ANZFA
Australia New Zealand **Food** Authority
TE MANA WHAKARITE KAI MŌ ĀHITEREIRIA ME AOTEAROA

09/02
8 May 2002

DRAFT ASSESSMENT REPORT
(FULL ASSESSMENT - S.15)

APPLICATION A436

OIL AND LINTERS DERIVED FROM INSECT-PROTECTED COTTON CONTAINING EVENT 15985

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:

19 JUNE 2002

(See 'Invitation for Public Submissions' for details)

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FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered an Agreement in December 1995 establishing a system for the development of joint food standards. On 24 November 2000, Health Ministers in the Australia New Zealand Food Standards Council (ANZFSC) agreed to adopt the new *Australian New Zealand Food Standards Code*. The new Code was gazetted on 20 December 2000 in both Australia and New Zealand as an alternate to existing food regulations until December 2002 when it will become the sole food code for both countries. It aims to reduce the prescription of existing food regulations in both countries and lead to greater industry innovation, competition and trade.

Until the joint *Australia New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, as gazetted in New Zealand, or the New Zealand *Food Regulations 1984,* but not a combination thereof. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the New Zealand *(Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.*

- **Food imported into Australia other than from New Zealand** must comply solely with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, but not a combination of the two.

- **Food imported into New Zealand from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* as gazetted in New Zealand, but not a combination thereof. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

- **Food imported into Australia from New Zealand** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, but not a combination of the two. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may **also** be imported into Australia from New Zealand provided it complies with the New Zealand *Food Regulations 1984.*

- **Food manufactured in Australia and sold in Australia** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* but not a combination of the two. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act 1986* and all food sold in Australia must comply with the Australian *Trade Practices Act 1974,* and the respective Australian State and Territory *Fair Trading Acts.*

Any person or organisation may apply to ANZFA to have the *Food Standards Code* amended. In addition, ANZFA may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. ANZFA can provide advice on the requirements for applications to amend the *Food Standards Code*.

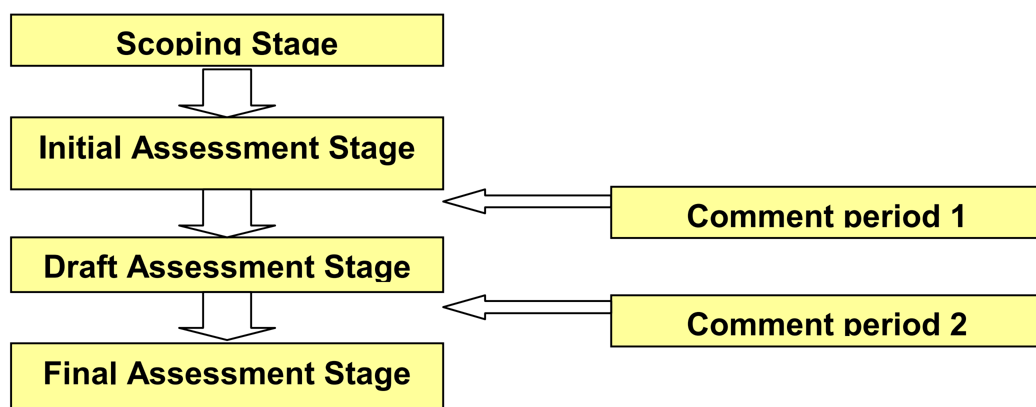
INVITATION FOR PUBLIC SUBMISSIONS

The process for amending the *Australia New Zealand Food Standards Code* (the Code) is prescribed in the ANZFA Act 1991. Open and transparent consultation with interested parties is a key element in the process involved in amending or varying the Code.

Any individual or organization may make an 'application' to the Australia New Zealand Food Authority (the Authority) seeking to change the Code. The Authority itself, may also seek to change the Code by raising a 'proposal'. In the case of both applications and proposals there are usually two opportunities for interested parties to comment on proposed changes to the Code during the assessment process. This process varies for matters that are urgent or minor in nature.

Following the initial assessment of an application or proposal the Authority may decide to accept the matter and seek the views of interested parties. If accepted, the Authority then undertakes a draft assessment including, preparing a draft standard or draft variation to a standard (and supporting draft regulatory impact statement). If a draft standard or draft variation is prepared, it is then circulated to interested parties, including those from whom submissions were received, with a further invitation to make written submissions on the draft. Any such submissions will then be taken into consideration during the final assessment, which the Authority will hold to consider the draft standard or draft variation to a standard.

Comment opportunities in the usual assessment process
to change the Australia New Zealand Food Standards Code
(Note: this process may vary for matters that are urgent or minor)



Content of Submissions
Written submissions containing technical or other relevant information which will assist ANZFA in undertaking an assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organizations. 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 presented should be in sufficient detail to allow independent scientific assessment.

Submissions may provide more general comment and opinion on the issue although those framing their submissions should bear in mind ANZFA's regulatory role specifically relates to food supplied for human consumption in Australia and New Zealand. The ANZFA Act 1991 sets out the objectives of the Authority in developing food regulatory measures and variations of food regulatory measures as:

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

In developing food regulatory measures and variations of food regulatory measures The Authority must also have regard to the following:

(a) the need for standards to be based on risk analysis using the best available scientific evidence;
(b) the promotion consistency between domestic and international food standards;
(c) the desirability of an efficient and internationally competitive food industry;
(d) the promotion of fair trading in food.

Submissions addressing the issues in the context of the objectives of the Authority as set out in the *ANZFA Act 1991* will be more effective in supporting their case.
Written submissions containing technical or other relevant information which will assist the Authority in undertaking a final assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

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

Following its draft assessment of the application the Authority may prepare a draft standard or draft variation to a standard (and supporting draft regulatory impact statement), or decide to reject the application/proposal. If a draft standard or draft variation is prepared, it is then circulated to interested parties, including those from whom submissions were received, with a further invitation to make written submissions on the draft. Any such submissions will then be taken into consideration during the inquiry, which the Authority will hold to consider the draft standard or draft variation to a standard.

Transparency
The processes of ANZFA are open to public scrutiny, and any submissions will ordinarily be placed on the public register of ANZFA and made available for inspection. If you wish any confidential information contained in a submission to remain confidential to ANZFA, you should clearly identify the sensitive information and provide justification for treating it in confidence. The *Australia New Zealand Food Authority Act 1991* requires ANZFA 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 reasonable be expected to be destroyed or diminished by disclosure.

Contact details for submitters are recorded so that the Authority can continue to keep them informed about progress of the application or proposal.

Deadlines
The deadlines for submissions are clearly indicated in the advertisements calling for comment and in the relevant Assessment Reports. While the Authority often provides comment periods of around 6 weeks, the periods allowed for comment may vary and may be limited to ensure critical deadlines for projects can be met. Unless the Project Manager has given specific consent for an extension, the Authority cannot guarantee that submissions received after the published closing date will be considered.

