FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

5-05
3 August 2005

DRAFT ASSESSMENT REPORT

APPLICATION A553

FOOD DERIVED FROM GLYPHOSATE-TOLERANT COTTON LINE MON 88913

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 14 September 2005
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

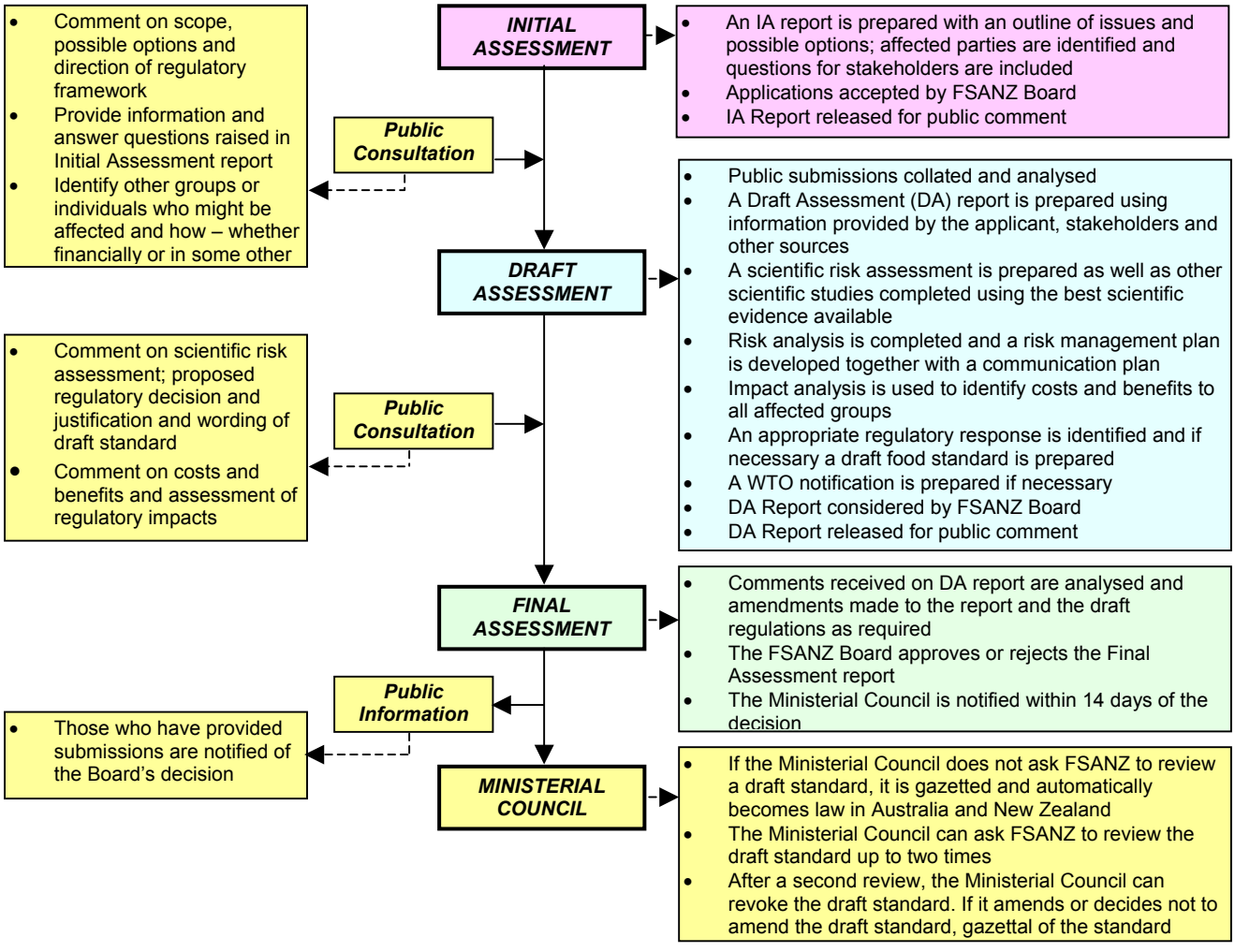
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Australian Government; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Australian Government, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Australian Government, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A533; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
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NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 14 September 2005.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ Website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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Executive Summary and Statement of Reasons

An Application was received from Monsanto Australia Limited on 17 November 2004 seeking approval for food derived from genetically modified (GM) cotton line MON 88913 under Standard 1.5.2 – Food Produced Using Gene Technology of the *Australia New Zealand Food Standards Code* (the Code). Standard 1.5.2 requires that GM foods (refer to Standard 1.5.2 for the definition of a GM food) undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

The new genetic trait in cotton line MON 88913 (Roundup Ready® Flex cotton) confers increased tolerance to glyphosate herbicide during reproductive phases of growth. Tolerance to the herbicide is achieved by expression in the plant of a bacterially derived enzyme that specifically inactivates the herbicide, allowing the plants to survive and grow following herbicide application.

Cotton line MON 88913 has been developed for cultivation in the major cotton growing regions of the world and has recently received approval for commercial release in the United States. In Australia, Roundup Ready® Flex cotton has been planted in contained field trials under the OGTR licence DIR 035/2003 since October 2003, and recently, the OGTR completed a full risk assessment and risk management plan (DIR 055/2004) for a large scale field trial. The Applicant intends to release cotton line MON 88913 for commercial production in Australia in the future. Roundup Ready® Flex cotton will not be grown in New Zealand.

Food derived from this cotton could enter the market in Australia and New Zealand via imported and domestic products, once it is grown on a commercial scale and approved for use under the Code.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from cotton line MON 99813 as required under the Act. The assessment included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of any new proteins; and (iii) the composition and nutritional adequacy of the food, including whether there had been any unintended changes.

No potential public health and safety concerns were identified in the assessment of food derived from cotton line MON 88913. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from cotton line MON 88913 is as safe and wholesome as food derived from other soybean varieties.

Labelling

Under Standard 1.5.2, GM food must be labelled if novel DNA and/or protein are present in the final food and also where the food has altered characteristics.

The only food uses of cotton are derived from cottonseed oil and linters, neither of which contained DNA or novel protein. Foods containing these ingredients would therefore not be required to be labelled.

Impact of regulatory options

Two regulatory options were considered in the assessment: either (1) no approval; or (2) approval of food from cotton line MON 88913 based on the conclusions of the safety assessment. Following an assessment of the potential impact of each of the options on the affected parties (consumers, the food industry and government), Option 2 is the preferred option as it potentially offers significant benefits to all sectors with very little associated cost. The proposed amendment to the Code, giving approval to food from cotton line MON 88913, is therefore considered of net benefit to both food producers and consumers.

Consultation

In response to the invitation to comment on the Initial Assessment Report, eighty-four submissions were received from the public, of these eighty were not in favour of approving cotton line MON 88913. The remaining submitters expressed support for the Application, contingent on a satisfactory safety assessment.

Statement of Reasons

An amendment to the Code to give approval to the sale and use of food derived from cotton line MON 88913 in Australia and New Zealand is recommended on the basis of the available scientific evidence for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce herbicide tolerant cotton line MON 88913;

- food derived from cotton line MON 88913 is equivalent to food from other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from cotton line MON 88913 will be required if novel DNA and/or protein is present in the final food;

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

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

It is proposed that the draft variation come into effect on the date of gazettal.

1. Introduction

An Application was received from Monsanto Australia Limited on 17 November 2004 seeking approval for food derived from herbicide-tolerant cotton line MON 88913 under Standard 1.5.2 – Food Produced Using Gene Technology.

The genetic modification involved the transfer into the cotton plant of the *epsps* gene derived from the bacterium *Agrobacterium* sp. Strain CP4. The *epsps* gene encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which confers tolerance to the herbicide glyphosate.

A Draft Assessment of the Application, including a detailed safety assessment of food from herbicide-tolerant cotton line MON 88913, has been completed and public comment is now being sought to assist in the Final Assessment of the Application.

2. Regulatory Problem

Standard 1.5.2 requires that a GM food undergo a pre-market safety assessment before it may be sold in Australia and New Zealand. Foods that have been assessed under the Act, once fully approved, are listed in the Table to clause 2 of Standard 1.5.2.

Monsanto Australia Ltd. has developed a new variety of herbicide-tolerant cotton, cotton line MON 88913. Before food derived from this cotton can enter the food supply in Australia and New Zealand, it must first be assessed for safety and an amendment to the Code must be approved by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted once the Ministerial Council process has been finalised.

Monsanto Australia Limited has therefore applied to have Standard 1.5.2 amended to include food derived from cotton line MON 88913 in the Table to clause 2.

3. Objective

The objective of this Application is to determine whether it is appropriate to amend the Code to approve the use of food derived from cotton line MON 88913 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 of the FSANZ Act. These are:

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

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;

- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

In addressing the issue of approving the sale and use of food from cotton line MON 88913, the key objectives are the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, FSANZ will also have regard for the need for standards to be based on risk analysis using the best available scientific evidence and the desirability of an efficient and internationally competitive food industry.

4. Background

The Applicant has developed cotton plants that are genetically modified for tolerance to the herbicide glyphosate. These cotton plants are referred to as cotton line MON 88913 (Roundup Ready® Flex). The purpose of the modification is to provide growers with an expanded window of application of the glyphosate herbicide and enhanced flexibility in weed control options, relative to the current Roundup Ready® cotton product.

Cotton line MON 88913 has been developed by the insertion of the *cp4 epsps* gene, derived from the soil bacterium *Agrobacterium*. The modification did not involve the transfer of any antibiotic resistance genes.

Glyphosate is the active ingredient of the proprietary herbicide Roundup® which is used widely as a non-selective agent for controlling weeds in primary crops. The mode of action of glyphosate is to specifically bind to, and block, the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme involved in the biosynthesis of aromatic amino acids in all plants, bacteria and fungi. Glyphosate-tolerance is conferred by the introduction of a bacterial gene (from *Agrobacterium* sp. strain CP4), which produces an EPSPS enzyme with a reduced affinity for glyphosate. The resultant level of enzyme activity is sufficient to produce the aromatic amino acids essential for growth and thus sustain the plant in the presence of the herbicide

Cotton is primarily grown for its fibre, however the cottonseed can be processed into a number of important by-products, some of which are used in food. The major by-products are oil, meal, hulls and linters with only the oil and the linters being used for human consumption. Cottonseed oil is used in a variety of food including cooking, salad and frying oils: mayonnaise, salad dressing, shortening, margarine and packaging oils. Cotton linters are used in high fibre dietary products as well as viscosity enhancers in toothpaste, ice cream and salad dressing. Cottonseed meal is primarily used for stock food, and is not currently sold for human consumption in Australia or New Zealand.

Cotton line MON 88913 has been developed for cultivation in the major cotton growing regions of the world and has recently received approval for commercial production in the United States. The anticipated commercial launch of Roundup Ready® Flex is for the 2006 growing season.

In Australia, Roundup Ready® Flex cotton has been planted in contained field trials under the OGTR licence DIR 035/2003 since October 2003, and recently, the OGTR completed a full risk assessment and risk management plan (DIR 055/2004) for a large scale field trial over two seasons on up to 91 sites (1811 ha.) from 2005-6. The Applicant intends to release cotton line MON 88913 for commercial production in Australia in the future. Roundup Ready® Flex cotton will not be grown in New Zealand.

5. Relevant Issues

5.1 Safety assessment of food from cotton line MON 88913

Food from cotton line MON 88913 has been evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The safety assessment included the following:

- a detailed characterisation of the genetic modification to the plant;
- characterisation of any novel proteins, including their potential toxicity and allergenicity;
- a consideration of the composition and nutritional adequacy of the food, including whether there had been any unintended changes to the food.

The Applicant submitted a comprehensive data package in support of their application and provided studies on the molecular characterisation of cotton line MON 88913, the potential toxicity and allergenicity of the EPSPS protein, compositional analyses of food derived from cotton line MON 88913. In addition to information supplied by the applicant, the evaluation also had regard to other available information and evidence, including from the scientific literature, general technical information, other regulatory agencies and international bodies.

No potential public health and safety concerns were identified in the assessment of food from cotton line MON 88913. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from cotton line MON 88913 is as safe and wholesome as food derived from other cotton varieties. The full safety assessment report is at **Attachment 2** to this document.

5.2 Labelling

Under Standard 1.5.2, GM food must be labelled if novel DNA and/or protein are present in the final food and also where the food has altered characteristics. The only food uses of cotton are derived from cottonseed oil and linters, neither of which contained DNA or novel protein. Foods containing these ingredients would therefore not be required to be labelled.

5.3 Issues arising from public submissions

In addition to the specific issues addressed below, FSANZ has also developed a Fact Sheet: *Frequently Asked Questions on Genetically Modified Foods – August 2002*, which responds to many of the general issues raised in connection with GM foods. The Fact Sheet may be obtained from the FSANZ website[2].

[1] http://www.foodstandards.gov.au/_srcfiles/ACF6A6.pdf

[2] www.foodstandards.gov.au/mediareleasespublications/factsheets/factsheets2002/index.cfm

5.3.1 Adverse effects on rat organs and the survival of cp4 epsps transgenes in the intestine

GE Free New Zealand raised the issues that glyphosate-tolerant canola, expressing the EPSPS protein, could have an adverse effect on rat organs and that *cp4 epsps* transgenes could be transferred to human gut microflora.

5.3.1.1 Response

FSANZ has addressed issues regarding animal feeding studies and adverse effects on rat organs arising from Glyphosate-tolerant canola in the GM fact sheet[2] and more specifically, in *Genetically Modified or GM Foods: Safety of Genetically Modified Foods*[3].

GE Free New Zealand referred to a study by Netherwood et al. (2004), for which the fate of the epsps gene from GM soy was traced in seven ileostomy patients.

