events 10211 and 10222.

Table 6: The percentages of bromoxynil-sensitive plants found in the field of T_6 plants

		Open-pollinated[a]		Self-pollinated[b]	
Line	**Field**	**Population size**	**% susceptible**	**Population size**	**% susceptible**
10211-1	Empire 1874#3	153 536	0.5	412 508	0.02
10211-20	Harlan Bohne	1 093 251	0.97	670 057	0.07
10211-1	Somerset	482 853	0.22	875 172	0.05
10211-20	Indianola	446 533	1.09	210 133	0.04
10222-1	Empire 1074			1 822 538	0.001

[a] open-pollinated in South Africa 1991-92 nursery
[b] self-pollinated in South Africa 1991-92 nursery

The populations of T_6 plants were split into open pollinated and self-pollinated. This refers to pollination done three generations (T_3) earlier in a counter season location. Rows from self-pollinated seed of individual plants in the T_3 generation were then grown at T_4 progeny rows in the US nursery without self-pollination in the following season. T_5 bulk seed harvested from these rows were planted under isolation from other cotton in the next counter season. The T_6 generation was then grown in several different field locations in the United States. Self-pollinated seed should not produce any susceptible plants. The small number of bromoxynil susceptible plants found in the self-pollinated lots most likely came from crossing which occurred in the T_4 generation grown in the US nursery. The number of bromoxynil susceptible plants found in the open pollinated populations is still carryover from the nursery in the counter season when the lines were T_3s.

These data are consistent with the conclusion that the BXN tolerance trait is stably inherited and maintained in BXN cotton.

Conclusion

Stability of the transferred *oxy* gene was studied by backcrossing of plants containing transformation events 10211 and 10222 with commercially available cotton varieties and by self-crossing followed by propagation. The BXN gene was determined to be stable over at least six generations through observed tolerance to bromoxynil treatment. Additionally, Southern blot analysis demonstrated that both the *oxy* and *nptII* genes were stably transferred from generation to generation in a variety of genetic backgrounds.

3. GENERAL SAFETY ISSUES

3.1 History of use

Cotton is grown primarily for the value of its fibre; cottonseed (and its processed products) is very much a by-product of the crop. Cottonseed itself is not used as a food for human consumption because it contains naturally occurring toxic substances known as gossypol and the cyclopropenoid fatty acids. These harmful substances can however be removed or reduced with processing which means that a number of products derived from cottonseed are suitable for animal as well as human food uses. The four main products derived from cottonseed are oil, meal, hulls and linters. Processing of cottonseed typically yields by weight: 16% oil, 45% meal, 9% linters, and 26% hulls, with 4% lost during processing (Cherry and Leffler 1984).

The main products destined for human consumption are the oil and linters. These products are routinely used in foods and have a history of safe use. Cottonseed oil has been in common use since the middle of the nineteenth century (Jones and King 1990) and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958. Cottonseed meal and hulls are typically used for livestock feed. Cottonseed oil is premium quality oil that is used in a variety of foods including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine, and packing oil. Linters are a major source of cellulose for both chemical and food uses. Food uses include as a thickener in products such as ice cream and salad dressings.

Some human food uses for cottonseed flour have been reported, particularly in Central American countries and India where it is used as a low cost, high quality protein ingredient in special products to help ease malnutrition where cottonseed meal is inexpensive and readily available (Ensminger 1994, Franck 1989). Cottonseed flour is also permitted for human consumption in the United States, provided it meets certain specifications for gossypol content, although no products are currently being produced.

Cottonseed processing steps

After the majority of the fibre is removed at the cotton gin, a significant amount of "fuzzy" fibre remains associated with the seed. These short fibres, known as linters, are removed from the seed during de-linting. After extensive processing at alkaline pH and high temperatures, the linters can be used as a high fibre dietary product. After this processing, the fibre does not normally contain any detectable genetic material or protein. Once the lint is removed from the seed, the hulls are cut and separated from the seed. After hulling, the cottonseed is flaked by a rolling process to facilitate oil removal. Prior to oil extraction, the flakes are heated to: (i) break down the cell walls; (ii) reduce the viscosity of the oil; (iii) coagulate the protein; (iv) inactivate proteins and kill any microbial contamination; (v) detoxify gossypol by the combination of heat and moisture; and (vi) fix certain phosphatides in the cake to minimise refining losses.

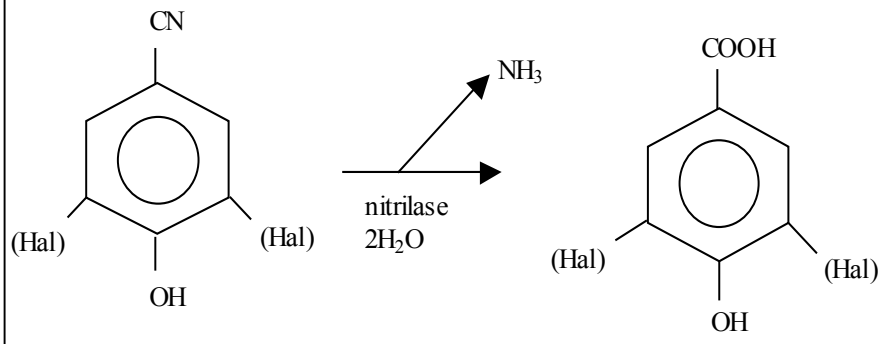
After cooking, the oil is typically removed from the meal by direct solvent extraction with hexane. The material left over after the crude oil is extracted is the cottonseed meal. After extraction the gossypol levels in the oil are reduced by about half. Crude cottonseed oil is then further processed, depending on the end use of the product. A winterisation step is added to produce cooking oil, whereas for solid shortening, a hydrogenation step is added to transform the liquid oil into a solid fat. Further processing (refining) for all the uses of cottonseed oil includes deodorization and bleaching. Deodorization greatly reduces the cyclopropenoid fatty acid content of the oil due to the extreme pH and temperature conditions and the resulting oil generally contains no detectable protein (Jones and King 1990).

3.2 Nature of the novel protein

Nitrilase

The *oxy* gene was isolated from *Klebsiella pneumoniae* subspecies *ozaenae* (McBride *et al* 1986, Stalker and McBride 1987, Stalker *et al* 1988) and encodes a 37 kDa nitrilase (EC. 3.5.5.6). This enzyme hydrolyses the oxynil herbicides into non-phytotoxic compounds: 3.5-dibromo-4-hydroxybenzoic acid or 3,5-diiodo-4-hydroxybenzoic acid and ammonia (Figure 2).

Figure 2: Oxynil degradation by the oxy-encoded nitrilase. (Hal is either Br or I)



Oxynils

3,5-dibromo-4-hydroxybenzoic acid
3,5-diiodo-4-hydroxybenzoic acid

Purified nitrilase has optimal activity at pH 9.2 and at a temperature of 35˚C. The pH optimum remains relatively constant at different substrate concentrations. Nitrilase activity declines to 15% at pH 7.0 and also in temperatures of 10 and 55˚C. The *oxy*-encoded nitrilase is highly specific for its substrates, exhibiting a K_m of 0.31nM and a V_{max} of 15µmole of NH_3 released/min/mg protein for bromoxynil.

Neomycin phosphotransferase II

NPT II (also known as aminoglycoside 3'-phosphotransferase II) is an enzyme with a molecular mass of 29 kDa that catalyses the transfer of a phosphate group from adenosine 5'-triphosphate (ATP) to a hydroxyl group of aminoglycoside antibiotics, including neomycin and kanamycin, thereby inactivating the antibiotics (Davies *et al* 1986). The enzyme is encoded by the *nptII* gene, which is derived from transposon Tn5 from the bacterium *E. coli* (Beck *et al* 1982).

3.3 Expression of novel protein in the plant

All of the plants used for the analyses had been sprayed with an agronomic dose of Buctril® to monitor seed purity.

Nitrilase

The concentration of the nitrilase enzyme was determined in leaves, acid delinted cottonseed, decorticated cottonseed kernels, cottonseed hulls, processed cottonseed meal and crude oil using Western blot analysis. This assay detects both active and inactive nitrilase protein. Protein extractions were made of each of the tissues or fractions and these were separated electrophoretically on an SDS-polyacrylamide gel.

A positive nitrilase signal on the Western blot consists of a single band at 37 kDa. The protein level was quantitated by comparing the intensity of the signal in the tissue extracts from plants containing transformation events 10211 and 10222 with the extracts from the non-transgenic control, Coker 315 spiked with purified nitrilase of known concentrations.

Each assay was repeated at least three times to obtain an estimate of the maximum nitrilase concentration in each of the transformation events. A summary of the protein expression data is provided in Table 7 below.

Table 7: Summary of nitrilase expression data for BXN cotton

Sample	Event 10211	Event 10222
Leaf tissue		
µg/g total protein	20	20
% total protein	0.002%	0.002%
Seed, kernels, hulls		
µg/g total protein	< 0.6	max. of 0.6
% total protein	<0.00006%	0.00006%
Meal		
µg/g total protein	0.6	0.12
% total protein	0.00006%	0.000012%
Crude oil		
µg/g total protein	Not detected[1]	Not detected[1]
% total protein		

[1] limit of detection was 0.1 ppm.