Delivery of Submissions
Submissions must be made in writing and should be clearly marked with the word 'Submission' and quote the **correct project number** and **title**. Submissions may be sent by mail to the **Standards Liaison Officer** at one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2258
email: slo@anzfa.gov.au

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
email: anzfa.nz@anzfa.gov.au

Submissions should be received by the Authority by: 19 JUNE 2002

Submissions may also be sent electronically through the submission form on the ANZFA website www.anzfa.gov.au. Electronic submissions should also include the full contact details of the person making the submission on the main body of the submission so that the contact details are not separated.

FURTHER INFORMATION
Further information on this and other matters should be addressed to the Standards Liaison Officer at the Australia New Zealand Food Authority at one of the above addresses.

Assessment reports are available for viewing and downloading from the ANZFA website or alternatively paper copies of reports can be requested from the Authorities Information Officer at info@anzfa.gov.au.

EXECUTIVE SUMMARY

ANZFA began assessment on a new genetically modified cotton line on 27 April 2001 after receiving an application from Monsanto Australia Limited. The Application seeks approval under Standard A18/1.5.2 – Food Produced Using Gene Technology, for foods derived from cotton lines containing event 15985. The presence of this event in cotton confers protection against insect attack and such lines are known commercially as Bollgard II® cotton. Cotton containing this event is not currently commercially grown but an application to grow it commercially in Australia has been lodged with the Office of the Gene Technology Regulator.

This cotton variety was developed from an already genetically modified cotton line (cotton line 531), the oil and linters from which were approved in July 2000, as part of Application A341 – Insect-Protected Cotton, also known as Ingard® cotton. Cotton line 531 contains three transferred genes, the primary gene of interest being the *cry*1Ac gene conferring insect protection to the cotton plant. Oil and linters from this line were found to be as safe as those from conventional cotton varieties.

Insect-protected cotton containing event 15985 was developed from cotton line 531 by the introduction of two additional genes - *cry*2Ab (another *Bt* gene) and *uid*A. The *cry*2Ab gene encodes an insecticidal protein that, like other *Bt* proteins, is highly selective in controlling Lepidopteran insects. The use of two *Bt* genes results in increased protection against insect attack and may also delay the development of resistance to *Bt* pesticides. The *uid*A gene is a marker gene that allows selection of transformed tissue based on the presence of a colour marker.

Oil and linters derived from insect-protected cotton containing event 15985 have been evaluated according to the safety assessment guidelines prepared by ANZFA. The assessment considered the following aspects of the food: (1) the nature of the genetic modification; (2) general safety issues such as history of use and the potential for transfer of antibiotic resistance genes to microorganisms in the human digestive tract; (3) characterisation of novel proteins including toxicological and allergenicity issues; and (4) comparative analyses and nutritional impact of the food. On the basis of the available information, it is concluded that oil and linters derived from cotton event 15985 are as safe and wholesome as those produced from other commercial cotton varieties. A detailed food safety report on cotton containing event 15985 has been prepared.

Changes to the labelling requirements of Standard A18/1.5.2 came into effect on 7 December 2001. Under the revised standard, oil and linters manufactured from cotton lines containing event 15985 will likely be exempt from labelling given that such highly processed foods do not contain protein or DNA. No additional labelling subject to clause 7 is required.

ANZFA undertook the first round of public consultation in relation to this Application on 17 July 2001. In response, 57 submissions were received. The majority of these opposed the approval of oil and linters from insect-protected cotton containing event 15985 primarily on the basis that all foods produced from GM crops are perceived to be unsafe, that not all foods will be labelled and that the process for assessment is flawed. Many submissions raised environmental concerns regarding GM crops. The food safety concerns raised in submissions have been addressed by the draft safety assessment report.

Conclusions/Statement of Reasons

- Based on the available information, there are no public health and safety concerns associated with the genetic modification used to produce insect-protected cotton containing event 15985.

- Oil and linters derived from insect-protected cotton lines containing event 15985 are equivalent to those derived from other commercially available cotton varieties in terms of their safety for human consumption and nutritional adequacy.

- Oil and linter products derived from lines containing cotton event 15985 are exempt from labelling unless it can be shown that novel DNA and/or protein is present in the final food; and.

- The benefits of permitting oil and linters from cotton containing event 15985 primarily accrue to industry and are considered to outweigh the costs to government, consumers and industry, since the safety assessment has not identified any public health and safety concerns; and

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

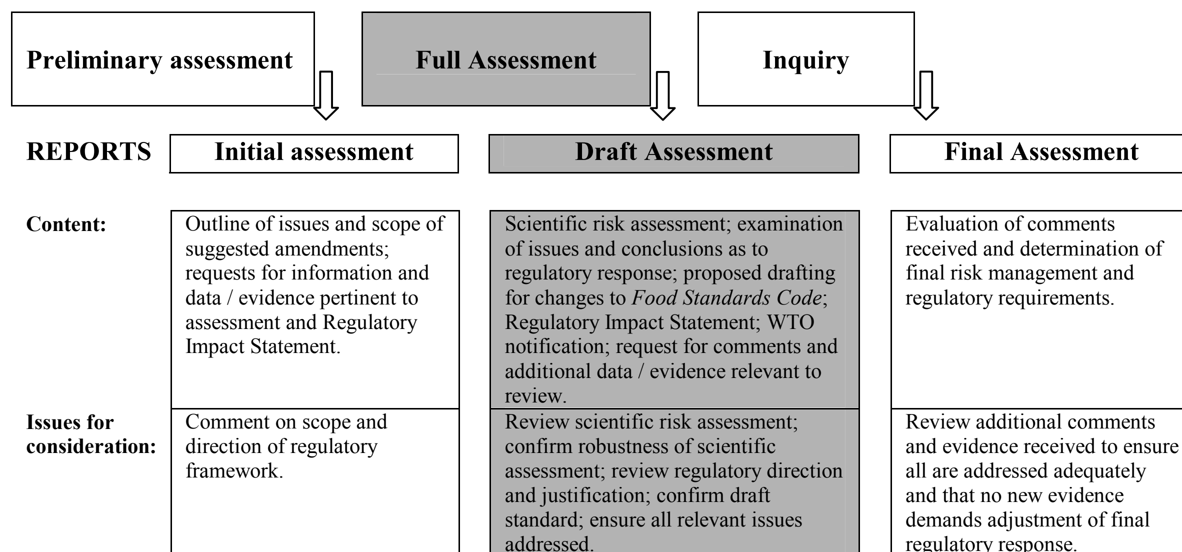
1. INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a bi-national statutory body responsible for developing draft food standards and draft variations of standards, in order to make recommendations to the Australia New Zealand Food Standards Council (Ministerial Council), and to review standards. The Ministerial Council may then decide to adopt the draft standards or draft variations of standards, which results in their incorporation into food laws of the Australian States and Territories, and New Zealand.