In the subjects with intact gastrointestinal tracts, none of the endogenous bacteria in the faeces were found to contain the *epsps* gene from the GM soy. This indicates that either the *epsps* -containing bacterium in the small bowel of the ileostomists did not survive passage through the human colon or that in intact digestive systems gene transfer from plant material to the intestinal microflora does not occur at the same frequency as in the ileostomists. Furthermore, no intact novel DNA was found in the faeces of volunteers with intact gastrointestinal tracts. The authors conclude that the data presented in this study support the view that GM foods do not represent a significant risk to human health through gene transfer to either the intestinal epithelium or the microflora within the human intestine. Furthermore, due to the refined nature of cottonseed oil, it is highly unlikely to contain any detectable DNA and therefore there is negligible chance for the presence of *cp4 epsps* transgenes in cottonseed oil to be transferred to human gut microflora.

6 Regulatory Options

6.1 Option 1 – not approve food from cotton line MON 88913

Maintain the *status quo* by not amending the Code to approve the sale of food derived from cotton line MON 88913.

6.2 Option 2 – approve food from cotton line MON 88913

Amend the Code to permit the sale and use of food derived from cotton line MON 88913, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

- Consumers, particularly those who have concerns about biotechnology;
- Food importers and distributors of wholesale ingredients;

[3] http://www.foodstandards.gov.au/whatsinfood/gmfoods/index.cfm

- The manufacturing and retail sectors of the food industry; and

- Government generally, where a regulatory decision may impact on trade or WTO obligations and enforcement agencies in particular who will need to ensure that any approved products are correctly labelled.

7.2 Impact of regulatory options

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts.
The following is a draft assessment by FSANZ of the costs and benefits of the two regulatory options.

This is based on information supplied by the applicant, issues raised in the first round of public comment on the Application and experience FSANZ has gained from consideration of previous applications relating to GM foods. Your further comments are invited on the costs and benefits identified for the options below.

7.2.1 Option 1

Consumers: Cost in terms of a possible reduction in the availability of certain food products.

Cost associated with higher retail prices for segregated foods.

No impact on consumers wishing to avoid GM foods, as food derived from cotton line MON 88913 is not currently permitted in the food supply.

Government: This decision may impact on monitoring resources as it could be necessary to test imported cotton products to ensure an unapproved cotton line was not being sold in Australia and New Zealand

Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Industry: Cost to the food industry to source either segregated or non-GM supplies.

Potential longer-term impact - any successful WTO challenge has the potential to impact adversely on food industry.

7.2.2 Option 2

Consumers: Benefit of lower prices, to the extent that savings from production efficiencies are passed on.

Benefit of access to a greater range of products including imported food products containing ingredients derived from cotton line MON 88913.

Unlikely to impact negatively on consumers wishing to avoid GM food as food from other varieties of GM cotton is already permitted in the food supply.

Government: Possible impact. This decision may impact on monitoring resources.

Industry: Benefit to importers and distributors of overseas food products as the product range is extended.

Benefit for food manufacturers in that the choice of raw ingredients is extended.

Benefit to food retailers in an increased product range.
Benefit to farmers in an increased crop range with improved crop management issues.

7.2.3 Discussion

Option 1 would impose significant costs, particularly on consumers and the food industry sector, without offering any commensurate health benefit. This option is also likely to be inconsistent with Australia and New Zealand's obligations under the WTO. This option would also offer very little benefit to those consumers wishing to avoid GM foods, as food from other GM cotton varieties is already permitted in the food supply.

Option 2 is the preferred option as it potentially offers significant benefits to all sectors with very little associated negative impact.

The proposed amendment to the Code, giving approval to food from cotton line MON 88913, is therefore considered necessary, cost effective and of net benefit to both food producers and consumers.

8. Consultation

8.1 Public submissions

The Initial Assessment of this Application was advertised for public comment between 9 February 2005 and 23 March 2005. A total of eighty-five submissions were received during this period and a summary of these is included in **Attachment 3** to this Report.

FSANZ carried out an assessment of the Application, including a safety assessment of the food, taking into account the comments received in the first round of consultation.

In assessing the safety of the food, specific issues relating to cotton line have been addressed in the report.

8.2 WTO notification

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are no relevant international standards for GM foods, however the proposed amendment to the Code to allow food derived from cotton line MON 88913 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade.

For these reasons, FSANZ will be recommending to the agencies responsible that the WTO be notified under the Sanitary and Phytosanitary Measure (SPS) Agreement, in order to enable other member nations to comment on the proposed changes to standards that may have a significant impact on them.

9. Conclusion and Recommendation

An amendment to the Code to give approval to the sale and use of food from cotton line MON 88913 in Australia and New Zealand is recommended on the basis of the available scientific information for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce cotton line MON 88913;
- food derived from cotton line MON 88913 is equivalent to food from other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from cotton line MON 88913 will be required if novel DNA and/or protein is present in the final food;
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the amendment to the Code is necessary, cost effective and of net benefit to both food producers and consumers; and
- the proposed draft amendment to the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

The proposed draft variation is provided in **Attachment 1**.

10. Implementation and review

It is proposed that the draft variation come into effect on the date of gazettal.

Attachments

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Draft safety assessment report
3. Submission summary

ATTACHMENT 1

DRAFT VARIATION TO THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE

To commence: On gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2 –

Food derived from glyphosate-tolerant cotton line MON 88913

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT REPORT

APPLICATION A553 – FOOD FROM GLYPHOSATE-TOLERANT COTTON LINE MON 88913

SUMMARY AND CONCLUSIONS

Background

Food derived from genetically modified (GM) cotton line MON 88913 has been assessed for its safety for human consumption. This cotton line has been genetically modified to be tolerant to the herbicide glyphosate. The line in this application will be known commercially as Roundup Ready® Flex cotton.

A number of criteria have been addressed in the safety assessment including: a characterisation of the transferred genes, their origin, function and stability; changes at the DNA, protein and whole food levels; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic to humans.

History of Use

Cotton is grown primarily for the value of its fibre, with cottonseed and its processed products being a by-product of the crop. Humans have consumed cottonseed oil, the major product of cottonseed, for decades. Cottonseed oil is considered to be premium quality oil, valued for its high-unsaturated fatty acid content. The other food use of cottonseed is the linters, which are composed of greater than 99% cellulose. Cottonseed itself and the meal fraction are not presently used in Australia and New Zealand as a food for human consumption because they contain naturally occurring toxic substances. These toxins are essentially removed in the production of oil and linters, making them fit for human consumption. The types of food products likely to contain cottonseed oil are frying oils, mayonnaise, salad dressing, shortening, and margarine. After processing, linters may be used as high fibre dietary products and thickeners in ice cream and salad dressings.

Description of the Genetic Modification

Cotton line MON 88913 was generated using an Agrobacterium-mediated transformation system through the transfer of the *epsps* gene to a line derived from the cultivar Coker 312. The *epsps* gene encodes the protein 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme involved in the biosynthesis of aromatic amino acids in all plants, bacteria and fungi. Glyphosate-tolerance is conferred by the introduction of a bacterial gene (from *Agrobacterium* sp. strain CP4), which produces an EPSPS enzyme with a reduced affinity for glyphosate. The resultant level of enzyme activity is sufficient to produce the aromatic amino acids essential for growth and thus sustain the plant in the presence of the herbicide.

No functional antibiotic resistance genes were transferred to cotton line MON 88913; selection was through resistance to glyphosate. Detailed molecular and genetic analyses of cotton line MON 88913 indicate that the transferred *epsps* gene is stably integrated into the plant genome at a single insertion site and is stably inherited from one generation to the next.

Characterisation of Novel Protein

Cotton line MON 88913 expresses a single novel protein – EPSPS. Protein expression analyses indicate that EPSPS is expressed at low levels or is undetectable in the cotton and their processed fractions and therefore exposure to the protein through consumption of food derived from cotton line MON 88913 would be negligible, if at all. In cotton line MON 88913, EPSPS was found in leaves, roots, seed and pollen at the following (mean) levels: 170, 31, 310 and 4 μg/g fresh weight respectively. The amount of CP4 EPSPS protein in the total cottonseed protein was approximately 0.12 %. Previous studies have shown that no EPSPS protein is present in cottonseed oil and that linter food products are protein-free.

The safety of EPSPS has been assessed on numerous previous occasions by FSANZ. In all instances it has been concluded that EPSPS is non-toxic to humans and has limited potential as a food allergen.

Compositional Analyses

Compositional analyses were done to establish the nutritional adequacy of cotton line MON 88913, and to compare it to the isogenic control line, which does not express the *cp4 epsps* gene, and commercial varieties of cotton. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, minerals and the anti-nutrients, gossypol, cyclopropenoid fatty acids and aflatoxins.

No differences of biological significance were observed between the cotton line MON 88913 and the isogenic control line. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very small percentage changes and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it was concluded that food from cotton line MON 88913 is equivalent in composition to that from other commercial cotton varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that food derived from cotton line MON 88913 is equivalent in composition to food from non-GM cotton varieties. The introduction of food produced from cotton line MON 88913 into the food supply is therefore expected to have minimal nutritional impact. The nutritional adequacy of food produced using glyphosate-tolerant cotton (Roundup Ready® cotton) has been demonstrated in recent research with lactating Holstein cows.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food from cotton line MON 88913.

On the basis of the data provided in the present application, and other available information, food from this cotton line can be considered as safe and as wholesome as food produced from other cotton varieties.

1. INTRODUCTION

Monsanto Australia Limited submitted an application to FSANZ seeking approval for food derived from herbicide-tolerant cotton line MON 88913 under Standard 1.5.2 - Food Produced Using Gene Technology in the *Australia New Zealand Food Standards Code* (the Code).[4]

Cotton line MON 88913 has been genetically modified to be tolerant to the herbicide glyphosate. Glyphosate, the active ingredient in the herbicide Roundup®, is a broad-spectrum contact herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. Glyphosate is a reversible competitive inhibitor of the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS), however it does not inhibit any other phosphoenolpyruvic acid dependent enzymatic reactions (OECD, 1999).

Tolerance to glyphosate is conferred though the expression in the plant of the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS) encoded by the *epsps* gene from the bacterium *Agrobacterium* sp. strain CP4. The production of EPSPS by cotton line MON 88913 enables the post emergence use of glyphosate herbicides without risk of damaging the crop. The applicant has stated that development of glyphosate tolerant cotton will provide increased tolerance to glyphosate and will enable the application of a Roundup® herbicide over the top of the cotton crop at later stages of development than is currently possible with Roundup Ready® cotton.

Cottonseed is processed into four major by-products: oil, meal, hulls and linters. Only the oil and the linters are used in food products in Australia and New Zealand. Cottonseed oil is used in a variety of foods including cooking, salad and frying oils: mayonnaise, salad dressing, shortening, margarine and packaging oils. Cottonseed oil is the third major vegetable oil produced in the U.S., behind soybean and corn oil (NCPA 1999). It is considered to be premium quality oil, due to its balance in unsaturated fatty acids and high tocopherol (Vitamin E) content and stability when used as frying oil. Cotton linters are used as a cellulose base in high fibre dietary products as well as viscosity enhancers in toothpaste, ice cream and salad dressing. Linter fibre is also used to improve the viscosity of dressings and is commonly used to bind solids in pharmaceutical preparations such as tablets. Linter pulp also has diverse uses in the paper industry, in fingernail polishes and printed electrical board circuits for use in the computer and electronics industry (NCPA, 1999). Cottonseed meal is primarily used for stock food and is not currently sold for human consumption in Australia or New Zealand.

Cotton line MON 88913 has been developed for cultivation in the major cotton growing regions of the world and in March 2005 received U.S.- FDA approval for use in food. Field trials of Roundup Ready® Flex are currently in progress in Mexico and applications for field trials in South Africa and Costa Rica have been made.

[4] For the purpose of this assessment "line" denotes a plant (cotton) containing a particular genetic modification derived from a unique transformation event. The usage is intended to be inclusive of the introduction of the genetic modification into other plant (cotton) backgrounds by conventional breeding. For the legal definitions of "line" and "transformation event" refer to the drafting in Attachment 1.

In Australia, Roundup Ready® Flex cotton has been planted in contained field trials under the OGTR licence DIR 035/2003 since October 2003, and recently, the OGTR completed a full risk assessment and risk management plan (DIR 055/2004) for a large scale field trial over two seasons on up to 91 sites (1811 ha.) from 2005-6. The applicant intends to release cotton line MON 88913 for commercial production in Australia in the future. Roundup Ready® Flex cotton will not be grown in New Zealand.

2. HISTORY OF USE

2.1 Donor Organisms

Agrobacterium sp. strain CP4

The species *Agrobacterium tumefaciens* is a Gram-negative, non-spore forming, rod-shaped bacterium commonly found in the soil. It is closely related to other soil bacteria involved in nitrogen fixation by certain plants.

Agrobacterium naturally contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its genome into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing root deformation mainly with sugar beets, pome fruit and viniculture crops. However, adaptation of this natural process has now resulted in the ability to transform a broad range of plant species without causing adverse effects in the host plant.

Agrobacterium sp. strain CP4 was chosen as the donor organism as it exhibited tolerance to glyphosate by naturally producing the protein EPSPS, which endows glyphosate-tolerance (Padgette et al., 1996a). Genes for many EPSPSs have been cloned (Padgette et al., 1996b) and active site domains are conserved among the known EPSPSs. The bacterial isolate CP4, was identified by the American Type Culture Collection as an *Agrobacterium* species, and was found to show higher tolerance and increased catalytic activity, compared to other glyphosate-tolerant EPSPSs (Barry et al., 1992; Padgette et al., 1996b). *Agrobacterium* species are not known for human or animal pathogenicity, and are not commonly allergenic (FAO/WHO, 1991). There have been no incidences of a population of individuals with sensitisation to bacterial proteins (FAO/WHO, 2001).