Neomycin phosphotransferase II (NPTII)

Western blot analysis was also used to determine the level of NPTII expressed in leaf tissue, cottonseed, meal and crude oil. The NPTII protein is a monomer of 29 kDa. NPTII was not detected in protein extracts from the non-transformed control, Coker 315.

The results of these studies are summarised in Table 8 below.

Table 8: Summary of NPTII expression data for BXN cotton

Sample	Event 10211	Event 10222
Leaf tissue		
µg/g total protein	80	80
% total protein	0.008%	0.008%
Seed, kernels, hulls		
µg/g total protein	< 30	max of 27
% total protein	< 0.003%	0.0027%
level in cottonseed	6.6 ppm	5.9 ppm
Meal		
µg/g total protein	14	7
% total protein	0.0014%	0.0007%
level in meal	5.7 ppm	2.9 ppm
Crude oil		
µg/g total protein	Not detected[1]	Not detected[1]
% total protein		

[1] limit of detection was 0.1 ppm

Conclusion

The results show that the levels of nitrilase and NPTII are highest in cotton leaf tissue, the levels being about 80µg/ g total protein for NPTII (equivalent to 0.008% of total leaf protein) and about 20µg/ g total protein for nitrilase (equivalent to 0.002% of total leaf protein). The levels of both proteins decline in the seed and meal. In the crude oil fraction, which is the fraction destined for human consumption, neither proteins can be detected at a limit of detection of 0.1 ppm.

Therefore, as it is known that the refining process further removes any protein, it can be concluded that the refined oil produced from BXN cotton is extremely unlikely to contain any detectable nitrilase or NPTII.

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

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

This section of the report will therefore concentrate on evaluating the human health impact of the potential transfer of antibiotic resistance genes from BXN cotton to microorganisms present in the human digestive tract.

In transformation events 10211 and 10222, Southern analysis (Section 2.3) demonstrated that a single antibiotic resistance gene has been transferred – the *nptII* gene. Both transformation events contain the *nptII* gene under the control of the 35S promoter. The gentamicin resistance gene, which was also present in plasmid pBrx75, was not transferred to the cotton genome in the transformation process.

The first issue to be considered is the probability that the *nptII* gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract. There are two considerations in relation to this issue.

Firstly, DNA is not present in refined oil and linters, which are the only products intended for human consumption. Processed linters are essentially pure cellulose (>99%) and are subjected to heat and solvent treatment that would be expected to remove and destroy DNA. The refining process for cottonseed oil also includes heat, solvent and alkali treatments that would be expected to remove and destroy DNA, and intact fragments of the *nptII* gene are unlikely to survive the processing steps. The processing steps can also lead to the release of cellular enzymes (nucleases) that are responsible for degrading DNA into smaller fragments.

[3] Food and Agriculture Organization.

Refined oil from another genetically modified cotton – glyphosate-tolerant cotton line 1445 – assessed under Application A355[4], was analysed by the applicant (Monsanto) to ascertain if any intact DNA could be detected using a highly sensitive technique called the Polymerase Chain Reaction (PCR). No DNA could be detected in refined oil produced from the cotton. The detection limit of the assay was 1ng of DNA.

The lack of intact DNA in the intended food products, cottonseed oil and cellulose from linters reduces any risk of horizontal transfer of genetic material to cells in the human digestive tract as a result of the ingestion of these foods.

The second consideration is the steps necessary for horizontal DNA transfer to occur. These are:

- excision of DNA fragments containing the *nptII* gene;
- survival of DNA fragments containing the *nptII* gene in the digestive tract;
- natural transformation of bacteria inhabiting the digestive tract;
- survival of the bacterial restriction system by the DNA fragment containing the *nptII* gene;
- stable integration of the DNA fragment containing the *nptII* gene into the bacterial chromosome or plasmid;
- maintenance and expression of *nptII* gene by the bacteria

The transfer of the *nptII* gene from refined BXN cotton seed oil or cellulose from linters to microorganisms in the human digestive tract is therefore considered to be highly unlikely because: (i) DNA would not be present in the food as consumed; and (ii) because of the number and complexity of the steps that would need to take place consecutively.

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

The human health impacts are considered to be negligible. The *nptII* gene occurs naturally in bacteria inhabiting the human digestive tract therefore the additive effect of an *nptII* gene entering the human gastrointestinal flora from a genetically modified plant would be insignificant compared to the population of kanamycin resistant microorganisms naturally present.

The transfer of other novel genetic material is 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.

[4] ANZFA (2000) *Final Risk Analysis Report*. Application A355: food produced from glyphosate-tolerant cotton line 1445.

Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in genetically modified foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Conclusion

It is extremely unlikely that the *nptII* gene would transfer from BXN cotton to bacteria in the human digestive tract because of the number and complexity of steps that would need to take place consecutively and because the food products, refined oil and linters, are unlikely to contain any DNA. In the highly unlikely event that the *nptII* gene was transferred, the human health impacts would be negligible because kanamycin resistant bacteria are already commonly found in the human digestive tract and in the environment. It is also equally unlikely that other novel genetic material from BXN cotton would be transferred to human cells via the digestive tract for the same reasons.

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally-occurring toxins

Cotton contains two naturally occurring toxic compounds – gossypol and cyclopropenoid fatty acids.

Gossypol is a biologically active terpenoid aldehyde that exists within the puncta or 'glands' found in all parts of the cotton plant, including seeds (Abou-Donia 1976). Gossypol can cause toxic effects such as reduced appetite, body weight loss, and dyspnoea (difficult and laboured breathing) (Berardi and Goldblatt 1980) and also has adverse effects on the protein nutritive value of food by rendering lysine metabolically unavailable (Yannai and Bensai, 1983). The presence of gossypol limits the use of cottonseed as a protein source for humans or in animal feed, except for ruminants where bacteria in the rumen are able to detoxify gossypol (Randel *et al* 1992, Poore and Rogers 1998, Nikokyris *et al* 1991).

Several derivatives and isomers of gossypol have been described (Berardi and Goldblatt 1980, Altman *et al* 1989). The concentration of gossypol and related terpenoids varies in cotton depending on both genetic and environmental factors (Altman *et al* 1990, Dilday and Shaver 1980 and 1981, Hanny 1980). Unprocessed seed contains gossypol in the 'free' or unbound form, in the pigment glands (Jones 1991). Processing whole cottonseed into meal converts varying amounts of free gossypol to the bound form, thus eliminating much of its biological activity (Jones 1991). The removal or inactivation of gossypol during processing enables the use of some cottonseed meal in feed for fish, poultry and pigs. Some human food uses for cottonseed flour, derived from finely ground cottonseed meal, have also been reported, where the meal has been specially processed to minimise the toxicological properties of gossypol. The use of such products appears to be largely confined to Central American countries where it is used as protein enricher in special products to help ease malnutrition (Ensminger 1994, Franck 1989). Refined cottonseed oil is free of gossypol (Gunstone *et al* 1994). The gossypol that partitions into the oil is essentially completely eliminated during subsequent refining of the oil, through inactivation by heat and alkali treatment. The reduction of free gossypol in oil is a measure of the food quality and processing efficiency.

Cyclopropenoid fatty acids are naturally present in cottonseed, crude cottonseed oil and in the meal (because of the residual oil in the meal fractions). The principal forms of these fatty acids are sterculic and malvalic acid (Cherry and Leffler 1984). These fatty acids produce undesirable biological effects, including: the inhibition of biodesaturation of stearic to oleic acid affecting phospholipid biosynthesis (Rolph *et al* 1990; Cao *et al* 1993, Gunstone *et al* 1994); and have been reported to induce termination of embryo development in sheep through inhibition of progesterone production in the *corpus luteum* (Tumbelaka *et al* 1994). In two studies of cyclopropenoid fatty acids from several domestic varieties, ranges were found of 0.56 to 1.17% in crude oil (Bailey *et al* 1966), and 0.07 to 0.32% in refined oil (Lawhon *et al* 1977). In another study cyclopropenoid fatty acids were found at levels up to 2% of crude oil, and 0.64% of refined oil (Jones and King 1990).

Gossypol

Free and, in some cases, total gossypol levels were measured in de-linted whole cottonseed samples taken from homozygous BXN cotton, and from the Coker 315 control line which were grown in the field in the United States in 1991 and 1993, and in Spain in 1997. The values obtained were compared to values obtained for common commercial varieties of cotton grown at the same site. Data was obtained for both bromoxynil-sprayed and unsprayed cotton. The data are presented below in Tables 9 – 11 below.

1991 field trial data

The samples taken from the BXN cotton for this study were from plants that had been sprayed with Buctril® once at 1.5 lb.ai/acre at the two and six-leaf stages. Free and total gossypol measurements were done on whole seed samples by Woodson-Tenant Laboratories, Inc using standard procedures. Four separate replicated field plots, planted in a randomised complete block design, were harvested from each genotype at each of three locations.