On 24 November 2000, the Ministerial Council adopted the *Australia New Zealand Food Standards Code* (known as Volume 2 of the *Food Standards Code*) that applies in both Australia and New Zealand. A two-year transitional period has been implemented at the conclusion of which Volume 2 of the *Food Standards Code* will be the sole code for both countries. In the interim, for the majority of food standards, there are two standards operating in Australia and three in New Zealand (including the New Zealand Food Regulations).

Standard A18 – Foods Produced Using Gene Technology in Volume 1 and Standard 1.5.2 in Volume 2 of the *Food Standards Code* regulate the sale of genetically modified food in Australia. An application from Monsanto Australia Ltd to amend the standard has been received by ANZFA.

Under the requirements of the ANZFA Act, this is the Full Assessment of this Application (referred to as Draft Assessment) as indicated below.



2. REGULATORY PROBLEM

Standard A18 / 1.5.2 requires that genetically modified (GM) foods undergo a pre-market risk assessment through an application to ANZFA before being offered for sale in Australia and New Zealand. Foods that have been assessed under the standard and subsequently approved by the Ministerial Council are listed in the Table to the Standard.

Monsanto Australia Ltd has developed a new genetically modified variety of insect-protected cotton containing event 15985. Before food from insect-protected cotton containing event 15985 can be sold in Australia or New Zealand it must first be assessed as safe and approved by the Ministerial Council. Monsanto Australia Ltd have therefore applied to have Standard A18 – Food Produced Using Gene Technology (Standard 1.5.2 of Volume 2 of the *Food Standards* Code) amended to include food from insect-protected cotton containing event 15985.

3. OBJECTIVES

The objectives, in addressing the issue of approving the sale and use of food from insect-protected cotton containing event 15985 are (in descending priority order):

- 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 fulfilling these objectives, ANZFA will also have regard to 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 cotton plant has been genetically modified to produce a naturally occurring insecticidal protein that is specific to Lepidopteran insects pests and is effective against the major pests of cotton. The insecticidal protein (CRY2Ab) is one from a family of proteins that are produced by the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*). *Btk* strains are widely used as biopesticides on a variety of cereal and vegetable crops. These proteins are commonly referred to as *Bt* proteins and cotton lines containing event 15985 produce the *Bt* protein CRY2Ab.

This cotton variety has been developed from a GM cotton line, the food from which was previously assessed by ANZFA under *Application A341 – Oil and linters derived from insect-protected cotton lines 531, 757 and 1076*. Insect-protected cotton containing event 15985 was developed from the GM cotton line 531, which already contains the *Bt* protein CRY1Ac. Cotton containing event 15985 therefore contains two *Bt* genes, the presence of the second gene conferring additional protection against the cotton pests. These two insecticidal proteins each exhibit a similar mode of action but interact with different receptor sites in the target insects thus conferring two avenues of protection against insect attack. The use of more than one of these *Bt* proteins may also reduce the development of resistance to *Bt* proteins.

The safety of the first genetic modification and associated new proteins will not be re-evaluated in this assessment. The Final Assessment (i.e. Inquiry) Report and Technical Report including the safety assessment of this parental GM line can be obtained from the ANZFA website (www.anzfa.gov.au).

Cotton is one of Australia's major agricultural crops with 461 900 hectares planted in 1999 (Cotton Yearbook 2000) and 484 000 hectares in the 2000-2001 season (CRDC, 2001). An increase in acreage of approximately 4% is expected in the 2001-2002 season. Of the acreage planted in 1999, approximately 25% (120 000 ha) was Ingard cotton (the food from which was approved as part of Application A341), including cotton line 531 from which the line in this Application was developed. Cotton is not grown in New Zealand.

Although oil and linters derived from the cotton lines assessed in Application A341 (i.e. lines 531, 757 and 1076) are approved for food use and are grown commercially in Australia, cotton containing insect-protected event 15985 (Bollgard II®) are currently not approved for commercial planting in either Australia or New Zealand. The Applicant has indicated that they intend to apply to the Office of the Gene Technology Regulator for commercial release approval in 2001.

Insect-protected cotton containing event 15985 is currently awaiting approval for commercial planting and for food use in the USA, Canada and Mexico. It is also undergoing assessment for feed and/or food use approval in Japan, the European Union, Argentina and South Africa.

5. ISSUES RELATED TO THIS APPLICATION

Safety assessment

Oil and linters derived from insect-protected cotton lines containing event 15985 have been evaluated according to the safety assessment guidelines prepared by ANZFA[1]. The assessment considered the following aspects: (1) the nature of the genetic modification; (2) general safety issues such as history of use and the potential for transfer of antibiotic resistance genes to microorganisms in the human digestive tract; (3) characterisation of novel proteins including toxicological and allergenicity issues; and (4) comparative analyses and nutritional impact of the food. On the basis of the submitted scientific data and other available information, ANZFA concluded that oil and linters derived from cotton containing event 15985 is as safe and wholesome as those produced from other commercial cotton varieties.

The full safety assessment report is at **Attachment 2** to this document.

Labelling of food derived from cotton containing event 15985

On 28 July 2000 the Ministerial Council agreed to a revised standard which requires labelling of food where novel DNA and/or protein is present in the final food and also where the food has altered characteristics. The revised standard (A18 in Volume 1 / 1.5.2 in Volume 2 of the *Food Standards Code*) came into effect on 7 December 2001.

Under these new provisions, oil and linters derived from insect-protected cotton containing event 15985 will require labelling if novel protein and/or novel DNA are present. However, as both food products from cotton are not be expected to contain any plant protein or DNA, due to their high degree of refinement, they may be exempt from labelling. No additional labelling subject to clause 7 is required.

[1] ANZFA (2001) Information for Applicants – Amending Standard A18/Standard 1.5.2 – Food Produced Using Gene Technology.

Issues arising from public submissions

General issues

The majority of submissions received in the first round of public comment raised matters of a general nature relating to gene technology or reiterated issues that were addressed in the safety assessment report (see **Attachment 3**) rather than any specific comment on the cotton variety in this Application. A discussion of some of these general issues, raised in connection with GM foods as a whole, is included at **Attachment 4**.

In light of rapid developments in the field of biotechnology in food production, the discussion of the general issues in Attachment 4 has been updated since consideration of previous applications to reflect more recent outcomes of intensive deliberations on gene technology issues in the international arena. This includes matters such as the publishing of the report of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on "New Biotechnology Food and Crops: Science, Safety and Society", and the deliberations of various international committees and taskforces including those of the Codex Alimentarius Commission, the OECD and FAO/WHO Expert Consultations.

Specific issues

The Authority received 57 submissions in response to the invitation for the first round of public comment on this Application. Although most of the submissions provided general comments relating to gene technology, several issues were identified to relate more specifically to this Application. These issues are addressed below.