Agrobacterium tumefaciens is not used in the food industry, however the *cp4-epsps* gene has been transferred into a number of other crop plants, including sugarbeet (A378) soybean (A338), canola (A363), corn (A416), and cotton (A355), to establish glyphosate tolerance, and has similarly been included in cotton line MON88913.

Cauliflower mosaic virus

The *35S* promoter element is derived from the plant virus CaMV and controls the expression of the *epsps* gene. CaMV is a double stranded DNA caulimovirus with a host range restricted primarily to cruciferous plants.

Although CaMV is a known plant pathogen, only a single DNA fragment of the CaMV genome corresponding to a promoter, has been transferred into cotton (Odell et al., 1985). No other DNA fragments, including the genes that code for the pathogenicity of the virus, have been transferred into cotton line MON 88913.

Host Organism

Gossypium hirsutum L.

Cotton (*Gossypium hirsutum* L.) is grown as a commercial crop worldwide and has a long history of safe use for both human food and stock feed.

Cotton is grown typically in arid regions of the tropics and sub-tropics. It is primarily grown as a fibre crop with the resulting cottonseed being processed as a by-product. Cottonseed is processed into four major by-products: oil, meal, hulls and linters (Figure 1), but only the oil and the linters are used in food products. Food products from cottonseed are limited to highly processed products due to the presence of the natural toxicants, gossypol and cyclopropenoid fatty acids in the seed. These substances are removed or reduced by the processing of the cottonseed into oil and linters.

Cottonseed oil is regarded as premium quality oil and has a long history of safe food use. It is used in a variety of foods including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine and packing oil. It is considered to be healthy oil as it contains predominantly unsaturated fatty acids. Cottonseed oil has been in common use since the middle of the nineteenth century (Jones and King 1990; Jones and King 1993) 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. In the US, it ranks third in volume behind soybean and corn oil, representing about 5-6% of the total domestic fat and oil supply.

Cotton linters are short fibres removed from the cottonseed during processing and are a major source of cellulose for both chemical and food uses. They are used as a cellulose base in products such as high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste.

The other major products cottonseed is processed into are meal and hulls, which are used as stock feed. Cottonseed meal is not used for human consumption in Australia or New Zealand. Although it has permission to be used for human food (after processing) in the US and other countries, it is primarily sold for stock feed. Human consumption of cottonseed flour has 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. In these instances, cottonseed meal is inexpensive and readily available (Ensminger 1994, Franck 1989). Cottonseed flour is also permitted for human consumption in the US, provided it meets certain specifications for gossypol content, although no products are currently being produced.

In Australia, the area of cotton harvested in 2004 – 5 was 315,000 hectares and the predicted harvested area for 2005 – 2006 is 341,000 hectares (ABARE, 2005.) Cotton is not grown in New Zealand.

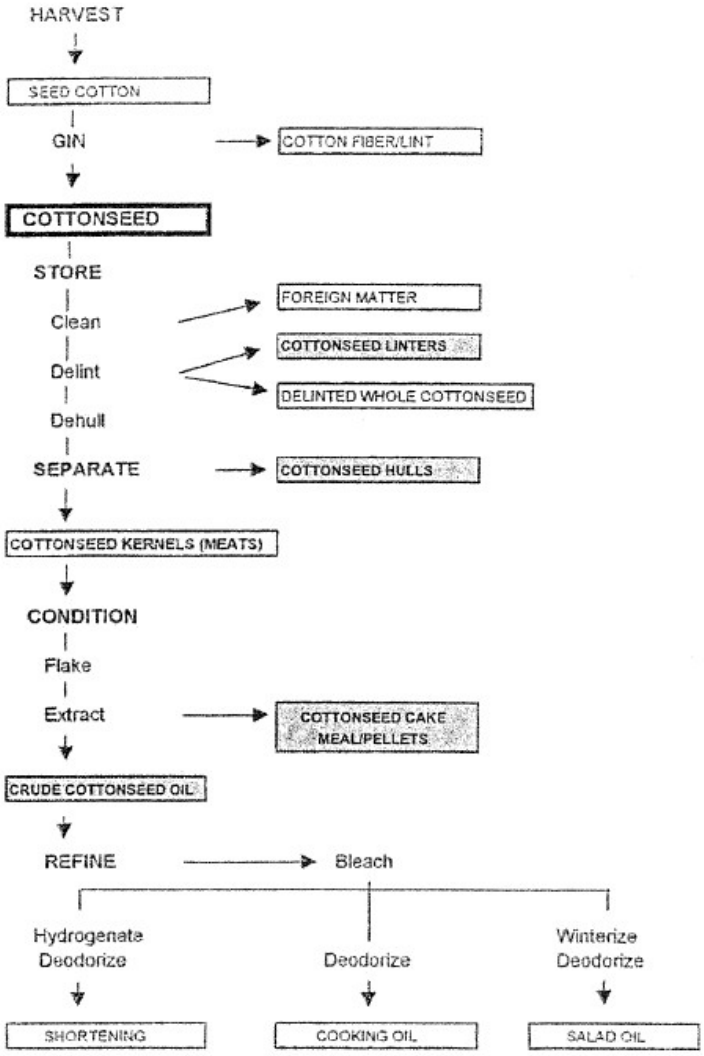


Figure 1: Processing steps of cottonseed, from harvest to products (NCPA, 2000)

3. DESCRIPTION OF THE GENETIC MODIFICATION

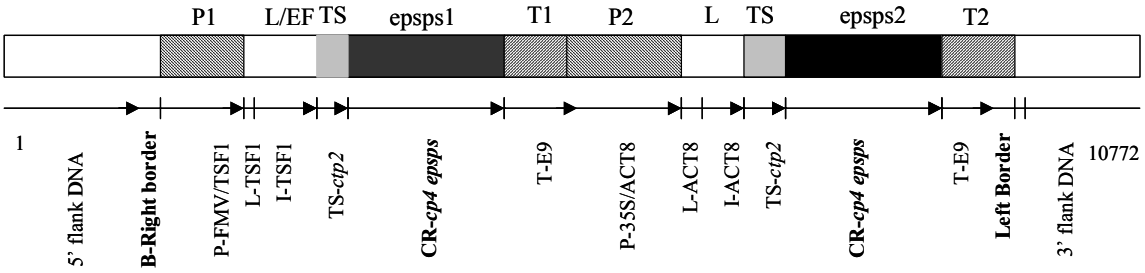
3.1 Method used in the genetic modification

The new gene was introduced into the cotton plant (*Gossypium hirsutum* L, Coker 312 va), by *Agrobacterium*-mediated transformation (Zambryski, 1992). *Agrobacterium tumefaciens* strain AB1, a derivative of *A. tumefaciens* strain C58 harbouring the plasmid PV-GHGT35, was used as the transformation vector; this strain contains a disarmed Ti plasmid (Koncz and Schell, 1986). This vector was co-cultured with hypocotyl explants of *in vitro* cotton seedlings, which were then used to generate somatic embryogenic cotton callus. The callus was selected *in vitro* for the desired sectors by incorporating glyphosate into the culture medium. The *Agrobacterium* vector was eliminated from the cultures by incorporating antibiotics (carbenicillin and cefotaxime) into the culture medium.

The method is analogous to the transformation method used to develop Roundup Ready® cotton, however cotton line MON 88913 was selected *in vitro* using glyphosate as the selective agent, whereas Roundup Ready® cotton was selected *in vitro* using kanamycin.

3.2 Function and regulation of novel genes

The section of plasmid (the expression cassette) transferred into cotton line MON88913 is illustrated in Figure 2; the T-DNA is approximately 8.2 kb long. The double-border, binary vector PV-GHGT35 contains two tandem *cp4 epsps* gene expression cassettes delineated by left and right border regions. From the right border region, the first *cp4 epsps* coding sequence is under the regulation of a chimeric transcriptional promoter P-FMV/TSF1, L-TSF1 leader and intron sequences, a chloroplast transit peptide (TS-*ctp2*) sequence and a T-E9 polyadenylation sequence. The second *cp4 epsps* coding sequence, is regulated by a P-35S/ACT8 chimeric transcriptional promoter, L-ACT8 leader and intron sequences, and the same chloroplast targeting and polyadenylation sequences as used in the first *cp4-epsps* gene expression cassette. The *cp4 epsps* coding sequence used to produce cotton line MON 88913, is identical to that in the current Roundup Ready® cotton product. All the genetic elements present in the expression cassette are described in Table 1.



P1 promoter for *epsps* gene 1
L/EF Leader sequence encoding elongation factor
T1 3' untranslated transcriptional termination sequence and polyadenylation signal sequences for epsps gene1.
P2 promoter for *epsps* gene 2
T2 3' untranslated transcriptional termination sequence and polyadenylation signal sequences for epsps gene 2.

Figure 2: Linear map of insert in cotton line MON 88913

The cp4 epsps gene

The *cp4 epsps* gene from *Agrobacterium* species strain CP4 has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996a). The native *cp4 epsps* gene from *Agrobacterium* CP4 has a codon usage that might impair its expression in plants. A plant-preferred version of the CP4 EPSPS gene was synthesised in order to substitute plant preferred codons while producing an identical CP4 EPSPS protein (Harrison *et al* 1996). The activity of the resultant CP4 EPSPS enzyme was unaltered. The endogenous EPSPS enzyme of plants is located within chloroplasts, the site of aromatic amino acid biosynthesis in plant cells.

Many proteins with subcellular locations are synthesised as pre-proteins and directed to a particular organelle by a transit peptide at the end of the mature protein. Following delivery to the organelle, the short transit peptide is cleaved from the mature protein and is rapidly degraded.

The CP4 EPSPS enzyme is targeted to the plastid by a chloroplast transit peptide sequence derived from the *Arabidopsis thaliana* EPSPS (CTP 2, Klee *et al* 1987). The *ctp2* gene sequence was fused to the 5' end of the *cp4 epsps* gene. The CTP2 peptide sequence has been shown to deliver bacterial EPSPSs to the chloroplasts of higher plants where the aromatic amino acid biosynthetic pathway and endogenous EPSPS activity is located (Della-Cioppa *et al*, 1986). The *ctp2* present in PV-GHGT35 is the same as that used in the development of the existing cotton product Roundup Ready® cotton.

Regulatory sequences

The *cp4 epsps* gene is under the regulation of different elements, as outlined in Table 1. The *ctp2/cp4 epsps* coding sequence closest to the right border region, in the first gene expression cassette is under the regulation of the P-FMV/TSF1 transcriptional promoter. P-FMV/TSF1 is a chimeric promoter containing the *A .thaliana* TSF1 gene promoter (encoding elongation factor EF-1 alpha (Axelos et al., 1989) and enhancer sequences from the figwort mosaic virus 35S promoter (Richins et al., 1987). Located between the P-FMV/TSF1 promoter and *ctp2/cp4 epsps* coding sequence are the nontranslated L-TSF1 leader sequence (exon 1) and I-TSF1 nontranslated intron (Axelos et al., 1989). The *ctp2/cp4 epsps* coding sequence is linked at the 3' end to the T-E9 DNA sequence derived from *P. sativum* , containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (rbc) E9 gene (Coruzzi et al., 1984) for transcriptional termination and polyadenylation of the *cp4 epsps* mRNA.

The second *ctp2/cp4 epsps* gene follows tandem to the first gene expression cassette, under the regulation of the P-35S/ACT8 transcriptional promoter. P-35S/ACT8 is a chimeric promoter containing the promoter of the ACT8 gene of *A. thaliana* (An et al., 1996) combined with the enhancer sequences of the cauliflower mosaic virus (CaMV) 35S promoter (Kay et al., 1987). Again, non-translated leader sequences are located between the promoter and the *ctp2/ cp4 epsps* coding sequence and this, in turn, is linked at the 3' end to the T-E9 DNA termination sequence.

T-DNA borders

Plasmid vector PV-GHGt35 contains respective right and left border regions that delineate the T-DNA to be transferred into cotton and are necessary for the efficient transfer of the T-DNA into the plant cell. These borders are derived from *Agrobacterium* (Depicker et al., 1982; Barker et al., 1983).

Genetic elements outside of the T-DNA borders

The genetic elements, OR-ORI V, CR-rop, OR-ORI-PBR322 and CR-*aad* (Table 1), are present on plasmid vector PV-GHGT35, but exist outside the T-DNA borders, and as such make up the "backbone" of the plasmid. They are not expected to be transferred into the cotton genome.

The aminoglycoside adenyltransferase (*aad*) gene is derived from bacterial Transposon Tn7 and confers resistance to the antibiotics spectinomycin and streptomycin. The *aad* gene was included in the construct as a marker to allow for selection of bacteria containing PV-GHGTO7 prior to transformation of the plant cells and is under the control of a bacterial promoter.

3.3 Characterisation of the genes in the plant

Study submitted:
Study number: 03-01-45-15. Groat, J.R., Palmer, G.M., Rice, J.F., Reiser, S.E. (2004) Molecular analysis of Roundup Ready® Flex cotton MON 88913.

Traditional molecular techniques were used to analyse the inserted DNA in cotton line MON 88913. A near isogenic line lacking the EPSPS-encoding gene cassettes, yet derived from the same line as the transformed line, Coker 312, was used as the control. This control line was termed the negative segregant, MON 88913 (-). Southern blot analysis was used to determine the insert copy number, intactness of the EPSPS coding region, intactness of the EPSPS expression cassette, and to assess whether vector backbone sequences were introduced during the transformation process. Table 2 outlines the analyses carried out to characterise the transformed plants.