Table 9: Free and total gossypol levels[1] in whole cottonseed in BXN cotton sprayed with Buctril® in 1991 field trials

	Total gossypol	Free gossypol
Coker 315 control	0.999	0.851
Event 10211	1.03	1.14
Event 10222	1.05	1.04
Natural range[2]	0.002-6.64	0.002-6.64

[1] Values presented are the percentage of free and total gossypol in whole seed and are the average of replicate samples, analysed in duplicate
[2] Price *et al* 1993

1993 field trial data

Additional studies were done on free gossypol levels in three lines derived from events 10211 and 10222 and the values compared to free gossypol levels in both the non-transformed control as well as current commercial varieties of cotton (DPL5415, LA 887 and Stoneville 453). In this study, the BXN cotton had not been sprayed with bromoxynil. The plants were grown in four separate replicated field plots planted in a randomised complete block design at three locations. The measurements were done by Dr Millard Calhoun from the Texas A+M University. The data from three field locations are presented in Table 10.

Table 10: Free gossypol levels[1] in BXN cotton and commercial varieties of cotton grown in the United States in 1993

Line	Mississippi	South Carolina	Arizona	Overall mean
10211-1	0.900 bc[2]	0.864 bc	1.019 bcd	0.93 bcd
10211-20	0.922 bc	0.869 bc	1.077 b	0.96 abc
10222-1	0.812 cd	0.756 cd	1.003 bcd	0.89 cd
C315 control	0.889 bc	0.788 cd	1.035 bc	0.90 cd
DPL5415	0.730 d	0.819 bcd	0.954 d	0.83 d
LA 887	0.968 b	0.963 a	1.169 a	1.03 ab
Stoneville 453	1.099 a	0.897 ab	1.198 a	1.06 a

[1] The values are the percentage of free gossypol in whole seed and are the average of four replicate samples analysed in duplicate

[2] lines within the same location containing the same letter are not significantly different at a 95% confidence level

The overall mean demonstrates that none of the BXN lines are significantly different in free gossypol from the non-transformed control, Coker 315. When a comparison of individual locations is done, no BXN line has a significantly greater level of gossypol than Coker 315. These results show that in general, growing regions have an impact on the free gossypol level of the seed produced but varietal rankings stay relatively consistent from location to location.

1997 field trial data

Samples were taken from OXY 47, which is a BXN cotton variety developed from transformation event 10222 in a Stoneville 474 genetic background. Free gossypol values for OXY 47 were compared to those obtained for Stoneville 474, which had been grown at the same sites. The BXN cotton had been sprayed with Buctril® at the rate of 563 g ai/hectare, which is representative of an agronomic dose. The plants were grown in two replicates planted in a randomised complete block design at two different field locations. The data are presented below in Table 11.

Table 11: Free gossypol levels[1] in BXN cotton sprayed with Buctril® and a commercial variety of cotton grown in Spain in 1997

Line	Rep. #	Site	Gossypol content[1]	Mean
OXY47	1	a	0.590	
OXY47	1	b	0.700	
OXY47	2	a	0.630	
OXY47	2	b	0.635	0.643[2]
Stoneville 474	1	a	0.520	
Stoneville 474	1	b	0.680	
Stoneville 474	2	a	0.580	
Stoneville 474	2	b	0.650	0.608

[1] The values are the percentage of free gossypol in seed and are the average of two replicate samples analysed in duplicate.

[2] There is no significant difference between the means at the 95% confidence level.

Conclusion

Data from field trials performed in the United States in 1991 and 1993, and in Spain in 1997 demonstrate that the transformation and line selection process have not caused gossypol levels to be increased in BXN cotton – the gossypol levels of the BXN cotton lines are equivalent to those of the non-transformed control line as well as current commercial varieties of cotton and also fall within the published ranges expected for cotton. The spraying of BXN cotton with a bromoxynil-containing herbicide does not result in significant increases in the levels of gossypol in the seed of BXN cotton.

Cyclopropenoid fatty acids

Cyclopropenoid fatty acid levels were determined for homozygous BXN cotton lines derived from transformation events 10211 and 10222. Cottonseed samples were collected from replicated field trials in the United States and South Africa in 1993 and in Spain in 1997. Oil extracted from the cottonseed samples was analysed for the cyclopropenoid fatty acids (dihydrosterculic, sterculic and malvalic) using a colourimetric reaction (modified Halphen reaction) based on the Association of Official Analytical Chemists (AOAC) International Method 974.19 and Bailey *et al* (1965). The values obtained for BXN cotton were compared to those obtained for the non-transformed control line and also with commercial cotton varieties. The BXN cotton grown in Spain was sprayed with Buctril® at the rate of 563 g ai/hectare.

1993 USA field trial data

The cotton plants were grown in three locations in the United States. Four separate replicated field plots, planted in a randomised complete block design, were harvested from each genotype at each location. The Engineering Biosciences Research Centre at the Texas A&M University performed small scale processing of the cottonseed samples under the United States Environmental Protection Agency Good Laboratory Practice protocols. This is a bench-top laboratory scale processing facility that is designed to produce oil (and meal) fractions comparable to what would be produced by large scale commercial processing. Data on cyclopropenoid fatty acid levels are presented in Table 12 below.

Table 12: Level of cyclopropenoid fatty acids[1] in oil extracted from cottonseed from BXN cotton and commercial varieties of cotton grown in the United States in 1993

Line	Mississippi	South Carolina	Arizona	Overall mean
10211-1	0.73 abc[2]	0.63 ab	0.81 a	0.73 a
10211-20	0.74 abc	0.60 a	0.78 a	0.72 a
10222-1	0.71 ab	0.59 a	0.81 a	0.71 a
Coker 315 control	0.67 a	0.70 abc	0.73 a	0.72 a
DP 5415	0.66 a	0.56 a	0.86 a	0.68 ab
LA 887	0.82 c	0.80 c	0.88 a	0.79 c
Stoneville 453	0.81 bc	0.75 bc	0.80 a	0.76 bc

[1] values presented are the percentage of cyclopropenoid fatty acids in oil and are means from four replicates analysed in duplicate

[2] lines within the same location containing the same letter are not significantly different at a 95% confidence level.

Significant differences were observed between locations, sample runs and lines, however none of the BXN cotton lines differed significantly in cyclopropenoid fatty acid levels compared to the parental control line Coker 315, grown at the same location. Two of the commercial varieties, LA 887 and Stoneville 453 were found to have the highest levels of cyclopropenoid fatty acids overall.

1993 South African field trial data

Cyclopropenoid fatty acid levels were determined in crude oil that had been produced from cottonseed collected from a homozygous line of OXY cotton, derived from transformation event 10222, and three Coker 315 control lines grown in the field in South Africa in 1993.

The OXY cotton had not been sprayed with bromoxynil. The values obtained were compared to those obtained for refined corn and cottonseed oils. The results are presented in Table 13 below.

Table 13: Cyclopropenoid fatty acid levels in oil extracted from BXN cotton and non-transformed control line grown in South Africa in 1993

Sample	Type of oil	Absorbance A_{547nm}
10222	Crude	0.69
Coker 315	Crude	0.65
Coker 315	Crude	0.70
Coker 315	Crude	0.73
Commercial corn oil	Refined	0.0
Commercial cotton oil	Refined	0.10

1997 Spanish field trial data

Cyclopropenoid fatty acid levels were determined in crude oil that had been produced from cottonseed taken from OXY 47, which is a BXN cotton variety developed from transformation event 10222 in a Stoneville 474 genetic background. These levels were compared to those obtained for crude oil produced from cottonseed taken from the Stoneville 474 variety which had been grown at the same site. The BXN cotton had been sprayed with Buctril® at the rate of 563 g ai/hectare, which is representative of an agronomic dose. The plants were grown in two replicates planted in a randomised complete block design at two different locations. The data are presented below in Tables 14a and 14b.

Table 14a: Cyclopropenoid fatty acid levels[1] in cottonseed oil extracts from BXN cotton sprayed with Buctril® and a commercial variety of cotton grown in Spain in 1997

Line	Rep. #	Location	Malvalic acid	Dihydrosterculic acid	Sterculic acid
OXY47	1	a	0.50	0.30	0.20
OXY47	1	b	0.50	0.30	0.20
OXY47	2	a	0.50	0.30	0.20
OXY47	2	b	0.50	0.30	0.25
Stoneville 474	1	a	0.50	0.30	0.20
Stoneville 474	1	b	0.50	0.30	0.30
Stoneville 474	2	a	0.50	0.30	0.30
Stoneville 474	2	b	0.40	0.20	0.20

[1] values presented are the percentage of cyclopropenoid fatty acids in oil and are the average of duplicate analyses

Table 14b: Comparison of means[1] for cyclopropenoid fatty acid levels

	OXY47	Stoneville 474	Literature range[3]
Malvalic acid	0.50 a	0.48 a	<0.1 – 1.9
Dihydrosterculic acid	0.30 a	0.28 a	0.2 – 0.8
Sterculic acid	0.21 a	0.25 b	0.3 – 0.7

[1] mean values across two field sites
[2] rows containing the same letter are not significantly different at a 95% confidence level.
[3] Wood 1986

The only significant difference is in relation to the levels of sterculic acid, which were found to be slightly decreased in BXN cotton compared to the isogenic control line. As the difference is minor, and both values are still within the published range for sterculic acid, this finding is not considered to have any biological or food safety significance.

Conclusion

In virtually all cases, the levels of cyclopropenoid fatty acids in oil produced from seeds of BXN cotton were lower or comparable to the levels in the controls. The levels reported are also within the literature reported ranges. It is therefore concluded that the transformation and line selection process has not resulted in an increase to the levels of cyclopropenoid fatty acids in oil from BXN cotton. The levels of cyclopropenoid fatty acids are unaffected by the spraying of the plants with a bromoxynil-containing herbicide.