(i) Two modifications of the same line

The National Council of Women of Australia raised concerns about engineering the cotton a second time to try and achieve the original objective of insect protection and that the original modification did not therefore achieve its objective.

Response

The purpose of the second modification was to introduce another mechanism into the cotton plant, of controlling insects and by doing so, potentially delaying the development of resistance to *Bt* proteins. The *Bt* gene introduced in the second modification (*cry*2Ab) gives rise to a different class of *Bt* protein, and as such, has a slightly different specificity to that produced from the gene introduced in the first modification (*cry*1Ac) in that the protein binds to different receptors in the insect midgut. The purpose of producing two different *Bt* proteins is twofold: to provide additional protection against insect attack and to delay the development of resistance to *Bt* pesticides. These are discussed further in the next response (*ii*).

(ii) Increase in the development of resistance to Bt

The National Council of Women of Australia were concerned that cotton plants containing two *Bt* genes would result in more *Bt* being used and increase the likelihood of resistance to *Bt*. Irmgard Habl was concerned that organic growers would lose the use of *Bt* pesticides as resistance to *Bt* develops due to GM crops.

Response

This is an issue that is frequently raised in submissions on *Bt* crops and is one that is assessed predominantly by the Office of the Gene Technology Regulator (OGTR) as well as other agencies such as the NRA and Environment Australia. ANZFA has notified the OGTR of the concern raised in submissions regarding environmental safety associated with the use of *Bt* crops. However, a brief response is given here.

The development of resistance to *Bt* is an ongoing issue for all agricultural sectors that use these pesticides, including growers of organic, conventional and GM crops. In Australia as well as in other countries, it is a requirement for insect resistance management plans to be implemented when *Bt* crops are grown. Such plans are designed specifically to minimise the build up of resistance in the pest population and take into consideration a range of factors (eg use of high doses and refuges, pest biology, ecology data, monitoring and surveillance and remedial action).

In this cotton line, the use of two *Bt* proteins is an attempt to delay and minimise the potential for resistance developing. The second *Bt* protein produced in cotton (CRY2Ab) has a different insect specificity to the *Bt* protein present in other cotton crops (CRY1Ac). This approach essentially harnesses the different specificities of each protein thus providing a two-pronged approach to controlling insects.

In the case of the *Bt* cotton varieties producing only one *Bt* protein, one mechanism for minimising the potential for development of resistance to *Bt* was to limit the acreage that could be planted to 30% of the total cotton crop. It is unlikely that such a cap will be required for Bollgard II cotton due to the presence of two *Bt* proteins, each having a different specificity.

(iii) Comparator for GM line

The National Council of Women of Australia sought clarification as to whether the cotton line will be compared to a GM parental line or a non-GM parental line.

Response

In the comparative analyses of cotton containing event 15985, the GM line was compared to both a non-GM parental control line as well as the GM parental control line. ANZFA's guidelines on the safety assessment of genetically modified food are based on the guidelines of international agencies such as the WHO/FAO and the CODEX Alimentarius Commission as well as the OECD Taskforce for Novel Foods and Feeds. Each of these bodies adheres to an underlying principle that the safety assessment of a genetically modified food includes the comparison between the genetically modified food and its closest *conventional or traditional* counterpart. The purpose of this comparison is to ensure that the genetically modified food is compared to one that has an acceptable level or benchmark for food safety in terms of a history of safe food use.

Accordingly, the levels of toxicants and nutrient parameters in cotton containing event 15985 are compared to both the non-genetically modified parental control line (DP50) and a GM parental control line (DP50B) as well as to several commercially available lines. These results are detailed in the safety assessment (Attachment 2).

(vi) New Zealand Royal Commission on Genetic Modification

The Environment and Conservation Organisation of New Zealand (ECO) and GE Free New Zealand considered that their submissions and issues raised at the New Zealand Royal Commission have been ignored.

Response

In September 2001, the New Zealand Royal Commission on Genetic Modification (http://www.genecommission.govt.nz/) reported their findings and concluded that the regulatory framework for the introduction of GM foods into the marketplace in New Zealand is 'appropriate' and that ANZFA, among other key institutions, carry out their functions 'conscientiously and soundly'. The Commission also stated "We have confidence in the ANZFA safety assessment process. We consider it unlikely that foods that have satisfied the food standard will have harmful effects", and "The Commission was reassured that ANZFA carries out its functions with an appropriate degree of independence not only from political influence but also from the influence of commercial interests."

In reaching this view, it should be noted that the Commission went to great lengths to gather, test and weigh up evidence and opinion from the wide diversity of relevant interest groups and experts including criticisms of ANZFA's processes in submissions such as those from ECO and GE Free New Zealand. Their conclusions provide support for ANZFA's approach in assessing the adequacy of the toxicological studies, use of substantial equivalence, sources and independence of data, antibiotic resistance marker genes etc. The Commission's final report is one that reflects a careful and balanced judgement of that material.

The Commission, among other international regulatory agencies and independent bodies regards the safety assessment process for GM foods established by ANZFA to be one of the most scientifically rigorous, comprehensive and transparent systems in place by any world standard.

6. RISK ANALYSIS

Under Standard A18 (Standard 1.5.2), a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines. On the basis of the conclusions of the safety assessment, together with a consideration of the public submissions, there are no public health and safety concerns associated with the use of insect-protected cotton containing event 15985. The labelling provisions of the standard require that foods derived from this line will be labelled if novel DNA or protein is present in the final food. However, as both products are highly processed and are unlikely to contain novel DNA and/or protein, they are likely to be exempt from labelling. No additional labelling subject to clause 7 is required.

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

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

In addition, in collaboration with Biotechnology Australia, ANZFA has produced an information pamphlet entitled *Genetically Modified Foods* that has been distributed throughout Australian supermarkets. Other government agencies such as the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand, and industry bodies are also addressing the broader concerns in relation to gene technology.

7. REGULATORY OPTIONS

Option 1 – no approval of insect-protected cotton containing event 15985
Maintain the *status quo* by not amending the *Food Standards Code* to approve the sale of oil and linters derived from insect-protected cotton containing event 15985.

Option 2 – approval of insect-protected cotton containing event 15985
Amend the *Food Standards Code,* as sought by the Applicant and approve the sale of oil and linters derived from insect-protected cotton containing event 15985, with or without listing special conditions in the Table to Clause 2.

8. IMPACT ANALYSIS

Affected parties
Parties affected by the options listed above include:

- consumers;
- Governments: State, Territory and New Zealand Health Departments, AQIS;
- industry: manufacturers, suppliers and importers and Australian cotton growers.

ANZFA is required, in the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, 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.

Option 1

Consumers: no impact
Consumers will not be exposed to this GM cotton as it is currently prohibited in foods.