Table 1 Outline of molecular methods used for characterisation of glyphosate-tolerant cotton line MON 88913

Analysis method	Purpose
Southern Hybridisation	- Determination of DNA insert number (number of integration sites into cotton genome) - Determination of copy number (the number of copies of the integrated DNA within one locus) - To show intactness of the two gene expression cassettes - To establish absence of plasmid backbone sequences in the plant - To show stability of the DNA insert over several generations
Polymerase Chain Reaction (PCR) and sequence analysis	- Identification of the 5' and 3' insert-to-genomic DNA junctions - Confirmation of the organisation of genetic elements within the DNA insert

Table 2: Summary of genetic elements in plasmid vector PV-GHGT35

Genetic Element	Fragment size (bp) .	Function and Source
Left Border region	441	DNA sequence derived from Agrobacterium containing the left border (LB) sequence for the efficient transfer of the T-DNA (Barker et al., 1983)
OR-OR1 V	637	Origin of replication for Agrobacterium derived from the broad host range plasmid RK2 (Stalker et al., 1981).
CR-rop	472	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
OR-OR1-PBR322	628	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978)
CR-*aad*	788	Coding sequence for Tn7 adenyltransferase conferring spectinomycin and streptomycin resistance (Fling et al., 1985)

Genetic Element	Fragment size (bp) .	Function and Source
Right Border region	330	DNA sequences derived from Agrobacterium containing right border (RB) sequences for the efficient transfer of the T-DNA (Depicker et al., 1982).
P-FMV/TSF1	1039	Chimeric promoter containing the *Arabidopsis thaliana tsf1* gene promoter (encoding elongation factor EF-1 alpha [Axelos et al., 1989]) and enhancer sequences from the Figwort Mosaic virus 35S promoter (Richins et al., 1987).
L-TSF1	45	Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1 alpha (Axelos et al., 1989).
I-TSF1	621	Intron from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1 alpha (Axelos et al., 1989).
TS-*ctp*2	227	DNA sequences derived from *Arabidopsis thaliana,* chloroplast transit peptide, derived from the *Arabidopsis thaliana epsps* gene, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee and Rogers, 1987).
cp4 epsps	1367	DNA sequence containing synthetic coding sequence for the CP4 EPSPS protein from *Agrobacterium sp*. strain CP4 (Padgette et al., 1996a; Barry et al., 1997).
T-E9	642	DNA sequences derived from *Pisum sativum*, containing the 3' nontranslated region of the pea ribulose-1,5-biphosphate carboxylase, small subunit (*rbc*) E9 gene (Coruzzi et al., 1984).
P-35S/ACT8	1174	Chimeric promoter containing the promoter of the *act8* gene of *Arabidopsis thaliana* (An et al., 1996) combined with the enhancer sequences of the Cauliflower mosaic virus (CaMV) 35S promoter (Kay et al., 1987).
L-ACT8	140	Leader sequence from the *act8* gene of *Arabidopsis thaliana* (An et al., 1996).
I-ACT8	471	Intron and flanking exon sequence from the *act8* gene of *Arabidopsis thaliana* (An et al., 1996).
TS-*ctp* 2	227	DNA sequences derived from *Arabidopsis thaliana,* chloroplast transit peptide.
Genetic Element	**Fragment size (bp) .**	**Function and Source**
CR-*cp4 epsps*	1997	DNA sequence containing synthetic coding sequence for the CP4 EPSPS protein from *Agrobacterium sp*. strain CP4
T-E9	642	DNA sequences derived from *Pisum sativum*, containing the 3' nontranslated region.

Insert and copy number

Southern hybridisation was used to confirm the number and nature of the DNA insertions in cotton line MON 88913. Genomic DNA isolated from seeds of cotton line MON88913 and the negative segregant of MON 88913 was digested with five different restriction enzymes, or combination of restriction enzymes. The digested DNA was processed by gel electrophoresis, transferred by blotting to nylon membranes and then probed with different radioactively –labelled probes hybridising to different sequences within the T-DNA region.

DNA from cotton line MON 88913 digested with *Spe I* produced a single band of ~13 kb, indicating that there is only one insert. When this DNA was digested with a combination of *Spe I* and *Sca I*, two bands of ~12 kb and 1.2 kb were produced representing the two border fragments and confirmed that only a single copy of DNA is present in cotton line MON88913.

The restriction enzyme *Xho*I was used separately and in combination with *Bgl*II, to investigate the intactness of the two gene cassettes containing *cp4 epsps*. Both the gene expression cassettes gave identical bands showing that they were intact.

PCR and sequence analysis

The organisation of the genetic elements within the insert in cotton line MON 88913 was evaluated using PCR analysis by amplifying six overlapping regions of DNA, spaning the length of the insert. The order of the different elements was confirmed and the 5' and 3' insert-to-genomic DNA junctions were identified.

Six PCR fragments covering the T-DNA insert were sequenced and aligned to the sequence of the transcription vector PV-GHGT35 using the BestFit function in the SeqLab program.

Confirmation of absence of plasmid backbone

Southern blot analysis was used to investigate whether there was any detectable backbone sequence from the transformation vector PV-GHGT35 integrated into the plant genomic DNA. There was no evidence of backbone sequences with any of the three backbone probes used.

Conclusion

Detailed molecular analyses have been carried out on cotton line MON88913 to characterise the inserted DNA. Results indicate that one copy of the T-DNA was introduced, containing two intact *cp4 epsps* gene expression cassettes at a single locus in the cotton genomic DNA.

The organisation of the genetic elements within the T-DNA was confirmed and there were no inversions or deletions of sequences during transformation. The T-DNA sequence showed 100% identity with the transcription vector PV-GHGT35, indicating that there were no changes to the sequence during the transformation process. In addition, backbone sequence from plasmid PV-GHGT35 was not detected. No functional antibiotic resistance genes (i.e. the aad gene) were transferred to cotton line MON 88913; selection was through resistance to glyphosate

3.4 Stability of the genetic changes

Breeding process

Figure 3 represents the development of cotton line MON88913. The glyphosate tolerance of individual plants was determined by antibody strip test for the CP4 EPSPS protein and/or tolerance to a Roundup agricultural herbicide spray.

The original transformed plant (RO) was a selected hemizygous progeny line of the non-transgenic line, Coker 312. When this line was self pollinated, the progeny were derived according to Mendelian inheritance, so that 25% were homozygous for the transgene, 50% were hemizygous for the transgene and 25 % were homozygous and did not contain the inserted DNA. A seed from the latter group was used as the parent line for the negative segregant of cotton line MON88913 (i.e. MON 88913 (-).

As this negative segregant came from the same seed as the transformed line, it can be considered a near-isogenic line and as such, is the most appropriate comparator, or control, to cotton line MON 88913.

When the control line MON 88913 (-) was probed for elements of the transformation vector PV-GHGT35, no exogenous DNA was located. Exogenous DNA was also undetectable in the control using PCR amplification.

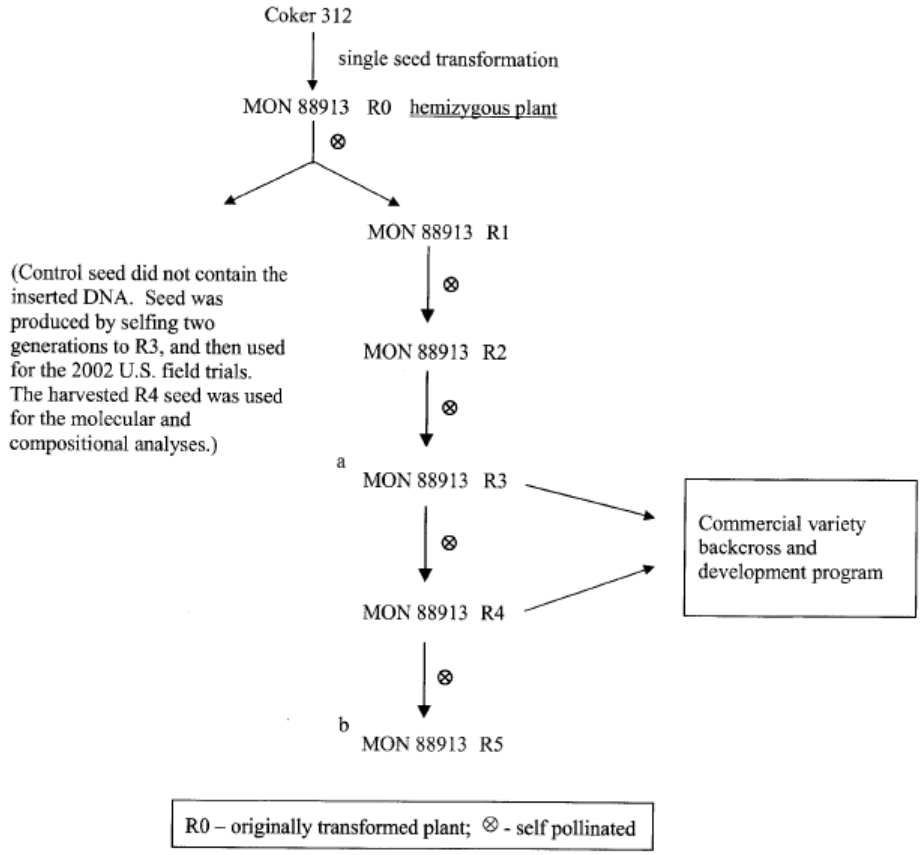


Figure 2: Breeding tree for MON 88913 and MON 88913 (-)

Segregation data

Segregation data comparing the frequency of the observed-to-expected numbers of progeny expressing the EPSPS protein were analysed statistically using the Chi-squared analysis. As referred to above, the R1 plants segregated in the expected 3:1 ratio in favour of the glyphosate-tolerant phenotype as a single, dominant trait loci. In the R1 plants, the calculated Chi-squared value for phenotype was less than the critical value of 3.84 at the 5% level of error.

Homozygous seeds could be identified at the R2 generation; individual R2 families were expected to segregate 1:2 for homozygosity after glyphosate-sensitive individuals were removed from the population. The Chi-squared analysis for R2 homozygote recovery was <3.84 at the 5% level of error. Therefore the expected number of homozygous families were recovered during the breeding process. Selection of homozygous plant seed lots continued in the R3 generation and was confirmed in generations R4 and R5. Homozygous cotton line MON88913 was expected to segregate 1:0 for glyphosate tolerance at R4 and R5 generations (Table 4).

Table 3: Inheritance of the glyphosate tolerance trait in cotton line MON88913

Generation	Phenotype	Expected Ratio	Expected no. of plants (E)	Observed no. of plants (O)	$(O-E)^2/E$
Segregation ratio for the MON88913 phenotype in the R1 generation:					
R1	Glyphosate tolerant	0.75	111.8	111	0.005
	Non-glyphosate tolerant	0.25	37.3	38	0.0151
		Total	149	149	0.0201
Homozygous recovery ratio for the MON88913 phenotype in R2 families					
R2	Homozygous	0.33	25.33	24	0.131
	Segregating	0.66	50.66	52	0.0675
		Total	76	76	0.1985

Table 4: Confirmation of homozygous status in the R4 and R5 generations

Generation	Number glyphosate tolerant	Number non-glyphosate tolerant	Test method
R4	322	0	Roundup spray
R5	310	0	Roundup spray

Genetic Stability

In order to show stability of the DNA insert in cotton line MON88913, Southern blot analysis was carried out using DNA from five generations (R1 – R5, Figure 3) digested with *Spe* I and *Sca* I. Two restriction fragments of ~12 kb and ~1.2 kb were obtained for the five generations of cotton line MON 88913.

Conclusion

The transformation event in cotton line MON88913 was shown to be stable over several generations. The segregation ratios shown for different generations in Table 3 and 4 confirm the homozygocity and generational stability of the T-DNA insert.

4. CHARACTERISATION OF NOVEL PROTEINS

A single novel protein is present in cotton line MON88913. The CP4 EPSPS protein is 47.6 kDa and consists of a single polypeptide of 455 amino acids.

4.1 Biochemical function and phenotypic effects

CP4 EPSPS

The EPSPS enzyme is essential in the biosynthesis of the aromatic amino acids, via the shikimate metabolic pathway, present in all plants, bacteria and fungi. In plants, the EPSPS enzyme is inhibited by glyphosate (Steinrucken and Armhein 1980), but bacterial EPSPSs, such as the CP4-EPSPS, have a reduced affinity for glyphosate.

In plants, EPSPS is found in the chloroplast. In cotton line MON 88913, the CP4-EPSPS gene was fused to the *Arabidopsis thaliana* EPSPS chloroplast transit peptide (CTP), which targets the protein to the chloroplast. In vitro chloroplast uptake assays have shown that the *A. thaliana* EPSPS CTP delivers mature CP4-EPSPS to the chloroplast, following cleavage from the pre-protein. (Della Cioppa et al 1986). The chloroplast transit peptide is rapidly degraded after cleavage *in vivo* by cellular proteases.

Upon glyphosate treatment, plants or plant cells expressing the CP4 EPSPS protein are unaffected since the continued action of the glyphosate-tolerant EPSPS enzyme enables the biosynthesis of aromatic compounds.

4.2 Protein characterisation

The plant-produced CP4 EPSPS protein was identified and characterised using *E. coli*-produced CP4 EPSPS protein as a reference standard. The following analytical tests were used: immunoblot analysis and densitometry, matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry, N-terminal sequence analysis, SDS PAGE and densitometry, CP4 EPSPS enzymatic activity assay and glycosylation analysis.

On the basis of western blot analysis, the electrophoretic mobility and immunoreactive properties of the plant-produced CP4 EPSPS protein were shown to be comparable to those of the *E. coli*-produced CP4 EPSPS reference standard. MALDI-TOF mass spectral analysis showed that the peptide masses and molecular weight of the plant-produced CP4 EPSPS protein was consistent with the CP4 EPSPS protein calculated from the amino acid sequence. This was also confirmed with N-terminal sequencing. The functional activities of the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were also found to be functionally equivalent. In addition, similar to bacterially produced CP4 EPSPS protein, the plant-produced CP4 EPSPS protein showed no evidence of glycosylation.

4.3 Protein expression analysis

Studies submitted:
MSL study number: 18859. Karunanandaa, K., Thorp, J.J., Lee, J.L. and Silanovich, A. (2003) Characterisation of the CP4 EPSPS protein purified from the seed of Roundup Ready® Flex cotton MON 88913 produced in year 2002 and assessment of the physiochemical and functional equivalence of the plant and *E.coli*-produced CP4 EPSPS proteins.