4.2 Potential toxicity of novel proteins

The protein expression data demonstrates that transformation events 10211 and 10222 express two novel proteins – nitrilase and neomycin phosphotransferase II. This section of the report will therefore assess the potential toxicity of these two proteins.

Presence of the novel proteins in the food as consumed

It should be noted that the products intended for human consumption – refined cottonseed oil and cellulose from the linters – do not normally contain any detectable amounts of protein (see Section 3.1). Furthermore, when crude cottonseed oil from BXN cotton was analysed for the presence of both nitrilase and neomycin phosphotransferase II neither could be detected at a detection limit of 0.1 ppm. Therefore, it is highly unlikely that humans ingesting refined oil or cellulose products derived from BXN cotton would be exposed to any appreciable amounts of the two novel proteins.

Potential toxicity of nitrilase

Studies submitted by applicant:

Dange, M. (1996) Nitrilase: sub-acute oral toxicity study in the mouse. Rhône-Poulenc Study SA 96267.

Astwood, J.D. (1997). *Klebsiella ozaenae* nitrilase (BXN) has no significant sequence similarity to known allergens or toxins. Monsanto Study Report No. MSL-15120.

Sub-acute oral toxicity study in mice

To obtain sufficient quantities of nitrilase for toxicity testing, the enzyme was expressed in *Escherichia coli* BL21 and subsequently purified as an inclusion body pellet.

The applicant reports that an acute oral toxicity study was planned to be performed using doses up to 2000mg/kg body weight, using a suspension of nitrilase at 200mg/ml. However, the consistency of the suspension once prepared did not allow the total dose to be administered at one time. Therefore, the suspension was administered over four consecutive days at 500mg/kg body weight/day.

Four consecutive oral doses (500mg/kg body weight) of nitrilase (Batch No. JHJ0001) were administered to groups of OF1 mice (5/sex) at a dose volume of 20ml/kg. The purified nitrilase was suspended in 0.25% methylcellulose in distilled water.

All animals were checked daily for clinical signs over a period of 15 days, and their body weight recorded weekly. At termination of the study period, all animals were killed and subject to necropsy. The necropsy included the macroscopic examination of abdominal and thoracic cavities, major organs and tissues.

No clinical signs were observed during the study and there were no unscheduled deaths. The body weight gain of the animals was unaffected by the treatment and no gross findings were recorded at necropsy. The LD_{50} was designated as >500mg/kg body weight.

Similarity with known protein toxins

A database of protein toxin amino acid sequences was assembled from the public domain genetic databases, which included GenPept ver. 92 (a protein database extracted from GenBank and EMBL), PIR ver. 45, and SwissProt ver. 31. Amino acid sequences were retrieved from the databases using the STRINGSEARCH program supplied with the GCG sequence analysis package version 7 (Devereux *et al* 1984). Using the DATASET program, the sequences of toxins were combined into a single database called TOXIN3.

The keyword "toxin" identified and retrieved 2662 amino acid sequences from the public domain genetic databases – this comprised the TOXIN3 database. There were no toxins in the TOXIN3 database that showed significant similarity to nitrilase.

History of human exposure to nitrilases

Nitrilase enzymes, similar to that encoded by the *oxy* gene from *Klebsiella pneumonia,* have been found in a number of plant and microbial species. Although substrates and pathways differ, it appears as though nitrilases share common functions such as hydrolysis of nitriles to carboxylic acids. Plant nitrilases can also confer resistance to some of the nitrile containing herbicides. Nitrilases have been found in a number of important food crops such as wheat, cabbage, barley, and bananas (Buckland *et al* 1973, Thimann and Mahadevan 1964), therefore, humans have a history of exposure to similar types of proteins with no apparent ill effects ever being documented.

Potential toxicity of bromoxynil metabolites

Bromoxynil has recently been re-registered for use in the United States as a contact herbicide to control broadleaf weeds in BXN cotton (US EPA 1998). The bromoxynil-tolerant plants hydrolyse bromoxynil to 3,5-dibromo-4-hydroxybenzoic acid (DBHA), a carboxylic acid. It is reported that significant residues of DBHA can be present on BXN cotton as a result of the enzymatic activity of the bacterial-derived nitrilase (US EPA 1998). As this metabolite is a by-product resulting from the activity of an introduced enzyme it is important that a consideration of its toxicity be included in any safety evaluation of BXN cotton.

The US Environment Protection Agency, in its evaluation of bromoxynil, stated that the human health risk from bromoxynil is negligible (US EPA 1998). As part of its evaluation of bromoxynil the US EPA also evaluated the toxicity of the DBHA metabolite of bromoxynil and concluded "there was no concern that DBHA would exhibit significant toxicity over that of the parent bromoxynil".

Bromoxynil and DBHA are extremely similar in structure, varying only in that bromoxynil has a cyano (-CN) group that has been converted to a carboxyl (-COOH) group in the DBHA metabolite. Conversion to a carboxyl group is generally considered to decrease the toxicity of a molecule (US EPA 1998). The conversion to the carboxyl group should cause the DBHA to be more polar and therefore more soluble in water and less in fats. Additionally, the presence of the carboxyl group will allow DBHA to combine with certain water molecules (such as glucuronic acid) which should further increase DBHA's water solubility and further decrease its solubility in fats. This increased water solubility, combined with the decreased fat solubility means that DBHA should be eliminated faster from the organism than its parent compound, bromoxynil. It is likely that these characteristics would also limit the amount of DBHA residue likely to be present in cottonseed oil.

To date, the US EPA has concluded that DBHA is likely to be no more toxic than bromoxynil, which the US EPA has recently determined poses negligible risk to human health at expected exposure levels.

Conclusion

The evidence from sub-acute toxicity studies in mice does not indicate that there is any potential for nitrilase from *Klebsiella pneumoniae* subsp. *ozaenae* to be toxic to humans. Furthermore, humans are extremely unlikely to be exposed to this enzyme through the consumption of refined oil and cellulose from BXN cotton as both food products are devoid of any detectable protein. The metabolite of bromoxynil, DBHA, also does not show any potential to be toxic to humans at the predicted exposure levels.

Potential toxicity of neomycin phosphotransferase II

The potential toxicity of neomycin phosphotransferase II (NPTII) has been investigated by ANZFA for a number of different applications for GM foods where acute oral toxicity studies in mice have been submitted for evaluation. The safety of this protein has also been considered on numerous occasions in the peer reviewed scientific literature (Flavell *et al* 1992, Nap *et al* 1992, Fuchs *et al* 1993a, Fuchs *et al* 1993b). In all instances it has been concluded that NPTII is non-toxic to humans. This conclusion also applies to NPTII in BXN cotton, which is identical to the NPTII assessed for toxicity on previous occasions. Furthermore, humans are extremely unlikely to be exposed to this enzyme through the consumption of refined oil and cellulose from BXN cotton as both food products are devoid of any detectable protein.

4.3 Levels of naturally-occurring allergenic proteins

Some common foods, e.g. cow's milk, soybeans and tree nuts, are known to elicit an allergic response in susceptible individuals. This response is primarily due to an immune reaction to a particular protein component of the food, whereas the components of fats or oils (such as fatty acids etc) are not generally associated with such reactions. Moreover, refined cottonseed oil and cellulose from linters are devoid of protein therefore their consumption is unlikely to result in an allergic reaction.

There have been reported incidences of allergic reaction in humans in response to consumption of foods containing cottonseed protein (Atkins *et al* 1988, Malanin and Kalimo 1988). However, whole cottonseed, cottonseed meal and cottonseed flour are not used for human consumption in Australia and New Zealand.

4.4 Potential allergenicity of novel proteins

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

Presence of the novel proteins in the food as consumed

As humans would be extremely unlikely to be exposed to either nitrilase or NPTII through the consumption of refined oil or cellulose products derived from BXN cotton there is virtually no potential for the two novel proteins to become food allergens.

Potential allergenicity of nitrilase

Studies submitted by the applicant:

Astwood, J.D. (1997). *Klebsiella ozaenae* nitrilase (BXN) has no significant sequence similarity to known allergens and toxins. Monsanto Study Report No. MSL-15120.

Aasen, E., *et al* (1997). Assessment of the digestibility of purified BXN nitrilase protein *in vitro* using mammalian digestive fate models. Monsanto Study Report No. MSL-15148.

Similarity to known allergens and gliadins

A search for amino acid sequence similarity with known allergens and gliadins is a useful first approximation of potential allergenicity and potential association with coeliac disease (Fuchs and Astwood 1996, Metcalf *et al* 1996). Many protein allergens have been characterised and their amino acid sequences are known, and importantly, their IgE binding epitopes have been mapped (Elsayad and Apold 1983, Elsayad *et al* 1991, Zhang *et al* 1992). The binding epitopes are generally between 8 and 12 amino acids in length.

To undertake the amino acid sequence comparison between nitrilase and known protein allergens and gliadins, a database of allergen and gliadin sequences was assembled from the standard public domain databases containing protein sequences (GenPept ver. 86.0, PIR ver. 41, SwissProt ver. 30).