Government: no immediate impact
Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Industry: Cost in terms of restricting innovation in food production for both growers and other sectors of food industry
Potential longer term impact; any successful WTO challenge has the potential to impact adversely on food industry.

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 food products including imported food products using this GM cotton. Benefit to community in general as cotton lines containing event 15985 are likely to result in significant reductions in insecticide usage (based on field trial data). Cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or having to pay more for non-GM food, in cases where this GM cotton is substituted for conventional cotton.
Government:	no direct impact. This decision is unlikely to impact on monitoring resources.
Industry:	Benefit to growers in reduced exposure to agricultural chemical (Ingard cotton has already resulted in a reduction of up to 47% of the insecticides used when compared to conventional cotton. This reduction is expected to be even greater with Bollgard II cotton). Benefit in lower production costs when growing GM cotton by reduced agricultural chemical usage. Benefit for manufacturers in that they have an expected source or extended choice of cotton food products. Benefit to importers as they can use products made overseas with this GM cotton.

After consideration of the regulatory impact for food (i.e. primarily oil but also linters) derived from insect-protected cotton containing event 15985 it is concluded that the benefits of option 2, in permitting these products, outweigh the potential benefits identified in option 1. The benefits primarily accrue to the grower and food industry. These may have a flow on indirect benefit to the community in terms of reduced use of agricultural chemicals. These benefits are also considered to outweigh the identified costs to consumers and industry, given that the mandatory safety assessment did not identify any public health and safety concerns.

9. CONSULTATION

Public consultation

The Initial Assessment (formerly referred to as the Preliminary Assessment Report) of this Application was advertised for public comment between 17 July 2001 and 29 August 2001. A total of 57 submissions were received and a summary of these is included in this report at Attachment 3.

ANZFA has now carried out an assessment of the Application, including a safety evaluation of the food, taking into account the comments received. This Draft Assessment Report is the second stage of the assessment by ANZFA and now invites comments from the public on the both the safety and draft assessment report.

Notification to the WTO

During the ANZFA assessment process, comments are also sought internationally from other Members of the World Trade Organization (WTO). As Members of the WTO, Australia and New Zealand are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreements). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

As there is significant international interest in the safety of GM foods, and the proposed amendments are likely to have a liberalizing effect on international trade, this Application will therefore be notified to the WTO under both as a potential TBT or SPS matter.

10. CONCLUSIONS

- based on the available information, there are no public health and safety concerns associated with the genetic modification used to produce insect-protected cotton containing event 15985;

- oil and linters derived from insect-protected cotton lines containing event 15985 are equivalent to those derived from other commercially available cotton varieties in terms of their safety for human consumption and nutritional adequacy;

- oil and linter products derived from lines containing cotton event 15985 are exempt from labelling unless it can be shown that novel DNA and/or protein is present in the final food;

- the benefits of permitting oil and linters from cotton containing event 15985 primarily accrue to industry and are considered to outweigh the costs to government, consumers and industry, provided the safety assessment does not identify any public health and safety concerns; and

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

11. RECOMMENDATION

Based on the data supplied with the Application and other available information, ANZFA concludes that oil and linters derived from cotton lines containing event 15985 are as safe for human consumption as those from other commercial cotton varieties, and therefore recommends that the Australian *Food Standards Code* and the *Australia New Zealand Food Standards Code* be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard A18 / Standard 1.5.2 is provided in **Attachment 1**.

ATTACHMENTS

1. Draft variations to the *Food Standards Code*
2. Safety assessment report
3. Summary of first and second round public submissions
4. General issues raised in public submissions

ATTACHMENT 1

DRAFT VARIATIONS TO THE *FOOD STANDARDS CODE*

To commence : On gazettal

[1] ***Standard A18*** *of Volume 1 of the Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2, *immediately after the last occurring entry -*

Oil and linters derived from insect-protected cotton lines containing event 15895

[2] ***Standard 1.5.2*** *of Volume 2 of the Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2*, immediately after the last occurring entry -*

Oil and linters derived from insect-protected cotton lines containing event 15895

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT REPORT

APPLICATION A436

INSECT PROTECTED COTTON CONTAINING EVENT 15985

SUMMARY AND CONCLUSIONS

A variety of cotton has been developed that contains an insecticidal protein derived from the common soil bacterium *Bacillus thuringiensis*. The introduction of this protein provides a new avenue of protection against attack from the major Lepidopteran pests of cotton. Insect-protected cotton containing event 15985 has been genetically modified for improved control of such insects and its benefits accrue primarily to the grower through the reduction in pesticide usage and the potential delay in the development of resistance.

Nature of the genetic modification

This cotton variety was developed from an already genetically modified cotton line, the oil and linters of which have been assessed by ANZFA and approved for food use in Australia and New Zealand. Oil and linters from cotton line 531 were approved in July 2000, as part of Application A341 – Insect-Protected Cotton also known as Ingard® cotton. This first modification resulted in the introduction of three genes. The primary gene of interest is the *cry*1Ac gene from *Bacillus thuringiensis* which confers insect protection to the cotton plant through expression of the CRY1Ac protein. Two antibiotic resistance genes (*npt*II and *aad*) were also transferred. Oil and linters from Ingard cotton were found to be as safe and wholesome as that from other commercially available cotton varieties.

In cotton containing event 15985, the subject of this Application, another insect protection trait has been introduced into the cotton plant by the addition of a second gene from *B. thuringiensis.* This gene encodes the CRY2Ab protein, an insecticidal protein that, like the CRY1Ac protein, is highly selective in controlling Lepidopteran insects. These two insecticidal proteins each exhibit a similar mode of action but interact with different receptor sites in the target insects. Thus, a plant producing both insecticidal proteins will have increased protection against insect attack.

In addition to the primary gene, event 15985 also contains a visual marker gene, the *uid*A gene. The protein product of this gene, β-glucuronidase (GUS), can be used to catalyse a colourimetric reaction resulting in the production of a blue colour in transformed cells. No additional antibiotic resistance marker genes were transferred into cotton containing event 15985, although it still retains the two original antibiotic resistance genes transferred in the first round of transformation to produce line 531.

Both the *cry2Ab* and the *uidA* genes are stably integrated into the cotton genome as a single segment of DNA and the insect protection trait is stably maintained from one generation to the next.

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. Cottonseed oil, the major product of cottonseed, has been consumed by humans for decades. Cottonseed oil is considered to be a premium quality oil, prized largely 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.

Characterisation of novel proteins

Cotton containing event 15985 produces two new proteins, CRY2Ab and GUS. The presence of these two proteins was detected in leaf and seed, however it is unlikely that the refined oil contains any of the novel proteins since oil contains negligible protein. Additionally, both proteins have been derived from sources that humans are potentially exposed to either in or on raw food commodities.