Study number: 03-01-45-22. Bookout, J.T., Pineda, N.G., Nicholas, N.R. and Jennings, J.C. (2003) Assessment of CP4 EPSPS protein levels in leaf, root, seed and pollen tissues from Roundup Ready® Flex cotton MON 88913 produced in 2002 field trials.

Table 5 shows CP4 EPSPS protein expression levels for four typical cotton-growing regions in the U.S., Alabama, California, Georgia and Texas. The levels of CP4 EPSPS were determined by an enzyme-linked Immunoabsorbent assay (ELISA). The analytical accuracy of the ELISA was checked for each tissue type (leaf, root, seed and pollen) by spiking respective samples with known amounts of protein and determining the % extraction efficiency and % protein recovery. The extraction efficiency varied from 86 – 91% and spike and recovery varied from 93 – 118%, for the different tissues. The intra- and inter assay precision of ELISA protein concentration measurement showed a coefficient of variance (cv) of 6% and 22% respectively.

The limits of detection (LOD) were calculated as the mean value using the data from sample extracts for each tissue type plus three standard deviations. The limits of quantitation (LOQ) were calculated based on the lowest standard concentration. For each tissue, both LOQ and LOD values in "ng/ml" were converted to "µg/g fwt" using the respective dilution factor and tissue-to-buffer ratio (Table 5).

The levels of CP4 EPSPS protein in all tissue types from the control negative segregant MON 88913 (-), were less than the assay limits of quantitation (LOQ) presented in Table 5.

Table 5: Summary of CP4 EPSPS protein levels in tissue samples collected from Roundup Ready® Flex cotton MON 88913 produced in 2002 U.S. multi-site field trials

Tissue type	Mean CP4 EPSPS protein level in µg/g fwt (SD)[1]	Range[2] (µg/g fwt)	Mean CP4 EPSPS protein level in µg/g dwt (SD)[3]	Range (µg/g dwt)	LOQ/LOD (µg/g fwt)
Young leaf	170 (64)	64 – 260	970 (460)	279 – 1700	0.23/0.069
OSL1[4]	270 (99)	77 – 410	1400 (540)	480 – 2600	0.23/0.069
OSL2	170 (44)	63 – 260	690 (210)	290 – 1000	0.23/0.069
OSL3	160 (61)	66 – 260	630 (230)	290 – 1100	0.23/0.069
Root	31 (11)	19 – 64	99 (40)	57 – 200	0.23/0.073
Seed	310 (110)	67 – 550	340 (120)	72 – 580	2.7/1.7
Pollen	4 (0.22)	3.8 – 4.3	nd[5]	nd[5]	0.23/0.11

1. Protein levels expressed as µg of protein per gram of tissue on a fresh weight (fwt) basis. The arithmetic mean and standard deviation (SD) were calculated for each tissue type across sites.
2. Minimum and maximum values were determined for each tissue type across all sites.
3. Protein levels expressed as µg of protein per gram of tissue on a dry weight (dwt) basis. The dwt values were calculated by dividing the fwt values by the dry weight conversion factors obtained from moisture analysis data.
4. OSL1 – OSL3 represent overseason leaves collected at different time points throughout the growing season.
5. not determined

The only cotton plant-derived products entering the human food supply are cottonseed oil and cotton linters. Refined cottonseed oil was not tested for the presence of CP4 EPSPS protein, however the refining process (see Figure 1) removes protein and refined cottonseed oil is considered free of protein (Rogers, 1990). Cotton linters were also not tested for CP4 EPSPS protein, but the extensive processing of cotton linters (alkaline wash at temperatures >100^{o}C) has been shown to render linter food products protein-free (Sims et al., 1996). The presence of CP4 EPSPS protein in cottonseed oil and linters was investigated previously in Roundup Ready® cotton (application A355). Although the protein was detected in combed lint, it was not found in brown linter stock, the first product in the sequence of processing linters for cellulose (Sims et al., 1996).

Proportion of CP4 EPSPS protein in total protein

The CP4 EPSPS protein was detected at relatively low levels in various plant tissues. The mean average CP4 EPSPS protein level in cotton line MON 88913 seed was 340 µg/g dwt and the average percent dry weight of total protein was 28.23%. Therefore, the amount of CP4 EPSPS protein in the total cottonseed protein was approximately 0.12 %.

4.4 Potential toxicity of novel protein

Study submitted:
Study number: 03-01-45-26. McCoy, R.L. and Silanovich, A. Bioinformatics analysis of the CP4 EPSPS protein utilising the AD4, TOXIN5 and ALLPEPTIDES databases.

Acute toxicity studies

The acute toxicity of the CP4 EPSPS protein has been previously tested by acute gavage exposure in mice and no deleterious effects were observed (Harrison et al., 1996). The toxicity study was carried out using *E. coli*-produced CP4 EPSPS protein as it was demonstrated to be equivalent to the plant-produced protein. The CP4 EPSPS protein was administered at levels 1000 fold of those in anticipated consumption of food products; the no effect level (NOEL) for oral toxicity in mice is 572 mg/kg body weight, and was the highest dose tested.

Similarity to known protein toxins

Bioinformatic analyses were performed to assess the potential for toxicity or pharmacological activity of the of the CP4 EPSPS protein sequence. The comparison was performed with the toxin (TOXIN5) and public domain (ALLPEPTIDES) database sequences using bioinformatics tools.

The FASTA sequence alignment tool was used to assess structural similarity. This program directly compares amino acid sequences (primary protein structure) and may also be used to infer higher order structural similarities (i.e. secondary and tertiary structures). Proteins sharing a high degree of similarity throughout the entire length are often homologous, sharing secondary structure and common three-dimensional folds. The toxin sequence database (TOXIN5) was assembled from public sequence databases, including Genbank, EMBL release 124 and SwissProt release1. Protein searches were retrieved using the STRINGSEARCH function (keyword = toxin); the working database comprised approximately 12,771 sequence entries. Similarly, the ALLPEPTIDES sequence database was used to reveal potential similarity with pharmacologically active proteins.

The structural similarities of the CP4 EPSPS protein sequence, to sequences in each database, were assessed using the FASTA algorithm. FASTA comparisons were performed using the BLOSUM50 scoring matrix (Henikoff and Henikoff, 1992). The BLOSUM50 matrix identifies blocks of conserved residues that are at least 50% identical; it works well for identifying sequence similarities that include gaps, thus recognising evolutionary relationships.

Following assessment of the extent of similarity of sequence alignments, the most significant alignment was to *Bacillus cereus* shingomyelinase c precursor protein, with a 28.2% identity over a 131 aa overlap window and an alignment significance score (E) of 0.26. Only proteins with scores less than 1×10^{-5} were considered potentially homologous. This data indicates that the CP4 EPSPS protein in unlikely to share structural homology to any known protein toxins.

4.5 Potential allergenicity of novel protein

Studies submitted:

Study number: 01-01-62-09. Leach, J.N., Hileman, R.E., Throp, J.J., George, C. and Astwood, J.D. (2002) Assessment of the *in vitro* digestibility of purified *E. coli* produced CP4 EPSPS protein in simulated gastric fluid.

Study number: 92-01-30-15. Ream, J.E., Bailey, M.R., Leach, J.N. and Biest, N. (1993) Assessment of the invitro digestive fate of CP4 EPSPS synthase.

Similarity to known allergens

Bioinformatic analyses were also performed to assess the potential for allergenicity of the of the CP4 EPSPS protein sequence. The comparison was performed with the allergen (AD4) and public domain (ALLPEPTIDES) database sequences using bioinformatic tools.

Proteins homologous to allergens are more likely to share linear and/or conformational cross-reactive allergenic epitopes than unrelated proteins. So, in addition to the FASTA sequence alignment assessment, the CP4 EPSPS protein sequence was also screened against the AD4 database using a pair-wise comparison logarithm. In these analyses, any sequence of eight linearly contiguous amino acids found to be identical between the CP4 EPSPS protein sequence and proteins in the AD4 database were defined as immunologcially relevant. The presence of these contiguous sequences may indicate the presence of potentially cross-reactive allergenic epitopes.

Following assessment of the extent of similarity of sequence alignments, the most significant alignment was to the *Dermatophagoides farinae* allergen Der f 2 with a 30.5% identity over an 82 aa window, and with an alignment significance score (E) of 0.41. This alignment also consisted of gaps and the longest stretch of contiguous amino acid identities consisted of five amino acids. The length of the overlap is relatively short (18%) when compared to the full length (455 aa) of the CP4 EPSPS protein. There was no structural and/or functional homology between the CP4 EPSPS and the Der f 2 allergen. The minimum similarity required for allergenic cross-reactivity is >50% identity across the whole length of the protein.

It is therefore unlikely that there is cross-reactivity between the CP4 EPSPS protein and aligned allergens. No epitope sequences (eight contiguous amino acids) were detected when the CP4 EPSPS protein was compared to the AD4 database.

In vitro digestibility

Stability to digestion in simulated gastric and intestinal fluids has been considered an essential endpoint in assessing potential allergenicity, since several allergens are known to be stable for up to 24 hours in simulated gastric fluid. The simulated human gastric fluid method described in the U.S. Pharmacopeia has been used to systematically compare the relative stability of a number of common food allergens with common safe food proteins and with proteins engineered into plants (Fuch and Aswood, 1996). Allergens remain stable for at least 2 minutes with the major allergens being stable for at least 60 minutes in simulated gastric fluids, as demonstrated by gel electrophoresis.

The CP4 EPSPS protein used in this study was derived from a large-scale fermentation of *E. coli*. This material was characterised and found to be equivalent and functionally identical to plant-produced CP4 EPSPS protein (section 4.2). The CP4 EPSPS protein was incubated for different time periods (up to 60 minutes) in simulated gastric fluid (SGF; at pH 1.2 and 37^{o}C), in the presence and absence of pepsin, and +/- the test protein. The digestibility of purified *E. coli*-produced CP4 EPSPS protein in SGF was assessed using SDS-PAGE, western blot analysis and an EPSPS enzyme activity assay. At least 98% of the CP4 EPSPS protein was digested very rapidly (within 15 seconds) in SGF as determined by colloidal blue gel staining; likewise>95% of the protein was digested within 15 seconds as determined by western blot analysis. This was confirmed by >90% reduction of EPSPS activity within 15 seconds of incubation of the CP4 EPSPS protein in SGF. These results support earlier studies showing the rapid *in vitro* digestion of *E. coli*-produced CP4 EPSPS protein in SGF (and simulated intestinal fluid).

Glycosylation analysis

Many protein allergens are glycosylated, raising the possibility that the glycosyl groups may contribute to their allergenicity. In order to test whether there was post-translational glycosylation of the plant-produced CP4 EPSPS, the isolated plant protein was analysed for the presence of covalently bound carbohydrate. As found in previous assessments of the plant-produced CP4 EPSPS (eg. Application A355 – food produced from glyphosate-tolerant cotton line 1445), there was no evidence of typical glycosylation sequences (eg. histidine-aspartate-glutamate-leucine).

Stability to heat and processing

Many food allergens are stable to heat and processing. Although not tested in this study, CP4 EPSPS has been previously shown to be inactivated by heat treatment (Padgette et al., 1996a).

4.6 Residues of glyphosate or metabolites

Glyphosate is a herbicide commonly used on crops in the USA and Australia and is also used to desiccate plant tissues prior to harvest of grain. Maximum residue limits (MRLs) for glyphosate in grain crops have been set in the Food Standards Code (used by Australia, Standard 1.4.2 – Maximum Residue Limits, FSANZ 2005) and Codex (used by New Zealand). MRLs are set at levels well below those, which would represent a safety concern. Glyphosate has very low acute toxicity to mammals with an oral LD50 of >10,000 mg/kg in mice and >5,000 mg/kg in rats (Smith and Oehme 1992).

The MRL set for glyphosate in crude cottonseed oil is 0.1 mg/kg in the Food Standards Code and 0.05 mg/kg for edible cottonseed oil in Codex (FAO/WHO Codex 2000). The MRL is recommended by the Australian Pesticides and Veterinary Medicine Association on the basis of good agricultural practice and is the same for either GM or non-GM cotton plants.

The levels of glyphosate residues in refined cottonseed oil and processed linters would be expected to be very low because of removal in processing. Glyphosate is a very hydrophilic molecule (Malik *et al* 1989) and this would be expected to contribute to its removal in processing.

The residue levels in foods derived from glyphosate-tolerant cotton line MON 88913 would have to comply with either the Australian or CODEX MRL, depending on the jurisdiction.

Reports in the literature concentrate on the fate of glyphosate in weed plants killed by herbicide application, with data indicating that the primary removal of glyphosate is by bacterial activity (Smith and Oehme, 1992). Bacterial degradation results in the production of aminomethylphosphonate (AMPA) and glyoxylate, both non-toxic compounds (Smith and Oehme, 1992). An alternative pathway of degradation exists in many bacteria with glyphosate being converted to sarcosine and then to glycine and inorganic phosphate, all of which are non-toxic (Dick and Quinn 1995).

Data from the literature also suggest that glyphosate is not metabolised in plant tissues (Malik *et al* 1989, Wigfield *et al* 1994). CP4 EPSPS does not metabolise glyphosate. Therefore the novel CP4 EPSPS protein will not result in the production of any novel metabolites of glyphosate that would not otherwise be produced in a conventional plant sprayed with glyphosate.

4.7 Conclusion

Cotton line MON 88913 expresses one novel protein, CP4 EPSPS. Having ascertained that the *E. coli*-produced CP4 EPSPS was equivalent to the plant-produced CP4 EPSPS protein, a number of studies were carried out to determine its potential toxicity and allergenicity.