In addition, DNA sequences were retrieved from GenBank/EMBL ver. 86 as some allergen sequence entries do not appear in the protein sequence databases. The amino acid sequences of the allergens retrieved from the GenBank/EMBL database were either obtained from the GenEMBL flat files or were obtained by translation of the open reading frames in the DNA sequences. Therefore the assembled database consisted of two parts: (1) a dataset of protein sequences and (2) a supplemental database of protein sequences initially retrieved as DNA sequences. Duplicates were deleted from the assembled database and irrelevant sequences were identified by examining complete flat files or by reference to the scientific literature. The resulting database of 219 allergens and gliadins has been published in the scientific literature (Astwood *et al* 1996).

The allergen and gliadin database was then searched for sequences similar to nitrilase. A significant sequence similarity was defined as a sequence identity of greater than seven contiguous amino acids. No significant similarity between nitrilase and any of the known allergens or gliadins was identified.

Digestibility of nitrilase

If proteins are to be allergenic they must be stable to the peptic and tryptic digestion and acid conditions of the digestive system if they are to pass through the intestinal mucosa to elicit an allergenic response.

The digestibility of nitrilase was determined experimentally using *in vitro* mammalian digestion models. *In vitro* studies with simulated digestion solutions have been used as models for animal digestion for a number of years and have had wide application.

To obtain sufficient quantities of purified nitrilase for testing, the enzyme was expressed in *Escherichia coli* from a cloned *Klebsiella ozaenae* DNA fragment and purified to homogeneity (Stalker *et al* 1988). The coding region used to express nitrilase in *E. coli* was therefore identical to that transferred into BXN cotton. The molecular mass of nitrilase is approximately 37 kDa, however, the active form of the enzyme is as a dimer composed of two identical 37 kDa subunits.

Nitrilase was added to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) and incubated at 37ºC over a series of time points. The time points for SGF were 0 sec, 15 secs, 30 secs, 1 min, 5 mins, 10 mins, 30 mins, 1 hour and for SIF the time points were 0 sec, 1 min, 5 mins, 15 mins, 30 mins, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours.

Analysis of nitrilase after incubation in SGF showed that the protein is degraded to below the limit of detection within 15 seconds. Nitrilase was found to be stable in an inactive test system over the time period tested confirming that the degradation of nitrilase in the active test system is due to proteolytic activity, not to any molecular instability of nitrilase.

In SIF, nitrilase was degraded within 5 minutes of exposure. Once again, nitrilase was shown to be stable in an inactive SIF system.

The results of these studies demonstrate that nitrilase is rapidly degraded in conditions that mimic mammalian digestion, greatly minimising any potential for intact nitrilase to be absorbed by the intestinal mucosa.

Neomycin phosphotransferase II

The potential allergenicity of neomycin phosphotransferase II (NPTII) has been investigated by ANZFA for a number of different applications for GM foods where simulated mammalian digestion studies have been submitted for evaluation as well as studies where its amino acid sequence has been compared with known allergens. None of these has revealed any potential for NPTII to be a food allergen. In addition, the safety of this protein, including its potential allergenicity, has also been considered on numerous occasions in the peer reviewed scientific literature (Flavell *et al* 1992, Nap *et al* 1992, Fuchs *et al* 1993a, Fuchs *et al* 1993b). In all instances it has been concluded that NPTII has limited potential to be a food allergen. This conclusion also applies to NPTII in BXN cotton, which is identical to the NPTII assessed for potential allergenicity on previous occasions.

Conclusion

Humans are highly unlikely to be exposed to either nitrilase or NPTII through the consumption of refined cottonseed oil and cellulose products from BXN cotton. Moreover, neither of the proteins possesses any of the characteristics of known allergens. It is therefore concluded that nitrilase and NPTII have very limited potential to become food allergens.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

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

Three separate compositional analyses of the BXN cotton lines were done using cottonseed samples collected from three separate field trials. In all field trials, each replicate represents a field plot (at least 150 m^2) planted in a randomised complete block design.

For the first set of compositional analyses, T_3 cottonseed was collected from T_2 BXN cotton plants (derived from the transformation events 10211 and 10222) grown at a single location in the United States in 1991. Homozygous seed from the same transgenic event were pooled and processed as a single line. Bromoxynil treatment had been used to identify the homozygous seed lots but the seed samples used for the analyses had themselves been obtained from unsprayed plants. The seed was shipped to the Engineering Biosciences Research Centre at Texas A&M University for small scale processing under Good Laboratory Practice to obtain cottonseed meal and crude oil for the analyses. Control samples were bulk seed of the non-transgenic control Coker 315. Two seed sample lots of Coker 315 came from the same field as the BXN cotton and a third sample lot came from plants grown at a different site in the same year. Constituents analysed were: fatty acid composition of the crude oil; and protein, nitrogen, fibre, residual oil and amino acid content of the meal.

For the second set of compositional analyses, seed was harvested from a BXN cotton line (derived from transformation event 10222) and a number of commercial cotton varieties grown at four locations in the United States in 1996. The plants were grown in two replicated plots per location. The BXN cotton plants were unsprayed. Constituents analysed were: moisture, fat, protein and fibre content of the seed; amino acid content of the meal; and major fatty acid composition of the crude oil.

For the third set of compositional analyses, seed was harvested from a BXN cotton line (derived from transformation event 10222) and a commercial cotton variety grown at two locations in Spain in 1997. The BXN cotton plants had been sprayed with 563 g a.i./ha of Buctril®, which is representative of an agronomic dose. Constituents analysed were: moisture, ash, fat, protein, and fibre content of delinted seed; amino acid content of the meal; and major fatty acid composition of the crude oil.

Cottonseed

1991 field trial data - unsprayed

In samples collected from the 1991 field trial, the only constituent measured in whole cottonseed was the fibre content. Crude fibre, acid detergent fibre, and neutral detergent fibre provide measurements of relative digestibility and bioavailability for cottonseed products. The results of these analyses are presented in Table 15 below.

Table 15: Crude fibre, acid detergent fibre and neutral detergent fibre composition[1] of whole cottonseed from 1991 field trials in the United States

Sample	Crude fibre	Acid detergent fibre	Neutral detergent fibre
Coker 315	14.7	21.6	27.0
Event 10211	14.3	25.0	29.8
Event 10222	15.1	21.9	27.4

[1] values are percent of whole cottonseed

The levels of crude fibre, acid detergent fibre and neutral detergent fibre in the BXN cotton were comparable to the levels obtained for the Coker 315 control.

1996 field trial data - unsprayed

The BXN cotton line grown in this field trial was derived from transformation event 10222. Control samples were obtained from commercial cotton varieties (LA887, ST132 and ST474) grown at the same location. The constituents measured in cottonseed samples collected from the 1996 field trials were moisture, fat/oil, protein and crude fibre content. The results of these analyses are presented in Table 16 below.

Table 16: Major constituents[1] of cottonseed harvested from plants grown in the field in 1996 in the United States

Sample	Moisture content	Fat/oil content	Protein content	Crude fibre content
Event 10222	6.77 a[2]	16.18 b	20.27 ac	31.36 a
LA 887	6.49 a	16.94 a	20.09 a	31.59 a
ST132	6.88 a	16.08 b	20.79 b	31.02 a
ST 474	6.73 a	16.14 b	20.56 bc	32.06 a

[1] values are percent by weight of whole cottonseed and are the means of single analyses of two replicates from four locations

[2] values in a column marked with the same letter are not significantly different at a 95% confidence level

The levels of major constituents in the BXN cotton line are equivalent to those in standard commercial varieties of cotton.

1997 field trial data – sprayed with Buctril®

The BXN cotton variety (OXY47) grown in this field trial is a variety developed from transformation event 10222 in a ST474 genetic background. The control, ST474, is a current commercial variety of cotton. The constituents measured in delinted cottonseed samples collected from the 1997 field trials were moisture, ash, fat/oil, protein and crude fibre content. The results of the analyses are summarised in Table 17 below.

Table 17: Major constituents[1] of cottonseed harvested from plants grown in the field in 1997 in Spain

Sample	Moisture (% weight)	Ash	Fat/oil	Protein	Crude fibre
OXY47	10.23 a[2]	4.58 b	32.87 b	37.94 a	4.58 a
ST474	10.18 a	4.98 a	32.51 a	37.81 a	4.98 a

[1] except for moisture, values presented are percent dry weight of sample and are the average of two replicates from two sites

[2] values in a column marked with the same letter are not significantly different at a 95% confidence level

With the exception of ash and fat/oil, the levels of major constituents in OXY47 are equivalent to those measured for the parental cotton line. The differences in ash and fat/oil content are minor and have no biological significance.

Cottonseed meal

1991 field trial data - unsprayed

The constituents measured in meal obtained from cottonseed samples collected from the 1991 field trial were % total nitrogen, % total protein, % residual oil and amino acid content. Toasted cottonseed meal was analysed for % crude protein and residual oil content. Control values were obtained from meal produced from the non-transgenic control line, Coker 315. The results of these analyses are summarised in Table 18 below.