The absence of toxicity of both proteins has been confirmed through acute toxicity testing in mice, where no clinical signs of toxicity were observed. Furthermore, both proteins lack similarity to known allergens, are rapidly degraded in simulated digestive systems and occur at low levels in the seed. Thus, neither protein demonstrates any properties known to occur in protein toxins or food allergens and exposure to either protein through the consumption of cottonseed oil or linters is considered highly unlikely.

Comparative analysis

Detailed compositional analyses were done on both the cottonseed and more importantly, the refined oil. These studies establish the nutritional adequacy of the oil derived from cotton containing event 15985 and also demonstrate that unintended changes to the composition of the cotton plants had not occurred as a result of the second genetic modification of this line.

After assessment of the data, oil from cotton lines containing event 15985 was found to be compositionally similar to oil from other commercially available cotton varieties. In addition to the analysis on the food fraction, other components measured in the seed of cotton containing event 15985 were found to be similar to those measured from the parental or commercially available lines. Some statistically significant differences were observed between the test and control lines, particularly in the fatty acid profile of the seed (although these differences were not observed in the refined oil). These differences were small and in all cases, the values were within the ranges observed for commercially available lines. Thus, the differences were considered to reflect the variation expected in a natural environment and do not raise any concerns.

The levels of the naturally occurring toxins in cottonseed, cyclopropenoid fatty acids and gossypol, were assessed and compared to seed and refined oil from the control line. As confirmed in the analysis of the oil from cotton containing event 15985, refined cottonseed

oil is essentially free of gossypol but generally contains small amounts (typically <1.0%) of cyclopropenoid fatty acids.

The compositional analysis conducted on seed from cotton containing event 15985 demonstrates that the gossypol levels are similar to those of other commercially available cotton varieties. Some statistically significant differences between the test and control line were observed in the cyclopropenoid fatty acid levels of the seed. These differences were minor and in all cases, the values were within the ranges observed for other commercially available lines. The differences were therefore considered to be representative of the variation expected in a natural environment.
Given that the levels are similar to those in other varieties and that oil is refined to contain even lower levels of these toxins, the differences do not raise any food safety concerns.

Conclusion

Based on the available evidence, refined oil and linters from cotton containing event 15985 are compositionally similar to refined oil and linters from other commercially available cotton varieties in terms of their safety and nutritional adequacy. Oil and linters from cotton containing event 15985 are therefore considered suitable for human food use.

1. BACKGROUND DETAILS

Monsanto Australia Limited has submitted an application to ANZFA to vary Standard A18 of Volume 1 (Standard 1.5.2 of Volume 2) of the *Food Standards Code* to include oil and linters derived from an insect-protected cotton line containing event 15985, known commercially as BOLLGARD II®.

The insect-protected cotton has been genetically modified to produce a naturally occurring insecticidal protein that is specific to Lepidopteran pests. Such pests include *Helicoverpa punctigera*, *H. armigera* and *Heliothis virescens*, commonly known as the native budworm, cotton bollworm and budworm, respectively. The cotton plant described in this Application produces the CRY2Ab protein, which protects the plant from these major pests of cotton.

The CRY2Ab protein is one of a family of proteins that are produced by the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* and are commonly referred to as *Bt* proteins. These proteins are widely used in bio-pesticidal formulations on a variety of crops. Plants that express *Bt* proteins are protected against insect attack. The use of more than one of these *Bt* proteins may also reduce the development of resistance to *Bt* proteins.

This cotton plant has been developed from an approved GM cotton line that was previously assessed by ANZFA under *Application A341 – Oil and linters derived from insect-protected cotton lines 531, 757 and 1076*. Insect-protected cotton containing event 15985 was developed from the GM cotton line 531, which already contains a different *Bt* protein, CRY1Ac. Cotton containing event 15985 thus contains two *Bt* genes, the presence of the second gene conferring additional protection against the cotton bollworm. Each of the *Bt* proteins are structurally and functionally similar, but bind to different receptors in the midgut of the insect therefore conferring two avenues of protection against insect attack.

The safety of the genes introduced in the first modification and their protein products will not be reassessed in this assessment. The Technical Report for Ingard® Cotton lines 531, 757 and

1076, which includes the safety assessment of this parental GM line (i.e. event 531) can be obtained from the ANZFA website (www.anzfa.gov.au).

2. HISTORY OF USE

Cotton (*Gossypium hirsutum* L.) is grown worldwide, 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. 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 a 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 a 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, 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 USA, 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 USA 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 United States, provided it meets certain specifications for gossypol content, although no products are currently being produced.

In Australia, cotton was planted on 461 900 hectares in 1999 (Cotton Yearbook 2000) and 484 000 hectares in 2000-2001 season (CRDC, 2001) with an expected increase in acreage of approximately 4% in the 2001-2002 season. Of this, approximately 25% (120 000 ha) of the 1999 season was Ingard cotton, which includes cotton line 531 from which the line in this Application was developed. Cotton is not grown in New Zealand.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Methods used in the genetic modification

Studies submitted: Doherty, S, KA Hamilton, RP Lirette and I Borovkova. Amended Report for Molecular characterisation of BOLLGARD II Cotton Event 15985 and Molecular analysis of the stability of Cotton Event 15985. Monsanto Laboratory Project 99-01-36-04, MSL – 16620, April 2000.

Doherty S, DW Mittanck and RP Lirette. Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the *cry*2Ab2 insert in Bollgard II cotton event 15985. Monsanto Laboratory Project 00-01-36-28, MSL – 17099, completed January 2001.

Doherty S , DW Mittanck and RP Lirette. Demonstration by PCR that DNA sequences flanking the *cry*2Ab2 insert in Bollgard II cotton event 15985 are native to the cotton genome. Monsanto Laboratory Project 00-01-36-29, MSL – 17111, completed January 2001.

Bannon GA, M Ahibhai, R McCoy, A Reed, A Silvanovich and JD Astwood. 2002. Executive summary: safety assessment of β–glucuronidase E377K in Bollgard II cotton. Monsanto Co.

Bannon GA, M Ahibhai, R McCoy, A Reed, A Silvanovich and JD Astwood. 2002. Safety assessment of GUS E377K in Bollgard II cotton. Monsanto Technical Report, MSL – 17618.

Pineda NG, DW Mittanck, TA Cavato and RP Lirette. 2002. PCR and DNA Sequence analysis of the insert in Bollgard II cotton event 15985. Monsanto Technical Report, MSL – 17146.

Insect-protected cotton containing event 15985 was generated by the transfer of DNA into the genome of GM insect-protected cotton line 531 (i.e. a Delta and Pine Land Company commercial cotton variety containing event 531). Using the particle acceleration method of transformation, DNA was introduced into cotton meristems. This method of transformation allows for the selection and transfer of a specific segment of plasmid DNA to be incorporated into the genome of the cotton plant.
The specific segment of DNA from plasmid PV-GHBK11 has been purified by high pressure liquid chromatography (HPLC) and contains the gene of interest, the *cry*2Ab gene, together with its essential controlling elements (Figure 1, part A).