Bioinformatic data indicated there are no structural or immunologically relevant similarities between the CP4 EPSPS protein sequence and toxins, allergens or other pharmacologically active proteins that could adversely impact human or animal health. There is no evidence of acute toxicity from animal studies and *in vitro* digestibility studies confirmed that the CP4 EPSPS protein would rapidly break down once digested. This evidence indicates that the CP4 EPSPS protein is unlikely to be either toxic or allergenic to humans.

5. COMPOSITIONAL ANALYSES

A comparative approach focussing on the determination of similarities and differences between the GM food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods (WHO 2000). A compositional analysis of the food is one of the important elements of the comparative approach. The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO 1996).
The key nutrients and toxicants/anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins).

Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased).

Transgenic cotton line MON 88913, the negative segregant MON 88913 (-) and sixteen conventional cottonseed varieties were grown in 2002 at four U.S. locations, Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia and Hockley County, Texas.

These sites represent a variety of environmental conditions representative of regions where MON 88913 is expected to be grown commercially. At each site, cotton line MON 88913 was sprayed with Roundup Ultra MAX® three times throughout the growing season (at approximately the 3 node, 8 node and first flower stages) at an application rate of 40 - 42 ounces per acre (equivalent to 1.125 lb ae/A glyphosate).

Statistical analysis

A randomised block design was used at the four replicated sites; at each site MON 88913, MON 88913 (-) and conventional reference cottonseed (four per site) were planted in approximately 200 ft^2 plots in each of four replicated blocks.

All component values, except for moisture, were converted from a fresh weight basis to either a dry weight or % basis. The SAS® software GLM procedure (SAS Institute Inc., Cary, NC, USA) was applied to all data to detect potential outliers by screening studentised PRESS residuals. The model included cottonseed material, site and replication effects. SAS® software was used to generate all summary statistics and perform all analyses.

Statistical analyses were carried out on the converted values for each component in the cottonseed using a mixed model analysis of variance (ANOVA) for five sets of comparisons (i.e. an analysis for each of the four replicated trial sites and one for the combination of all four sites. A total of 53 components were statistically evaluated in raw cottonseed (representing the initial 69 analytes minus 16 for which >50% of the observations were below the LOQ and were excluded from the statistical analysis). A total of 265 comparisons were made, as there were 53 components with five statistical analyses of each (four sites individually plus all sites combined).

Individual replicated site analyses used the model:

$$Y_{ij} = U+T_i+B_j+e_{ij,}$$

Where Y_{ij} = unique individual observation, U=overall mean, T_i = plant material effect, B_j = random block effect and e_{ij} = residual error.

Combined site analyses used the model:

$$Y_{ij} = U+T_i+L_j+B(L)_{jk}+LT_{ij}+e_{ijk,}$$

Where Y_{ij} = unique individual observation, U=overall mean, T_i = plant material effect, L_j= random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by plant material interaction effect and e_{ijk} = residual error. MON 88913 was compared to MON 88913 (-) to determine statistically significant differences at $p \leq 0.05$.

Conventional analysis data from the conventional reference were used to determine a range of the reference values for each compositional analysis component. The reference data were also used to develop population tolerance intervals. For each component, tolerance intervals were calculated that were expected to contain, with 95% confidence, 99% of the values expressed in the population of conventional cotton. As negative quantities are not possible, calculated lower tolerance bounds that were negative were set to zero.

5.1 Nutrient analysis

The constituents analysed were selected on the basis that they comprise the important basic nutrients of cotton: these are proximates (protein, total fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude fibre, total dietary fibre (TDF), micro-nutrients such as minerals and vitamin E, amino acids and fatty acids (C8 – C22). In addition, a number of natural toxicants, that is, cyclopropenoid fatty acids (malvalic acid, sterculic acid and dihydrosterculic acid), and gossypol (free and total) were also analysed. Cottonseed was also analysed for the presence of aflatoxins.

Cottonseed

Compositional analysis of 69 different components was carried out on linted cottonseed (as described under statistical analysis). The composition and concentration of all the major constituents in the raw cottonseed are comparable to that of the whole cottonseed fraction profile and details of the amino acid profile in raw cottonseed are shown in Table 6. Tables 7 –11 refer to compositional analyses carried out on the delinted cottonseed fraction.

Of the 265 total comparisons made between raw cottonseed from cotton line MON 88913 and MON 88913 (-), there were 29 statistically significant differences, of these, 5% or approximately 13 (0.05 x 265) were expected based on chance alone.

Statistically significant differences ($p \leq 0.05$) between cotton line MON 88913 and MON 88913 (-) were observed in one of the five comparisons for tryptophan, glycine, 16:0 palmitic acid, 18:0 stearic acid, malvalic acid, sterculic acid, crude fibre, moisture, copper and zinc; in two of the five comparisons for phenylalanine, calcium, manganese and fat; in three of the five comparisons or sodium and 18:2 linoleic acid; and in all five comparisons for 18:1 oleic acid. Both 18:2 linoleic acid and 18:1 oleic acid showed a difference between MON 88913 and MON 88913 (-) at more than half the sites and were statistically different in the combined site analysis (Table 7).

However, the values were within the ranges for expected values for these components in conventional cotton. Those components with statistically significant differences between cotton line MON 88913 and MON 88913 (-) (Table 7) all fell within the 99% tolerance interval for the commercial conventional varieties planted alongside the experimental lines, and also within the literature range of values. The minor differences observed, were thought to reflect normal genetic variability and were not biologically meaningful.

Table 6: Amino acid content in cottonseed for cotton line MON 88913 and the control line MON 88923 (-) (n=16)

			Difference [MON 88913 minus MON 88913 (-)]			
Amino Acid (% Total AA)	**MON 88913 Mean[1] ± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E (Range)**	**95% Confidence Interval (Lower, Upper)**	***p* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
Alanine	4.28 ± 0.056	4.3 ± 0.056	-0.013 ± 0.03	-0.11, 0.081	0.691	(4.08 – 4.46)
	(4.09 – 4.51)	(4.15 – 4.46)	(-0.27 – 0.24)			[4.01,4.58]
Arginine	11.78 ± 0.17	11.77 ± 0.17	0.0033 ± 0.03	-0.39, 0.4	0.98	(11.08 – 12.77)
	(11.19 – 12.25)	(11.11 – 12.27)	(-0.81 – 0.99)			[10.57, 12.96]
Aspartic Acid	9.82 ±0.064	9.8 ± 0.064	0.02 ± 0.031	-0.08, 0.12	0.567	(0.97 – 10.38)
	(9.59 – 10.08)	(9.59 – 9.99)	(-0.13 – 0.29)			[9.48, 10.35]
Cysteine	1.89 ± 0.042	1.92 ± 0.042	-0.035 ± 0.029	-0.097, 0.027	0.243	(1.62 – 2.35)
	(1.69 – 2.1)	(1.76 – 2.1)	(-0.25 – 0.16)			[1.6, 2.14]
Glutamic acid	21.66 ± 0.13	21.55 ± 0.13	0.11 ± 0.096	-0.085, 0.31	0.253	(20.92 – 22.18)
	(21.08 – 22.14)	(21.1 – 21.96)	(-0.63 – 1.03)			[20.88, 22.49]
Glycine	4.42 ± 0.029	4.45 ± 0.029	-0.025 ± 0.018	-0.062, 0.012	0.171	(4.29 – 4.66)
	(4.33 – 4.56)	(4.33 – 4.64)	(-0.24 – 0.13)			[4.21, 4.64]
Histidine	3.15 ± 0.008	3.14 ± 0.08	0.06 ± 0.011	-0.022, 0.033	0.619	(3.01 – 3.22)
	(3.09 – 3.21)	(3.11 – 3.2)	(-0.07 – 0.1)			[3.04, 3.23]
Isoleucine	3.43 ± 0.02	3.43 ± 0.02	-0.004 ± 0.026	-0.086, 0.078	0.887	(3.19 – 4.49)
	(3.31 – 3.54)	(3.34 – 3.56)	(-0.25 – 0.12)			[3.13, 3.65]
Leucine	6.31 ± 0.048	6.27 ± 0.048	0.046 ± 0.026	-0.036, 0.13	0.169	(6.03 – 6.48)
	(6.14 – 6.52)	(6.1 – 6.48)	(-0.2 – 0.2)			[5.84, 6.66]
Lysine	4.99 ± 0.052	5.09 ± 0.052	-0.11 ± 0.053	-0.22, 0.002	0.053	(4.72 – 5.38)
	(4.77 – 5.23)	(4.89 – 5.48)	(-0.48 – 0.3)			[4.53, 5.43]
Methionine	1.65 ± 0.04	1.69 ± 0.04	-0.042 ± 0.043	-0.18, 0.094	0.397	(1.27 – 1.94)
	(1.47 – 1.9)	(1.49 – 1.95)	(-0.34 – 0.22)			[1.3, 1.93]
Phenylalanine	5.64 ± 0.014	5.6 ± 0.014	0.044 ± 0.019	0.0042, 0.083	0.031	(5.44 – 5.82)
	(5.53 – 5.75)	(5.45 – 5.72)	(-0.19 – 0.21)			[5.43, 5.82]
Proline	4.17 ± 0.045	4.16 ± 0.045	0.01 ± 0.028	-0.079, 0.0999	0.739	(3.97 – 4.49)
	(3.92 – 4.39)	(3.93 – 4.25)	(-0.18 – 0.2)			[3.91, 4.43]
Serine	4.88 ± 0.096	4.9 ± 0.096	-0.17 ± 0.054	-0.19, 0.15	0.773	(4.53 – 5.31)
	(4.35 – 5.32)	(4.65 – 5.32)	(-0.48 – 0.5)			[4.55, 5.42]
Threonine	3.19 ± 0.094	3.2 ± 0.094	-0.008 ± 0.067	-0.22, 0.21	0.91	(2.67 – 3.5)
	(2.61 – 3.49)	(2.7 – 3.45)	(-0.49 – 0.48)			[2.73, 3.74]
Tryptophan	1.1 ± 0.012	1.14 ± 0.012	-0.039 ± 0.016	-0.0074, -0.0044	0.029	(0.97 – 1.31)
	(1.03 – 1.23)	(1.09 – 1.25)	(-0.14 – 0.089)			[0.94, 1.26]
Tyrosine	2.79 ± 0.033	2.78 ± 0.033	0.017 ± 0.028	-0.071, 0.11	0.576	(2.63 – 2.93)
	(2.7 – 2.9)	(2.62 – 2.89)	(-0.085 – 0.18)			[2.61, 3.0]
Valine	4.84 ± 0.028	4.81 ± 0.028	0.032 ± 0.024	-0.019, 0.084	0.202	(4.57 – 2.02)
	(4.68 – 5.0)	(4.68 – 4.96)	(-0.12 – 0.22)			[4.48, 5.02]

Notes for Table 6:

1 Means in the table are least square means from SAS® . Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.

2 Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table 7: Summary of statistical differences ($p \leq 0.05$) in cottonseed for the comparison of MON 88913 to MON 88913 (-), plus commercial conventional varieties ;(combined site results)

Matrix/Component[a]	Mean [b] MON 88913	Mean MON 88913(-)	MON 88913 range	*p*-value	Commercial 99%[c]T.I.[Lower, Upper][b]	Literature values[d]
Phenylalanine (% Total AA)	5.64	5.6	5.53 – 5.75	0.031	[5.43, 5.82]	5.0 – 6.2[1]
Tryptophan (% Total AA)	1.1	1.14	1.03 – 1.23	0.0.029	[0.94, 1.26]	1.0 – 1.4[1]
18:1 Oleic acid (% Total FA)	18.61	20.94	16.35 – 20.72	0.003	[10.59, 21.29]	15.17 – 19.94[2]
18:2 Linoleic acid (% Total FA)	52.36	50.42	49.66 – 54.32	<0.001	[48.66, 61.11]	49.07 – 59.1[3]
Manganese (mg/kg dwt)	15.34	14.64	12.37 – 19.98	0.024	[4.69, 26.45]	10.0 – 20.1[4]
Moisture (%fwt)	6.39	6.22	5.65 – 7.34	0.013	[4.51, 7.21]	5.4 – 10.1[3]

[a] dwt =dry weight; AA = amino acids; FA = fatty acids

[b] Mean is the least squares mean; range is the range of the average duplicate analyses of single samples.

[c] Tolerance Interval: with 95% confidence, interval contains 99% or the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

[d] Range of values found in published literature for cotton varieties. [1] Lawhon et al., 1977; 2 Cherry, 1978; [3] Cherry, 1983; [4] Belyea et al., 1989.

Cottonseed products

Compositional analyses were conducted on the whole, unprocessed, delinted cottonseed in order to show that the cottonseed used for oil production was also compositionally equivalent to the control. Tables 8 – 10 detail the fatty acid composition, fibre and mineral composition and proximate, vitamin and gossypol composition of the whole cottonseed fractions. A compositional analysis of the fatty acids and vitamin E content of refined, bleached, deodorised cottonseed oil was also carried out (Table 11), in addition to an analysis of the amino acids, anti-nutritional fatty acids, fibre, minerals, proximates and gossypol in raw (untoasted) cottonseed meal.

For MON 88913 and MON 88913 (-), the data from two sites were combined and statistical analyses conducted using a mixed model ANOVA. Fifty-two comparisons were made in whole cottonseed fraction, forty-one in cottonseed meal and thirteen in cottonseed oil.

Four analytes were statistically different between MON 88913 and MON 88913 (-) in the processed fractions and are summarised in Table 12. The differences observed were for phenylalanine in the whole cottonseed fraction, total gossypol in raw cottonseed meal, and 14:0 myristic acid and 22:0 behenic acid in cottonseed oil. The range of values for these components fall within the 99% tolerance interval for commercial conventional cotton, and the differences observed were not biologically meaningful.