Table 18: Nitrogen, protein, residual oil and amino acid content[1] of cottonseed meal obtained from plants grown in the field in 1991 in the United States

Constituent	Event 10211	Event 10222	Coker 315	Literature values[2]
Untoasted meal:				
% total nitrogen	7.21	8.55	4.37 (2.96 - 5.77)	
% total protein[3]	45.06	53.41	27.31 (18.51 - 36.03)	(22 – 50)
% residual oil	1.74	3.78	1.92 (0.574 - 4.19)	
Toasted meal:				
% crude protein	53.73	40.08	47.62	45.2[4]
% residual oil	3.16	1.26	2.68	
Amino acids[5]:				
Cysteine	1.7	1.8	1.7	2.2 (1.7 – 2.6)
Proline	3.7	3.7	3.7	4.2
Aspartic acid	10.5	10.3	10.1	10.8
Serine	5.0	5.0	5.1	4.7 (4.2 – 5.0)
Threonine	3.5	3.5	3.6	3.5 (2.9 – 4.1)
Glutamic acid	21.5	21.8	21.5	24.8
Glycine	3.9	3.9	4.0	4.8 (4.0 – 5.6)
Alanine	3.8	3.9	4.0	4.6
Valine	5.2	5.2	5.6	5.1 (4.3 – 7.4)
Methionine	1.3	1.5	1.5	1.5 (1.4 – 1.9)
Isoleucine	3.6	3.4	3.6	3.7 (3.5 – 4.3)

Leucine	6.2	6.2	6.4	6.1 (4.5 – 6.8)
Tyrosine	3.3	3.6	3.1	3.0 (1.6 – 3.6)
Phenylalanine	5.8	5.7	5.8	5.5 (3.5 – 6.6)
Histidine	3.0	3.0	3.0	2.8 (2.4 – 3.3)
Lysine	4.8	4.9	5.1	4.3 (3.2 – 5.1)
Arginine	13.3	12.8	12.3	11.4 (9.1 – 13.5)

[1] values are presented as means with the range in parentheses (where provided)
[2] values presented as means with the range in parentheses, values taken from Ensminger *et al* (1990), McCarthy and Matthews (1984) and National Research Council (1982)
[3] calculated from % nitrogen
[4] solvent extracted
[5] values are percent by weight of amino acid in cottonseed meal protein

Some significant differences were observed between the BXN cotton and control lines with the untoasted meal from BXN cotton containing significantly increased levels of total protein (and hence total nitrogen) compared to the Coker 315 control. The total protein levels recorded for events 10222 and 10211 were however comparable to the literature reported range for total protein. As the refining process essentially removes all traces of protein from the food products in question (i.e. the oil and linters), this finding does not have any significance from a food safety perspective.

The levels of amino acids in meal derived from BXN cotton are equivalent to the levels measured for the control and are comparable to the literature values where these exist – the differences observed in total protein content of the meal are not reflected in the amino acid content because the levels of each amino acid were calculated as percentage of the crude protein.

1997 field trial data – sprayed with Buctril®

The BXN cotton line (OXY47) grown in this field trial was derived from transformation event 10222 and is in a ST474 genetic background. Control samples were obtained from the commercial cotton variety ST474, which was grown at the same location. Meal obtained from cottonseed samples harvested from the 1997 field trial were analysed for amino acid content. The results of these analyses are presented in Table 19 below.

Table 19: Mean amino acid content[1] of cottonseed meal from control and BXN cotton (sprayed with Buctril®) grown in the field in 1997 in Spain

Amino acid	Literature values[2]	ST474	OXY47
Cysteine	2.2 (1.7 – 2.6)	1.9	2.0
Proline	4.2	3.9	3.9
Aspartic acid	10.8	10.2	10.2
Serine	4.7 (4.2 – 5.0)	4.7	4.6
Threonine	3.5 (2.9 – 4.1)	3.5	3.5
Glutamic acid	24.8	21.2	21.1
Glycine	4.8 (4.0 – 5.6)	4.3	4.2
Alanine	4.6	4.1	4.1
Valine	5.1 (4.3 – 7.4)	4.6	4.8
Methionine	1.5 (1.4 – 1.9)	1.7	1.6
Isoleucine	3.7 (3.5 – 4.3)	3.3	3.3
Leucine	6.1 (4.5 – 6.8)	6.2	6.2
Tyrosine	3.0 (1.6 – 3.6)	3.2	3.2
Phenylalanine	5.5 (3.5 – 6.6)	5.8	5.8
Histidine	2.8 (2.4 – 3.3)	2.9	3.0
Lysine	4.3 (3.2 – 5.1)	4.6	4.7
Arginine	11.4 (9.1 – 13.5)	12.5	12.5
Tryptophan	1.4 (1.2 – 1.7)	1.4	1.3

[1] values are percent by weight amino acid in cottonseed meal protein and are the average of four samples, two from each field site
[2] values presented as means with the range in parentheses, values taken from Ensminger *et al* (1990), McCarthy and Matthews (1984) and National Research Council (1982)

The amino acid levels for OXY47 cotton sprayed with Buctril® were equivalent to those obtained for the ST474 parental control and are comparable to the literature values for amino acid levels.

Crude cottonseed oil

Crude cottonseed oil was analysed, rather than refined cottonseed oil, because of the small amount of BXN cottonseeds available for processing.

1991 field trial data – unsprayed

Fatty acid composition was determined for crude cottonseed oil obtained from seed harvested from BXN cotton plants grown in the field in the United States in 1991. The fatty acid levels obtained were compared to those measured in oil obtained from the control line, Coker 315 and in a commercial cottonseed oil product – House of Tsang wok oil. The results are summarised in Table 20 below.

Table 20: Fatty acid composition[1] of crude cottonseed oil obtained from BXN cotton plants and non-transformed control plants grown in the field in the United States in 1991

Fatty acid	Codex standard[2]	Wok oil	Coker 315	Coker 315	Coker 315	Event 10211	Event 10222
C <14	< 0.1	0.07	0.02	0.03	0.03	0.05	0.03
C 14:0	0.4-2.0	0.90	0.70	0.90	0.90	0.69	0.72
C 16:0	17.0-31.0	22.53	25.68	26.26	26.36	24.50	24.65
C 16:1	0.5-2.0	0.63	0.52	0.57	0.58	0.46	0.47
C 18:0	1.0-4.0	2.62	2.82	2.64	2.69	2.78	2.83
C 18:1	13.0-44.0	19.65	15.51	15.58	15.79	14.28	13.72
C 18:2	33.0-59.0	52.37	53.87	53.05	52.65	56.30	56.72
C 18:3	0.1-2.1	0.43	0.17	0.17	0.17	0.19	0.18
C 20:0	< 0.7	0.33	0.31	0.34	0.35	0.33	0.30
C 20:1	< 0.5	0.11	0.07	0.08	0.08	0.09	0.08
C 22:0	< 0.5	0.21	0.16	0.20	0.20	0.21	0.17
C 22:1	< 0.5	0.03	0.04	0.03	0.03	0.03	0.04
C 24:0	< 0.5	0.07	0.11	0.13	0.14	0.00	0.00

[1] values are percent of total lipids and are the average of six replicates
[2] ranges adopted by the FAO/WHO Codex Alimentarius Committee on fats and oils (Jones and King 1993)

The fatty acid levels determined for oil derived from BXN cotton are equivalent to those levels obtained for oil derived from the non-transformed control line and are comparable to the levels measured in a commercial cottonseed oil product. With the exception of palmitoleic acid (C 16:1), the fatty acid levels determined for BXN cotton are also all within the Codex specified ranges for cottonseed oil. The levels of palmitoleic acid in transformation events 10211 and 10222 are only marginally outside the Codex specified range and this finding is not considered to have any biological or food safety significance.

1997 field trial data – sprayed with Buctril®

Fatty acid and tocopherol content was determined for crude cottonseed oil obtained from seed harvested from BXN cotton line OXY47 grown in the field in Spain in 1997 and sprayed with Buctril®.

OXY47 is a BXN cotton variety developed from transformation event 10222 in a ST474 genetic background. The fatty acid and tocopherol levels obtained were compared to those measured in oil obtained from a current commercial cotton variety (ST474). The results are summarised in Tables 21 and 22 below.

Table 21: Fatty acid composition[1] of crude cottonseed oil from BXN cotton sprayed with Buctril®, and a commercial variety of cotton, grown in the field in Spain in 1997.