The introduced DNA also contained the *uid*A gene, which encodes the enzyme β-glucuronidase (GUS). GUS is a scorable marker that enables the selection of transformed plant tissue. Tissues containing the introduced DNA were detected by histochemical staining for the GUS protein. Non-transformed tissue was subsequently removed over time thus promoting growth only of meristems containing the introduced DNA.

3.2 Function and regulation of the introduced genes

The HPLC-purified fragment of DNA was approximately six kilobases and consisted of two adjacent gene cassettes, each containing separate controlling DNA elements essential for expression in the cotton plant cells. The first cassette contains a copy of the *cry*2Ab gene and the second gene cassette contains the colourimetric *uid*A marker gene. The purified segment of DNA transferred to the cotton genome does not contain any antibiotic resistance genes or bacterial origin of replication sequences.

Both of the *cry2*Ab and *uid*A genes are under the regulation of the enhanced cauliflower mosaic virus 35S promoter (e35S) and the 3'untranslated region of the nopaline synthase gene (NOS 3') for signal polyadenylation. The e35S promoter of the *cry*2Ab gene is also

fused to the 5' untranslated leader sequence from the petunia heat shock protein 70 (HSP70) and the chloroplast transit peptide from the *Arabidopsis thaliana* 5–enolpyruvyl shikimate–3–phosphate synthase (EPSPS) gene (CTP2) which is used to direct the protein to the green tissues of the plant.

Plasmid PV-GHBK11 contained other bacterial genes and controlling sequences for selection and replication in the laboratory. These sequences were not contained within the purified fragment used in the transformation and therefore are not present in the plant.

3.2.1 **cry2*Ab gene***

The *cry*2Ab is isolated from *B. thuringiensis* subsp. *kurstaki* (*Btk*). Different strains of *B. thuringiensis* have been shown to produce crystalline proteins (referred to as Cry or *Bt* proteins or delta-endotoxins), which are toxic to certain orders and species of insect pests. These proteins are produced as the bacterium enters the sporulation phase and can account for approximately one-third of the weight of the bacterial cell. The *Bt* proteins exhibit a complex, multi-component mode of action (English and Slatin, 1992). All *Bt* proteins tend to have a similar mode of action, whereby the proteins bind to specific receptors in the midgut epithelium cells of susceptible insects, opening cation-selective channels in the cell membrane. The cells swell due to an influx of ions and water, leading to lysis of the cells and eventually, death of the insect (Höfte and Whiteley, 1989).

The *cry*2Ab gene naturally occurs in *B. thuringiensis* subsp. *kurstaki* but is either not expressed or has low expression. The lack of expression in *B. thuringiensis* is due to an inefficient promoter (Dankocsik et al, 1990). The native *cry*2Ab gene in *B. thuringiensis* is thus a pseudogene, i.e. a gene that is very similar to a real or functional gene. It is very similar to the *cry*2Aa gene found in *Btk* (Donovan et al, 1988, 1989), but lacks similar regulatory sequences such as an effective promoter that are essential for gene expression. Due to the lack of expression in *Btk*, the *cry2*Ab pseudogene was cloned into a bacterial expression vector to improve production of this protein in *B. thuringiensis* and was subsequently fully characterised (Dankocsik et al, 1990; Widner and Whiteley, 1990). The gene was then re-cloned, introduced into a crystal-negative strain of *Bt* (i.e. one that doesn't produce any crystal proteins) and designated *Bt* strain EG7699. This approach ensured the expression of only CRY2Ab in the *Bt* strain. This gene sequence, together with a functional promoter (e35S) has been introduced into insect-protected cotton event 15895.

The DNA elements associated with the *cry2*Ab gene expression cassette are detailed in the table below.

Genetic element	**Source**	**Size (kb)**	**Function**
e35S	Cauliflower mosaic virus	0.6	The cauliflower mosaic virus (CaMV) promoter (Odell *et al*., 1985) with the duplicated enhancer region to drive expression.
PetHSP70-leader	Petunia	0.1	The heat shock protein 70 gene 5' untranslated leader sequence
CTP2	Arabidopsis thaliana	0.2	The N-terminal chloroplast transit peptide, isolated from the *Arabidopsis thaliana* EPSPS gene (Van den Broeck et al, 1985).
*cry*2Ab	*Bacillus thuringiensis* subspecies *kurstaki*	1.9	The synthetic *cry*2Ab based on sequence from *B. thuringiensis* (Widner and Whiteley, 1990) which confers insect protection.
NOS 3'	*Agrobacterium*	0.3	A 3' untranslated region of the nopaline

	tumefaciens		synthase gene from the T-DNA which ends transcription and directs polyadenylation of the mRNA (Fraley *et al*, 1983)

The gene encoding the CRY2Ab protein was engineered for plant expression by being completely re-synthesised to substitute the existing bacteria-preferred codons with plant-preferred codons, similar to previously described modifications on other *Bt* proteins (Perlak *et al* 1991). The synthetic *cry*2Ab gene expresses a protein that is 97% similar to the CRY2Aa protein produced in *Btk* and is present in various biopesticide formulations (e.g. Dipel, Cutlass OF, Crymax).

Chloroplast transit peptide

Transit peptides facilitate the intracellular transport of proteins between the different cellular compartments in the cell, e.g. between the cytoplasm and the chloroplast. The CTP is typically cleaved from the mature protein on uptake into the chloroplast, and then rapidly degraded.

The *cry*2Ab coding sequence is fused to a chloroplast transit peptide (CTP2) isolated from *Arabidopsis thaliana* EPSPS. The purpose of the CTP is to direct the new protein to the chloroplast, which is generally green leaf tissue – tissue most likely to be subject to insect attack.

3.2.2 **uidA** *gene*

The *uid*A gene is a marker gene that can be used to indicate if a plant cell has been transformed. It encodes the β-D-glucuronidase (GUS) protein and is derived from *Escherichia coli* strain K12 (Jefferson et al, 1986). *E. coli* is widely found in the environment and in the digestive systems of vertebrates, including humans.
The *uid*A gene is also known as the *gus* or *gus*A gene. The GUS protein is an enzyme that catalyses the hydrolysis of a range of chromogenic compounds permitting its use as a visible marker in plant transformation processes (Jefferson et al, 1987). The DNA sequence has been fully characterised and is available in the publicly accessible database, GenBank (Jefferson et al, 1986; Schlaman et al, 1994).

A summary of the DNA components associated with the *uid*A gene is given in the table below.

Genetic element	Source	Size (kb)	Function
e35S	Cauliflower mosaic virus	0.4	As above
*uid*A	*Escherichia coli* strain K12	1.8	Encodes for the β-D-glucuronidase (GUS) enzyme, which is used as a visible marker (Jefferson et al, 1986)
NOS 3’	*Agrobacterium tumefaciens*	0.3	As above

This gene and the organism from which it was derived have been considered by ANZFA in a previous assessment (A378 – Glyphosate-tolerant sugarbeet) and found not to pose a risk to human health and safety.