Table 8: Fatty acid content in the whole cottonseed fraction for cotton line MON 88913 and the control line MON 88923 (-) (n = 8)

			Difference [MON 88913 minus MON 88913 (-)]			
FattyAcid (% Total FA)	**MON 88913 Mean[1] ± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E (Range)**	**95% Confidence Interval (Lower, Upper)**	***p* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
14:0 Myristic	0.61 ± 0.03	0.62 ± 0.26	-0.018 ± 0.012	-0.17, 0.14	0.461	(0.51 – 0.74)
	(0.57 – 0.64	(0.58 – 0.66)	(-0.07– 0.003)			[0.19,1.12]
16:0 Palmitic	23.21 ± 0.8	23.43 ±0.8	-0.025 ± 0.052	-1.7, 1.27	0.316	(22.61 – 25.86)
	(22.38 – 24.07)	(22.53 – 24.49)	(-0.42 - -0.05)			[16.4, 30.85]
16:1 Palmitoleic	0.52 ± .022	0.53 ± 0.22	-0.006 ± 0.0056	-0.024, 0.012	0.359	(0.48 – 0.77)
	(0.51 – 0.59)	(0.5 – 0.59)	(-0.019 – 0.0079)			[0, 1.24]
18:0 Stearic	2.73 ± 0.18	2.71 ± 0.18	0.023 ± 0.0089	-0.09, 0.14	0.233	(2.41 – 2.68)
	(2.55 – 2.92)	(2.53 – 2.9)	(0.033 – 0.046)			[1.91, 3.2]
18:1 Oleic	19.92 ± 0.59	20.1 ± 0.59	-0.17 ± 0.066	-1.01, 0.67	0.231	(13.25 – 15.38)
	(19.21 – 20.67)	(19.42 – 20.85)	(-0.3 – -0.004)			[9.94, 18.9]
18:2 Linoleic	51.35 ± 1.56	51.06 ± 1.56	0.29 ± 0.24	-2.75, 3.34	0.437	(54.27 – 58.43)
	(49.71 – 53.02)	(49.04 – 52.82)	(-0.099 – 0.67)			[48.13, 65.04]
18:3 Gamma Linolenic	0.14 ± 0.019	0.095 ± 0.019	0.04 ± 0.023	-0.089, 0.17	0.256	(0.09 – 0.16)
	(0.12 – 0.15)	(0.048 – 0.13)	(0.0094 – 0.076)			[0, 0.26]
18:3 Linolenic	0.17 ± 0.015	0.17 ± 0.015	0.0012 ± 0.0091	-0.028, 0.03	0.901	(0.15 – 0.2)
	(0.14 – 0.19)	(0.14 – 0.19)	(-0.022 – 0.021)			[0.067, 0.27]
20:0 Arachidic	0.25 ± 0.0048	0.25 ± 0.0048	-0.0042 ± 0.0046	-0.062, 0.054	0.525	(0.22 – 0.26)
	(0.24 – 0.25)	(0.24 – 0.26)	(-0.015 – 0.0032)			[0.17, 0.32]
22:0 Behenic	0.099 ± 0.007	0.086 ± 0.007	0.013 ± 0.0079	-0.012, 0.038	0.205	(0.089 – 0.13)
	(0.08 – 0.12)	(0.16 – 0.19)	(-0.0016 – 0.032)			
Dihydrosterculic	0.19 ± 0.0056	0.18 ± 0.0056	0.0094 ± 0.0079	-0.025, 0.044	0.358	(0.14 – 0.019)
	(0.17 – 0.19)	(0.16 – 0.19)	(-0.0058 – 0.03)			[0.065, 0.27]
Malvalic	0.49 ± 0.038	0.44 ± 0.038	0.021 ± 0.04	-0.49, 0.53	0.691	(0.4 – 0.54)
	(0.39 – 0.51)	(0.42 – 0.45)	(-0.035 – 0.065)			[0.17, 0.78]
Sterculic	0.36 ± 0.02	0.34 ± 0.02	0.016 ± 0.028	-0.11, 0.14	0.621	(0.28 – 0.38)
	(0.32 – 0.41)	(0.32 – 0.36)	(-0.43 – 0.085			[0.087, 0.59]

1 Means in the table are least square means from SAS®. Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.

2 Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table 9: Fibre and Mineral content of the whole cottonseed fraction for cotton line MON 88913 and the control line MON 88923 (-)(n = 8).

			Difference [MON 88913 minus MON 88913 (-)]			
Analytical component	**MON 88913 Mean [1]± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E (Range)**	**95% Confidence Interval (Lower, Upper)**	***p* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
Fibre (% dwt)						
Acid detergent fibre	31.08 ± 2.3	33.37 ± 2.3	-2.28 ± 1.49	-21.23, 16.67	0.368	(31.2 – 33.71)
	(26.62 – 34.07)	(30.78 – 35.08)	(-6.44 - -0.057)			[27.02, 38.03]
Crude fibre	16.9 ± 1.98	18.1 ± 1.98	-1.29 ± 1.17	-16.15, 13.58	0.469	(19.04 – 21.01)
	(14.13 – 19.99)	(16.88 – 19.87)	(-2.76 – 0.086)			[15.19, 24.26]
Neutral detergent fibre	41.54 ± 3.6	41.35 ± 3.6	0.19 ± 1.83	-23.07, 23.45	0.932	(41.62 – 48.02)
	34.66 – 47.16)	(37.07 – 45.33)	(-5.83 – 2.55)			[29.88, 57.62]
Total Dietary fibre	38.32 ± 4	42.91 ± 4	-4.6 ± 1.97	-9.65, 0.46	0.066	(40.86 – 44.89)
	(34.55 – 42.7)	(38.86 – 51.29)	(-9.93 – 0.073)			[33.47, 52.34]
Mineral						
Calcium (%dwt)	0.17 ± 0.002	0.17 ± 0.002	-0.34 ± 0.0019	-0.0094, 0.0027	0.173	(0.13 – 0.15_
	(0.16 – 0.17)	(0.17 – 0.18)	(-0.0082 – 0.00009)			[0.093, 0.118]
Copper (mg/kg dwt)	8.56 ± 1.05	8.59 ± 1.05	-0.04 ± 0.1	-1.371, 1.29	0.769	(6.28 – 8.38)
	(.27 – 9.79)	7.24 – 9.75)	(-0.32 – 0.094)			[3.27, 11.71]
Iron (mg/kg dwt)	53.82 ± 6.52	62.3 ± 6.52	-8.48 ± 9.21	-49.8, 32.84	0.458	(43.43 – 54.44)
	(44.21 – 63.45)	(49.4 – 77.36)	(-32.33 – 1.95)			[23.72, 71.13]
Magnesium (% dwt)	0.48 ± 0.01	0.46 ± 0.01	0.012 ± 0.014	-0.049, 0.073	0.498	(0.42 – 0.46)
	(0.47 – 0.48)	(0.45 – 0.48)	(-0.011 – 0.033)			[0.35, 0.52]
Manganese (mg/kg dwt)	15.34 ± 0.73	16.1 ± 0.73	-0.77 ± 0.47	-2.26, 0.73	0.201	(14.23 – 16.31)
	(14.43 – 16.62)	(14.53 – 16.94)	(-2.05 – 0.13)			[10.72, 19.39]
Phosphorus (% dwt)	0.87 ± 0.034	0.84 ± 0.034	0.025 ± 0.035	-0.038, 0038	0.603	(1.19 – 1.37)
	91.23 – 1.38)	(1.24 – 1.4)	(-0.028 – 0.029)			[0.92, 1.65]
Potassium (%dwt)	1.31 ± 0.061	1.31 ± 0.061	0.0004 ± 0.012	-0.038, 0.038	0.972	(1.19 – 1.37)
	(1.23 – 1.38)	(1.24 – 1.4)	(-0.028 – 0.029)			[0.92, 1.65]
Zinc (mg/kg dwt)	40.4 ± 7.95	39.86 ± 7.95	0.54 ± 1.52	-18.82, 19.91	0.781	(29.73 – 44.31)
	(31.52 – 49.16)	(32.55 – 47.18)	(-1.34 – 2.31)			[3.47, 69.46]

1 Means in the table are least square means from SAS® . Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.

2 Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table 10: Proximate and Vitamin content in the whole cottonseed fraction for cotton line MON 88913 and the control line MON 88923 (-)(n = 8).

			Difference [MON 88913 minus MON 88913 (-)]			
Analytical component	**MON 88913 Mean[1] ± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E[1] (Range)**	**95% Confidence Interval (Lower, Upper)**	***p* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
Proximate						
Ash (% dwt)	4.88 ± 0.16	4.67 ± 0.16.	0.21 ± 0.2	-0.99, 1.41	0.436	(4.61 – 4.86)
	(4.54 – 5.13)	(4.45 – 4.88)	(-0.099 – 0.54)			[4.1, 5.29]
Calories (Kcal/100g dwt)	503.5 ± 5	500.28 ± 2.29	3.22 ± 2.79	-32.22, 38.66	0.454	(483.77 – 499.89)
	(498.26 – 507.25)	(499.42 – 501)	(-1.16 – 6.25)			[457.45, 527.86]
Carbohydrates (%dwt)	44.04 ± 2.93	45.95 ± 2.93	-1.92 ± 2.64	-35.43, 34.59	0.599	(46.65 – 49.61)
	(39.76 – 48.49)	(44.35 – 47.67)	(-5.2 – 1.66			[42.47, 54.05]
Moisture (% fwt)	10.5 ± 2.97	10.35 ±2.97	0.15 ± 0.48	-5.95, 6.24	0.813	(7.5 – 12.05)
	(6.95 – 13.7)	(7.38 – 13.2)	(-0.43 – 0.75)			[0.2, 51]
Protein (% fwt)	26.53 ± 2.25	25.62 ± 2.25	0.91 ± 1.66	-20.19, 22.01	0.68	(23.3 – 26.55)
	(23.52 – 25.39)	(24.24 – 26.98)	(-1.31 – 2.99)			[17.15, 32.56]
Total Fat (% dwt)	24.57 ± 0.57	23.76 ± 0.57	0.81 ± 0.72	-8.74, 10.36	0.477	(20.48 – 23.69)
	(23.52 – 25.39)	(23.61 – 23.94)	(-0.27 – 1.67)			[15.28, 29.21]
Vitamin						
Vitamin E (mg/kg dwt)	144.38 ± 9.02	137.25 ± 9.02	7.13 ± 3.46	-36.86, 51.12	0.287	(104.56 – 159.03)
	(135.88 – 152.95)	(125.96 – 152.07)	(-2.02 – 11.24)			[23.79, 238.24]

1. Means in the table are least square means from SAS® . Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.
2. Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table 11: Fatty acid and Vitamin E content in cottonseed oil for cotton line MON 88913 and the control line MON 88923 (-) (n=8)

			Difference [MON 88913 minus MON 88913 (-)]			
FattyAcid (% Total FA)	**MON 88913 Mean[1] ± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E (Range)**	**95% Confidence Interval (Lower, Upper)**	***P* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
14:0 Myristic	0.61 ± 0.026	0.62 ± 0.026	-0.013 ± 0.003	-0.023, 0.0025	0.029	(0.52 – 0.74)
	(0.57 – 0.064)	(0.58 – 0.66)	(-0.017 - - 0.0032)			[0.2, 1.13]
16:0 Palmitic	23.21 ± 0.83	23.33 ± 0.83	-0.025 ± 0.052	-0.19, 0.14	0.667	(22.51 – 25.61)
	(22.33 – 24.1)	(22.32 – 24.27)	(-0.17 – 0.066)			[16.41, 30.45]
16:1 Palmitoleic	0.53 ± 0.02	0.52 ± 0.02	0.00093 ± 0.0019	-0.015, 0.033	0.128	(0.49 – 0.78)
	(0.51 – 0.55)	(0.5 – 0.54)	(0.007 – 0.011)			[0, 1.24]
18:0 Stearic	2.53 ± 0.19	2.53 ± 0.19	-0.0025 ± 0.059	-0.76, 0.75	0.973	(2.26 – 2.59)
	(2.29 – 2.78)	(2.34 – 2.73)	(-0.11 – 0.12)			[1.69, 3.07]
18:1 Oleic	20.18 ± 0.63	20.15 ± 0.63	0.025 ± 0.055	-0.15, 0.2	0.681	(13.1 – 15.83)
	(19.41 – 20.82)	(19.46 – 20.83)	(-0.066 – 0.17)			[8.44, 20.6]
18:2 Linoleic	54.67 ± 1.66	51.72 ± 1.66	-0.045 ± 0.098	-1.29, 1.2	0.723	(54.7 – 59.69)
	(50 - 53.53)	(49.84 – 53.55)	(-0.27 – 0.16)			[46.72, 67.8]
18:3 Linolenic	0.15 ± 0.0067	0.16 ± 0.0067	-0.0072 ± 0.0033	-0.018, 0.0032	0.114	(0.13 – 0.17)
	(0.13 – 0.16)	(0.14 – 0.17)	(-0.013 – 0.0023)			[0.048, 0.24]
20:0 Arachidic	0.24 ± 0.0046	0.25 ± 0.0046	-0.0033 ± 0.0031	-0.013, 0.0066	0.369	(0.23 – 0.25)
	(0.24 – 0.26)	(0.24 – 0.25)	(-0.0086 – 0.0038)			[0.19, 0.3]
22:0 Behenic	0.11 ± 0.0016	0.12 ± 0.0016	-0.0052 ± 0.0016	-0.01, 0.0002	0.045	(0.11 – 0.13)
	(0.11 – 0.12)	(0.12 – 0.12)	(-0.0096 - - 0.002)			[0.08, 0.16]
Dihydrosterculic	0.19 ± 0.016	0.16 ± 0.016	0.027 ± 0.022	-0.007, 0.12	0.347	(0.12 – 0.16)
	(0.17 – 0.21)	(0.26 – 0.3)	(-0.014 – 0.084)			[0.058, 0.23]
Malvalic	0.31 + 0.031	0.28 + 0.031	0.031 + 0.03	-0.35, 0.42	0.489	(0.27 – 0.3)
	(0.26 – 0.37)	(0.26 – 0.3)	(-0.014 – 0.084)			[0.21, 0.38]
Sterculic	0.26 ± 0.02	0.25 ± 0.02	0.009 ± 0.026	-0.11, 0.13	0.758	(0.17 – 0.23)
	(0.22 – 0.29)	(0.22 – 0.29)	(-0.028 – 0.066)			[0.069, 0.34]
Vitamin						
Vitamin E (mg/kg FW)	464.38 ± 25.92	498.5 ± 25.92	-34.13 ± 12.24	-73.09, 4.84	0.068	(444 – 652)
	(406 – 507)	(454.5 – 532)	(-60.5 - -8)			[0, 1089.4)

1 Means in the table are least square means from SAS® . Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.

2. Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table 12: Summary of statistical differences (p <0.05) in combined site cottonseed, Cottonseed oil and cottonseed meal for the comparison of MON 88913 to MON 88913 (-) and commercial conventional reference varieties.