Fatty acid	Codex ranges[2]	Literature values[3,4]	OXY47	ST474
Myristic (14:0)	0.4-2.0	0.68-1.16	0.85	0.85
Palmitic (16:0)	17.0-31.0	21.63-26.18	22.68	22.70
Palmitoleic (16:1)	0.5-2.0	0.56-0.82	0.55	0.58
Stearic (18:0)	1.0-4.0	2.27-2.88	2.15	2.25
Oleic (18:1)	13.0-44.0	15.17-19.94	16.00	16.35
Linoleic (18:2)	33.0-59.0	49.07-57.64	55.58	55.10
Linolenic (18:3)	0.1-2.1	0.23	0.20	0.20
Arachidic (20:0)	< 0.5	0.41	0.28	0.30
Eicosenoic (20:1)	< 0.5		0.10	0.10
Behenic (22:0)	< 0.5		0.13	0.18
Lignoceric (24:0)	< 0.5		0.10	0.10

[1] values are percent of total lipids and are an average of 4 replicates
[2] ranges adopted by the FAO/WHO Codex Alimentarius committee on fats and oils (Jones and King 1993)
[3] Cherry and Leffler (1984), [4] Cherry (1983)

Table 22: Tocopherol levels[1] in crude cottonseed oil from BXN cotton sprayed with Buctril® and a commercial variety of cotton grown in the field in Spain in 1997

Line	Location[2]	α-tocopherol	δ-tocopherol	Total
OXY47 (U)	a	724	408	1131
OXY47 (T)	a	711	439	1150
ST474	a	770	400	1170
OXY47 (U)	b	810	377	1187
OXY47 (T)	b	816	375	1190
ST474	b	788	374	1162
Literature values[3]		402	572	1050.5

[1] values are expressed in mg tocopherols/kg oil extracted from whole cottonseed and are the average of duplicate analyses. OXY47 was either treated (T) with Buctril® at the agronomic dose of 563 g a.i./ha , or not treated (U).
[2] two replicates per location
[3] Jones and King (1990)

The fatty acid and tocopherol levels determined for OXY47 (both sprayed with Buctril® and unsprayed) are equivalent to those obtained for the parental cotton line. The fatty acid levels reported are also comparable to the Codex specified ranges for cottonseed oil. The levels reported for the α- and γ-tocopherols in both the OXY and control cottons however are significantly different compared to those reported in the literature for crude oil, although the total tocopherol levels are comparable. This difference is probably a reflection of agronomic conditions and has no relevance for food safety.

Conclusion

On the basis of the data provided in the present application, food from BXN cotton is compositionally no different to food from other commercial cotton varieties. The spraying of BXN cotton with a bromoxynil-containing herbicide does not result in any significant changes to the levels of the key nutrients.

5.2 Levels of anti-nutrients

In addition to its toxic effects the terpenoid gossypol, naturally occurring in cottonseed, has anti-nutritive characteristics through reducing the availability of lysine (Yannai and Bensai, 1983). The level of gossypol in events 10211 and 10222 are no different to the levels found in the non-transformed controls and are also comparable to levels found in commercial varieties of cotton. Furthermore, refined cottonseed oil is essentially free of gossypol.

5.3 Ability to support typical growth and well-being

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

The compositional and other data presented in the application are considered adequate for establishing the ability of oil and linters from BXN cotton to support typical growth and well-being. Additional studies are therefore not required.

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ATTACHMENT 3

REGULATORY IMPACT ASSESSMENT

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

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

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

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

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

Conclusion of the regulatory impact assessment

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

ATTACHMENT 4

WORLD TRADE ORGANIZATION AGREEMENTS

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

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

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

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

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

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

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

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

As members of the WTO both Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists). Matters raised in this proposal may be notified to the WTO as either SPS notifications or TBT notifications, or both.

SPS Notifications

These are primarily health related, and refer to any sanitary and phytosanitary 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 PUBLIC SUBMISSIONS

The draft risk analysis report for Applications A372, A375, A378 and A379 were advertised together on the 7 March 2001. Many submitters provided comment on the four applications in one submission or the submissions were general comment on GM foods, rather than specific comments on each individual application. Submissions from both rounds of consultation have been summarised in this attachment and a response to many of the general comments are addressed either in the safety assessment report or in **Attachment 6**.

A: First round submissions

1. National Genetic Awareness Alliance (Australia)

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

2. Pola Lekstan and Anna Clements (Australia)

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

3. Arnold Ward (Australia)

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

4. Australian GeneEthics Network

- Believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:

- Direct health effects of pesticide residues
- Possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
- The possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
- Insertion of viral DNA could create new and virulent viruses
- The possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
- The threat to GE-free status export markets

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

5. Public and Environmental Health Service (Australia)

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

6. David Grundy (Australia)

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

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

▪ Believes that:
  - Scientific research although limited, has brought concerns to light
  - Substantial equivalence is a subjective principal
  - Comprehensive and mandatory labelling must be urgently implemented

- The cauliflower mosaic virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
- Antibiotic marker genes could lead to increase in antibiotic resistance
- Several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

8. Australian Food and Grocery Council (AFGC)

- 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 Seymour, 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

B. Second round submissions

1. Australian Food and Grocery Council (AFGC)

- Supports the approval of the four applications:
  - A372 Oil derived from glufosinate ammonium tolerant canola lines Topas 19/2 And T45 and Oil derived from glufosinate-ammonium tolerant and pollination controlled lines Ms1, Ms8, Rf1, Rf2 And Rf3;
  - A375 Food derived from glufosinate ammonium tolerant corn line T35;
  - A378 Food derived from glyphosate-tolerant sugar beet line GTSB77; and
  - A379 Oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222.
- Submits that as ANZFA has concluded that foods encompassed by the four 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 GM foods.
- Supports the application of the revised labelling requirements of Standard A18 to the products encompassed by these four GM applications.

2. Bentleigh-Bayside Gene Alert, Campaign for Safe Food (Australia)

- Opposes all four of the GM food applications because of overwhelming concerns about the risks to health and the environment, particularly in the use of herbicides.
- Supports independent testing and questions the role and validity of overseas approvals of GM commodities in the Australian process.
- Contends that the safety assessments were questionable and scientifically unsound because of apparent inadequacies in the toxicity testing and in the conclusions drawn from the animal feeding studies.
- Considers that the assessment should include possible changes to the food product as it is metabolised by livestock that are bred for human consumption.
- Advises that the precautionary principle should be adopted in relation to the use of antibiotic resistance marker genes.

3. **New Zealand Ministry of Health**
   - Supports the conclusions of the ANZFA Draft Risk Analysis Reports for all four applications, that the foods are safe for human consumption.
   - Considers that the most important data are the molecular characterisation of the inserted DNA and compositional analyses, requiring presentation of as much raw data as possible, and that brief summaries of other issues are all that is required, especially where the same proteins have been previously assessed.

4. **Anne FitzSimon (New Zealand)**
   - Opposes the approval of all four applications primarily for ethical reasons and concerns about safety.
   - Demands detailed labelling of GM foods to enable consumer choice.

5. **Nelson GE Awareness Group (Susie Lees) (New Zealand)**
   - Do not support the approval of the four GM applications because they consider that GM foods pose unique public and environmental health risks.
   - Submits that there has been no independent scientific testing of the products.
   - Suggests complete removal of these foods from the market until safety testing and long term feeding studies of at least 12-18 years duration have been completed.
   - Submits that the new labelling provisions do not capture all foods produced using gene technology.
   - A372 – expresses grave concerns associated with the use of the *barnase*/*barstar* gene system (uses the term 'terminator technology'), and claims that whole canola seeds are used in certain bakery products.
   - Opposes the use of antibiotic resistance genes in all of the applications.

6. **Kate Clinch-Jones (Australia)**
   - Opposes all of the applications on the basis that the respective Draft Risk Analysis Reports do not address the potential public health and safety issues associated with the genetic modifications.
   - Claims that the safety assessments are not comprehensive, and lack adequate scientific evidence and peer review.
   - Opposes the use of the herbicides glyphosate and glufosinate-ammonium because of concerns relating to potential toxicity in humans and the environment.
   - Criticises the regulatory impact statement for each GM application. Contends that benefits of prohibiting the sale of GM foods include the protection of the integrity of the food chain, avoiding irreversible environmental damage, upholding the precautionary principle and meeting consumer demands.
   - Disagrees with government obligations in relation to the WTO.
   - Disagrees with ANZFA's assessment and discussion of the possibility for horizontal gene transfer and refers to supporting scientific articles.
   - Expresses concerns about food products derived from stock animals that consume GM crops.
   - States that because of the confidentiality of some of the information, potential hazards may not be identified by independent reviewers.
   - Suggests that ANZFA seek advice about antibiotic resistance genes from microbiology and infectious disease specialists.
   - Supports full proteome analysis on all GM foods.
   - Recommends that an expert team of advisors be established to design scientifically sound feeding studies that also consider ethical issues.

7. Food Technology Association of Victoria Inc.

- Supports approval of the four applications (A372, A375, A378 and A379) provided ANZFA is satisfied with their safety and that the foods will be appropriately labelled for the benefit of consumers.

8. Adrian Elliot (Australia)

- Supports the approval of the GM food applications and regards these as trailblazers.
- Claims that the new GM foods will assist in keeping Australian industry in step with developments made by the rest of the world.
- Considers that both industry and consumers benefit from the development of new varieties and new technology.
- Comments that the public would benefit from a national education campaign to provide greater awareness of the food supply and to promote public understanding of the technology, the safety and regulation of the products arising from this technology.

9. Aventis CropScience

- Suggests minor amendments and corrections to the Draft Risk Analysis Reports for each of the applications, which will be addressed in the respective Final Risk Analysis Reports.

10. GeneEthics Network (Australia)

- Opposes all four of the applications because of perceived adverse effects on the environment and public health.
- Opposes the use of the herbicides glyphosate, glufosinate ammonium and bromoxynil because of concerns about toxicity.
- States that ANZFA's regulatory impact assessment fails to acknowledge that primary production could be negatively affected by GM crops. ANZFA should consider the economic effects of its decisions.
- Considers that ANZFA's safety assessment process is too narrowly focussed and fails to consider environmental and animal health issues.
- Disagrees that ANZFA's assessments adopt a cautious approach.
- Considers that the safety assessment reports lack sufficient information to demonstrate food safety, and do not adequately consider the possibility of trace amounts of unintentional or unanticipated products.
- Expresses outrage that there is no post-market surveillance system in place to monitor any effects of crop release or GM food consumption.
- States that the new labelling regime is too lax and contravenes the rights of consumers to know whether foodstuffs have been genetically modified.