Matrix/Component[a]	Mean[b] MON 88913	Mean MON 88913(-)	MON 88913 range	p-value	Commercial 99%[c]T.I.[Lower, Upper][b]	Literature values[d]
Cottonseed (n=16)						
Phenylalanine (%Total AA)	5.53	5.49	(5.44 – 5.58)	0.015	[5.1, 6.02]	5.0 – 6.2[1]
Cottonseed Oil (n = 8)						
14:0 Myristic acid (% Total FA)	0.61	0.62	(0.57 – 0.64)	0.029	[0.2, 1.13]	0.5 – 2.5[3]
22:0 Behenic acid (% Total FA)	0.11	0.12	0.11 – 0.12_	0.045	([0.08, 0.16]	0.2[4]
Raw cottonseed meal (n = 8)						
Total Gossypol (% dwt)	1.32	1.48	(1.26 – 1.4)	0.006	[0, 3.35]	1.15 – 1.45[2]

[a] dwt =dry weight; AA = amino acids; FA = fatty acids
[b] Mean is the least squares mean; range is the range of the average duplicate analyses of single samples.
[c] Tolerance Interval: with 95% confidence, interval contains 99% or the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.
[d] Range of values found in published literature for cotton varieties. [1] Lawhorn et al., 1977; [2]Waldroup and Kersey, 2002; [3]Hui, 1996; [4]Rossell, 1991.

5.2 Key toxicants

Cotton contains two naturally occurring toxic compounds – gossypol and cyclopropenoid fatty acids. In addition, aflatoxins are potent exogenously formed fungal toxins, which can potentially be borne on cotton. These compounds have been analysed in cottonseed from cotton line MON 88913 and compared with the negative segregant line MON 88913 (-).

Gossypol

Gossypol is a biologically active terpenoid aldehyde that is present in discrete glands in all plant tissues, including seed (Abou-Donia, 1976; Jones, 1991). Gossypol can cause a number of toxic effects on mammals including reduced appetite, body weight loss, and dyspnoea (difficult and laboured breathing) (Berardi and Goldblatt 1980), adverse effects on the protein nutritive value of food by rendering lysine metabolically unavailable (Yannai and Bensai, 1983) and damage to normal mitochondrial functioning (Cuellar and Ramirez, 1993; Randel *et al.,* 1992, Risco *et al.,* 1993).

The levels of gossypol and related terpenoids in cottonseed varies with variety and environmental conditions, which can include factors as diverse as soil and air temperature, disease infections, moisture stress and the presence of chemicals (Bell, 1991). Any 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). Processing of cottonseed is therefore essential for it to have feed or food value.

Gossypol exists in two forms, free and bound. The free form is toxic, while the bound form is considered non-toxic since it is not released in the animal rumen. In whole unprocessed cottonseed almost all of the gossypol is in the free form. During processing, gossypol partitions into the meal and oil components. Although some of the gossypol in meal remains as the free form, much of it becomes bound to proteins and therefore detoxified. Gossypol in oil is eliminated during the refining process.

The levels of gossypol in processed, refined cottonseed oil was below the limit of detection for all samples. The levels of free gossypol recorded were in the mid-range of the standard values for cottonseed, whole cottonseed fraction (Table 13) and cottonseed meal (Table 12). The level of both free and total gossypol in the control line, MON 88913 (-), was higher than that found for the transgenic cotton line for cottonseed and cottonseed meal. One statistical difference was detected in the level of total gossypol in raw cottonseed meal, however the values fell within the range of values for reference cotton and within the 99% tolerance interval (Table 12).

Table 13: Gossypol content in the whole cottonseed fraction for cotton line MON 88913 and the control line MON 88923 (-)(n = 8).

			Difference [MON 88913 minus MON 88913 (-)]			
FattyAcid (% Total FA)	**MON 88913 Mean[1] ± S.E. (Range)**	**MON 88913 (-) Mean ± S.E. (Range)**	**Mean ± S.E (Range)**	**95% Confidence Interval (Lower, Upper)**	***P* - value**	**Commercial (Range) [99% Tolerance Interval[2]]**
Gossypol						
Free Gossypol (% dwt)	0.87 ± 0.024	0.86 ± 0.024	0.032 ± 0.027	-0.31, 0.37	0.447	(0.58 – 1.1)
	(0.84 – 0.94)	(0.8 – 0.87)	(-0.027 – 0.1)			[0, 1.97]
Total Gossypol (% dwt)	0.89 ± 0.03	0.86 ± 0.03	0.024 ± 0.03	-0.07, 0.12	0.47	(0.61 – 1.13)
	(0.86 – 0.92)	(0.77 – 0.91)	(-0.051 – 0.089)			[0, 1.99]

1. Means in the table are least square means from SAS®. Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.
2. Tolerance interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Cyclopropenoid fatty acids

Cyclopropenoid fatty acids are unique fatty acids that are naturally present in cotton, crude cottonseed oil and in the meal (because of the residual oil in the meal fractions). Refinement of cottonseed oil includes deodorisation and bleaching, which greatly reduces the cyclopropenoid fatty acid content of the oil due to extreme pH and temperature conditions. The major types are sterculic acid (C-17), malvalic acid (C-18) and dihydrosterculic acid (C-19).

Cyclopropenoid fatty acids are considered to be undesirable, anti-nutritional compounds of concern for food safety. They have unfavourable 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).

The cyclopropenoid fatty acids are destroyed either by hydrogenation or by heating the oil in the presence of free fatty acids for deodorisation purposes (Gunstone *et al.,* 1994).

The concentrations of cyclopropenoid fatty acids in cottonseed from both cotton line MON 88913 and its isogenic counterpart were in the mid-range of values compared to commercial varieties and there was no significant difference between the transgenic and control line for any of these compounds. The levels in the whole cottonseed fraction (Table 8), cottonseed meal and cottonseed oil (Table 11) were at the upper levels, however there was no statistical difference between cotton line MON 88913 and MON 88913 (-).

Aflatoxins

Aflatoxins are a group of mycotoxins produced by the *Aspergillus flavus* and *A. parasiticus* and are potent animal toxins and carcinogens and have been epidemiologically implicated as environmental carcinogens in humans. Cottonseed is one of the commodities most commonly contaminated by aflatoxins. Cotton that is damaged by moth larvae is more susceptible to infection by *Aspergillus* fungi. This infection is often initiated through larval damage that occurs in the field rather than in storage.

None of the four primary aflatoxins of cottonseed (B_1, B_2, G_1 and G_2) were detected at a sensitivity of 1 ppb in cottonseed from either cotton line MON 88913 or MON 88913 (-) at any of the experimental sites.

5.3 Conclusion

Detailed compositional analyses of key nutrients, anti-nutrients and toxicants were carried out on cottonseed and processed products, including refined oil, from cotton line MON 88913 and compared to control line, MON 88913 (-), as well as commercial cotton varieties. No meaningful differences ($p<0.05$) were observed in 51 of the 52 comparisons for cottonseed, 40 of the 41 comparisons for raw cottonseed meal, and 11 of the 13 comparisons for cottonseed oil. For all four statistically significant differences, all test values fell within the range of values expected in the commercial cotton population. It was concluded with 95% confidence that seed, meal and oil derived from cotton line MON 88913 are compositionally equivalent to those derived from conventional cotton varieties. .

6. NUTRITIONAL IMPACT

In assessing the safety and suitability of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. 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.

To date, all approved GM plants with modified agronomic production traits (e.g. herbicide tolerance) have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies with feeds derived from the approved GM plants have shown equivalent animal nutritional performance to that observed with the non-GM feed. Thus the evidence to date is that where GM varieties have been shown to be compositionally equivalent to conventional varieties, feeding studies using target livestock species will add little to a safety assessment and generally are not warranted.

For plants engineered with the intention of significantly changing their composition or nutrient bioavailability and thus their nutritional characteristics, however, it is recognised that suitable comparators may not be available for a nutritional assessment based solely on compositional analysis. In such cases, feeding trials with one or more target species may be useful to demonstrate wholesomeness in the test animals. In the case of corn line MON 88913, the extent of the compositional and other available data is considered to be sufficient to establish the nutritional adequacy of the food.

Although no feeding studies using corn line MON88913 have been undertaken, a number of feeding studies using glyphosate tolerant crops have been done in the past which are of general interest and relevance to this Application. For example, a number of feeding studies have been undertaken with Roundup Ready® soybeans (FSANZ application A338). Studies were conducted in rats (natural consumers of soybeans) with both processed and unprocessed soybean meal, in chickens (significant consumers of soybeans) with processed soybeans and, in dairy cattle (common consumers of soybeans) with raw soybeans. It was concluded that the wholesomeness of unprocessed meal from glyphosate–tolerant soybeans was similar to that from the parental, non-transgenic soybeans.

More recently, feeding studies with cows using both glyphosate-tolerant and insect-protected cotton varieties have been undertaken. Whole seeds of Roundup Ready® and Bollgard II cotton were used to supplement the diet of lactating Holstein cows at a concentration of about 10% of the total diet. Results showed that both the Roundup Ready® and Bollgard® II cotton wholeseed sources supported similar performance as the non-transgenic commercial varieties, as indicated by the dry matter intake, milk yield, milk composition, and body weight and body condition (Castillo et al., 2004).

7. REFERENCES

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Attachment 3

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

Submitter	Option	Comment
Queensland Department of Health (Gary Bielby)	-	Query regarding labelling if the final food product showed altered characteristics
Gene Ethics (Bob Phelps)	1	Concerned that it is premature to approve food derived from MON 88913 as it is not yet grown commercially.
Ivor Bell	1	Concerns regarding environmental issues and GM cotton generally and also royalty issues.
Frank Rowson	1	Concerns regarding the potential toxic effects of glyphosate
Australian Food and Grocery Council (Kim Leighton)	2	Supports the application with no intrinsic safety issues.
South Australia Department of Health (Kirsten Potoczky)	2	No intrinsic safety issues
Food Technology Association of Victoria (David Gill)	2	No intrinsic safety issues
GE Free New Zealand (Claire Bleakley)	1	Opposes the initial assessment on the grounds that no safety data was provided. Other specific concerns regarding the safety of MON 88913 are covered in section *5.3.1* in the draft assessment report.
GE Free New Zealand, Nelson branch (Susie Lees)	1	General concerns regarding GM foods: including lack of feeding studies, unstable GM varieties, toxic and allergenic side effects and environmental concerns.
Pam Parsons	1	Concerns regarding the policy and regulation of GM foods.
Shushila Ajani	1	Concerns with lack of labelling and consumer choice; environmental pollution by GM cotton and general safety issues.
Mike Trott	1	Concerns over ignoring the Precautionary Principle and general safety concerns.
Me Aroha Waiheke Foundation (C. Lehwenz)	1	General objection to GM crops, including lack of long-term feeding studies; escape of GM pollen; fear of exchange of genes with viral pathogens and the creation of new viral strains.
Hugh Halliday	1	General objections to GM crops and herbicides used in conjunction with GM crops
Hilary Jones, Chelsey Over	1	Concerns with inadequate labelling of GM foods
Gloria Varkoly, Annmarie Banchy	1	Concerns with allergenic potential of GM foods when used as additives.
Jews for GE-free food (Hilary Philips)	1	Objects to GE foods on the grounds that they are not kosher and a possible threat to human health.
Organic Food Producers (Betsy Kettle)	1	General objections to GM cotton for environmental (e.g. the development of Bt resistant strains of bollworm) and for economic reasons.

In addition, the following public submissions have shown a preference for option 1, and identified a general objection to GM crops:

Richard Dunwell, Donald McBride, Ruth Begg, L.K. Vasbenenter, Patricia Waugh, Johanna Metz, Lillian Fougere, Hans B. Grueber, Harold Curry, Jane Pearce, Ali Symmons, Rosemary R. Grueber, J.R. Collins, Anne Larsen, Simon, Ed Tye, Berthine Bruinsma, Wim Oosterhoff, Rosemary Bartle, Raymond Vogt, Paul Brimecombe, Joan Roesch; Sue Woledge, Sue Farland, Carol Mclean, Stephen Richardson, Colin Hewens, Mairead Ni Chonaola, Sue Ferrabee, Lesley Macdonald, Jane Landman, Anne Smith, Mr Royal, Daniel Meares, Kalani Bruce, Taleb Bench-Kanjou, Mary Madigan, Morag Brownlie, Pauline Bailey, Rose Mackinnon, Nerine Walbran, Linda Bench, Betty Wheeler, Liz Peters, Quentin Jamieson, Francisca Griffin, Sky Williams, Raylene Lodge, Shari French, G. Mabbs, J. Gerritsen, Dr Bruce Ross, Ian Roger, Robyn Mitchell, K Du Pont, Helen Varley Jamieson, Campbell Sturrock, Marion and Peter Corby, Amy Donovan, Mark Sidebotham, Lars Chresta, Mike McCree, J. Carapiet, Max Tobin.