11. Public Health Association of Australia Inc (PHAA)

- Asserts that ANZFA does not respond to all issues raised in their previous submissions.
- Expresses concerns on the use by ANZFA of the concept of substantial equivalence.
- Raises concerns on the use of antibiotic resistance marker genes during GM crop development.
- Claims that ANZFA does not require data in support of applications that is generated by independent laboratories other than the applicant.
- Raises concerns regarding the lack of detail in reporting of the parameters investigated in the acute toxicity tests on CP4 EPSPS, GUS and protein 34550.

A379:

- Raised concern about the adequacy of the toxicity studies
- Raised concerns about ANZFA's assessment of the toxicity of bromoxynil and its break down products
- Commented on the compositional differences between the GM versus control lines.

12. Consumers' Institute (New Zealand)

- Provides comments on the GM applications as a group, not as individual foods, stating that the regulatory process should take into consideration new scientific information or data as, or when, it becomes available and react accordingly.
- Favours ongoing monitoring of any long term effects
- States that consumers are primarily concerned with the apparent lack of independent verification of testing carried out by developers of the products, as well as the failure to do long term testing and animal testing of the products.
- Expresses a lack of confidence in the assessment process and in the principle of 'substantial equivalence' because of concerns that unexpected changes may not be identified.
- Considers that the system of regulation applying to new medicines, which require random controlled trials, is rigorous and the same has not been applied to GM foods.

13. Claire Bleakley (New Zealand)

- Believes that the foods should not be allowed on the market until the New Zealand Royal Commission has reported and labelling of GM foods is in place.
- Believes that there are risks that have not been fully considered:
  - effect of novel proteins on metabolic pathways e.g. over expression/inhibition of enzymes;
  - toxicity or allergenicity;
  - small compositional changes in the foods mean they are not substantially equivalent;
  - the risk of transfer of antibiotic resistant marker genes;
  - that gene sequences of genetic manipulations are prone to contain fragments of virulent genes that could be detrimental to human and environmental systems.
- Believes that previous decisions cannot be seen to be taking into account the "high degree of consumer confidence" as per the ANZFA Act.
- Believe that there hasn't been adequate consumer information made available in contravention to the ANZFA Act, i.e. provision of information to enable informed decision-making.
- Believe that long-term studies are required to show that the genetic constructs do not cause harm to the environment.

14. National Council of Women of Australia Inc

- Does not support the approval of any of the four applications due to concerns that GM foods have not been tested either adequately or appropriately.
- Provided comment on individual applications, which will be addressed within the specific issues section of the Final Risk Assessment Report.
- Raised concerns about the environmental impact as well as toxicity, neurotoxicity and teratogenicity of glufosinate ammonium and provided information about overdoses of glufosinate ammonium.

- Is concerned that GM applications for herbicide tolerant crops will result in the increasing use of herbicides.
- Considers that any health risk is not acceptable as the technology is not needed to feed the world or wanted by consumers.
- States that no further GM applications should be accepted until the Office of the Gene Technology Regulator has addressed the environmental, social and ethical issues, as ANZFA has no community consultative or ethics group to consider these issues.
- Considers that the benefits of the technology accrue to the applicant.
- Considers that ANZFA is not responding to objections raised previously and is repeating previous responses, leading to little desirable outcome from a community and public interest perspective.
- Believes that ANZFA is dismissing public opinion given that the majority of submissions are against approval of GM applications.
- States that the labelling laws are inadequate.

15. Consumers' Association of South Australia Inc

- Supports the submissions made by the National Council of Women.

16. GE Free New Zealand (RAGE)

- Opposes all four of the applications, A372, A375, A378 and A379.
- Provides a list of health and medical concerns that are claimed to be attributable to gene technology.
- Expresses grave fears about the possible health consequences of GM foods in general.
- Application specific concerns include:
  A379 – the use of the CaMV 35S promoter and the presence of antibiotic resistance genes
  A372 – the use of antibiotic resistance genes.

17. Sandra Jacobs (New Zealand)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.
- Considers that GE foods are polluting other crops, particularly GE canola containing the *barnase* gene.

18. Brian Lister and Lorraine Leader (New Zealand)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.
- Considers that the safety of GE foods cannot be guaranteed.

19. Paul Elwell-Sutton (New Zealand)

- Opposes application A372, because of a lack of confidence in the independence of the laboratories that generated the assessment data.
- Expresses concerns about the possible presence of novel substances or proteins in the canola meal that may enter the food supply.
- Considers that the labelling provisions are not adequate to ensure that consumers will be able to know about GE foods in products.

- Considers that ANZFA has not addressed the issue of the possible transfer of antibiotic resistance marker genes to gut microorganisms of stock, as animals are fed on canola meal and stubble.
- ANZFA's reports do not address the precautionary principle.
- Considers that GE food could have effects on the ageing process in animals, including humans, which ANZFA failed to consider in the assessment.
- Expresses concern that food approval will lead to planting of GE canola in New Zealand that will then lead to inevitable contamination of other crops.
- ANZFA has not adequately considered consumers in the assessment process.
- Opposes the remaining GM applications A375, A378 and A379 for the same reasons.

20. Julian Yates (New Zealand)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.

21. Oraina Jones (New Zealand)

- Opposes all four of the applications, A372, A375, A378 and A379 due to philosophical and ethical concerns relating to the environment and health.

ATTACHMENT 6

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received in response to the Section 14 Gazette Notice, express general views against the use of gene technology and assert that food produced using this technology is unsafe for human consumption, irrespective of the food concerned or the particular genetic modification. 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/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

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

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD). The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

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 express concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some reject 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. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

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

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for approximately ten years and has been an integral part of the safety assessment of some 40 products.

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

4. *The nutritional value of food produced using gene technology*

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

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

- *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 express 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 focus on this issue. Specifically, the submissions call for comprehensive labelling of foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters base their demands for full labelling on the presumption that all foods produced using gene technology are unsafe, even where no novel genes are present, and on consumer "right to know" arguments. It is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

- *Evaluation*

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard A18 (Volume 1) is now also known as Standard 1.5.2 in the joint Australia New Zealand Food Standards Code (Volume 2). 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.

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

Exempt from these requirements are:

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

In addition, the 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.

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

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[6], 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*

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

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

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

- the Australia New Zealand Food Authority (ANZFA)
- the Therapeutic Goods Administration (TGA)

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

- the National Registration Authority for Agricultural and Veterinary Chemicals (NRA)
- the National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
- the Australian Quarantine and Inspection Service (AQIS).

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

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

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

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

ATTACHMENT 7

STATEMENT OF REASONS

APPLICATION A379 - FOR RECOMMENDING A VARIATION TO STANDARD A18 OF VOLUME 1 AND STANDARD 1.5.2 OF VOLUME 2 OF THE *FOOD STANDARDS CODE* FOR THE APPROVAL OF OIL AND LINTERS FROM BROMOXYNIL-TOLERANT COTTON TRANSFORMATION EVENTS 10211 AND 10222

The Australia New Zealand Food Authority (ANZFA) has before it an application received on 30 April 1999 from Aventis CropScience Pty Ltd and the Stoneville Pedigreed Seed Company to amend Standard A18 of Volume 1 and Standard 1.5.2 of Volume 2 of the *Food Standards Code* for the approval of oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222.

ANZFA recommends the adoption of the draft variation for the following reasons:

- There are no public health and safety concerns associated with the two genes introduced into bromoxynil-tolerant cotton transformation events 10211 and 10222.

- Oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222 are as safe and wholesome as that from other commercially available cotton.

- On 7 December 2001, food products containing oil or linters from bromoxynil-tolerant cotton will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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

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

REGULATION IMPACT

ANZFA has undertaken a regulation impact assessment process, which also fulfils the requirement in New Zealand for an assessment of compliance costs. That process concluded that the amendment to the Code is necessary, cost effective and of benefit to both producers and consumers.

WORLD TRADE ORGANIZATION (WTO) NOTIFICATION

Australia and New Zealand are members of the WTO and are bound as parties to WTO agreements. In Australia, an agreement developed by the Council of Australian Governments (COAG) requires States and Territories to be bound as parties to those WTO agreements to which the Commonwealth is a signatory. Under the agreement between the Governments of Australia and New Zealand on Uniform Food Standards, ANZFA is required to ensure that food standards are consistent with the obligations of both countries as members of the WTO.

In certain 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. 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).

This matter was notified to the WTO because there is significant international interest in the safety of GM foods and the proposed amendments are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters.

DRAFT VARIATION TO VOLUME 1 AND 2 OF THE *FOOD STANDARDS CODE*

A379 – OIL AND LINTERS DERIVED FROM BROMOXYNIL-TOLERANT COTTON TRANSFORMATION EVENTS 10211 AND 10222

To commence: on gazettal

The Food Standards Code is varied by:

[1] *Standard A18 of Volume 1 and Standard 1.5.2 of Volume 2 are varied by inserting in Column 1 of the Table to clause 2 -*

Oil and linters derived from bromoxynil-tolerant cotton transformation events 10211 and 10222.