As both introduced genes are under the control of the constitutive e35S promoter, expression is expected in all parts of the plant. Thus, this cotton variety is likely to be protected against insect attack throughout the plant.

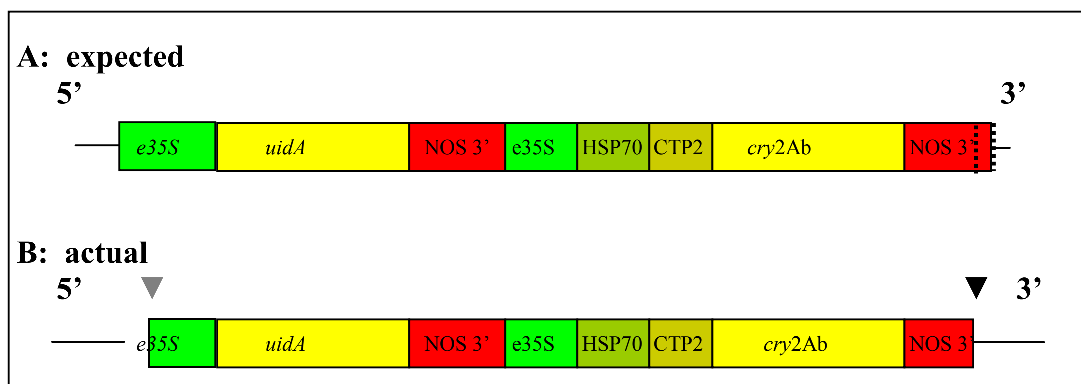
3.4 Characterisation of the genes in the plant

A range of molecular tools, including Southern blot and PCR analyses were used to characterise the inserted DNA in cotton containing event 15985. Genomic plant DNA was analysed using Southern blot analysis to determine the insert and copy number, the integrity of coding regions of both genes and their respective regulatory sequences as well as confirming the absence of any of the backbone sequences derived from plasmid PV-GHBK11. Polymerase chain reaction (PCR) and sequence analysis was used to verify the sequences at the 5' and 3'ends of the inserted DNA segment at the junctions where it integrates into the plant DNA. The Applicant has also provided full sequence data of the inserted DNA, which permit confirmation of the exact nature of the DNA insert.

DNA was extracted from leaf tissue of the test cotton containing event 15985, the parental line DP50B (which contains the *cry*1Ac event 531 but is negative for *cry*2Ab) and a negative or non-transgenic isogenic control cotton line DP50. The leaf tissue was taken from R3 plants grown in the glasshouse under normal growing conditions.

The data from the molecular analyses are consistent with a single DNA insertion from PV-GHBK11 plasmid into the genome of cotton containing event 15985 as shown in Figure 1.

Figure 1. Schematic representation of the plasmid DNA introduced into cotton event 15985.



Additional Southern blot analyses involved digesting genomic DNA with a range a restriction enzymes and probing with each individual element present on the plasmid (i.e. *cry*2Ab coding region, *uid*A coding region, e35S promoter, NOS 3' polyadenylation sequence and plasmid backbone fragments). These molecular analyses support the conclusions that only one insert from plasmid PV-GHBK11 is present in the cotton plant and that within this insert, there is only one copy of each of the *cry*2Ab and *uid*A gene cassettes. Additionally, analyses using the plasmid backbone sequences as a probe, support the conclusion that cotton containing event 15985 does not contain any detectable backbone from plasmid PV-GHBK11 and therefore no antibiotic resistance marker genes were transferred in this transformation round.

The Southern blot analyses also confirm the presence of event 531 (i.e. the first round modification). The data indicate that the genes in the insert are in the same orientation and

order as described previously by the Applicant. Therefore, event 531 appears physically stable over multiple generations and through more than eight seasons of commercial Ingard cotton production.

3.4.1 **cry***2Ab cassette integrity*

Results from Southern blot analyses indicate that the *cry*2Ab coding region and the gene cassette are complete. However, a restriction site following the NOS 3' polyadenylation sequence in the cassette is not present (Figure 1, part B). PCR and DNA sequencing analyses on genomic DNA from cotton containing event 15985 verified the exact nucleotide sequence at the 5' and 3' ends of the newly inserted segment.

These data indicate there is a 66 bp deletion at the 3' end of the gene cassette, which does not include any of the NOS 3' polyadenylation sequence. These deleted nucleotides are polylinker DNA (i.e. restrictions sites in cloning vectors used for the insertion of DNA fragments) and are not associated with the function of the *cry*2Ab gene cassette (Figure 1, part B, denoted as ▼).

*3.4.2 uid***A** *cassette integrity*

The Southern blot analysis of the *uid*A gene cassette indicated that the *uid*A coding region and the NOS 3' polyadenylation sequence in the cassette are complete. However, part of the enhanced CaMV 35S promoter has been truncated. The junction between the plant genomic DNA and the 5' end of the insert (determined by genome walking) was verified by PCR and sequence analysis.
These results establish that the *uid*A cassette is missing approximately 260 bp of the 5' promoter sequence (i.e. ~40%) and 24 bp of polylinker DNA (Figure 1, part B, denoted as ▼). It has previously been shown that such a deletion should not affect accurate transcription initiation (Odell et al, 1985) and as expected, despite the truncation, the e35S promoter is still functional as demonstrated by the production of the GUS protein.

3.4.3 DNA sequence of the insert

In addition to these analyses, overlapping PCR products were generated that spanned the length of the entire DNA insert. These PCR products were subsequently sequenced in order to obtain the sequence of the entire DNA insert within cotton containing event 15895. These data confirmed the exact nature of the insert, the gene sequences and the order and orientation of each element within the insert as determined by the Southern blot and PCR analyses. The sequence analyses also define the 5' and 3' ends of the insertion and provide plant genomic sequence extending to approximately 200 nucleotides upstream and 869 nucleotides downstream of the introduced DNA.

This sequence analysis has also shown that the plant-expressed version of the *uid*A gene encodes a single amino acid change relative to the protein produced by *E. coli* fermentation (discussed under characterisation of protein).

3.5 Stability of the genetic changes

To determine if the transferred genes are stable across generations, a series of tests were conducted with the progeny. Segregation data across four generations, including two generations backcrossed to commercial cotton cultivars (R1, R2, BC1F1 and BC2F2), were statistically analysed, comparing the frequency of observed to expected numbers of progeny

that expressed the CRY2Ab protein. Expression of CRY2Ab was assessed by enzyme linked immunosorbent assay (ELISA). Presence of the *cry*2Ab gene was confirmed using Southern blot analysis.

All generations segregated as expected for a single insertion site of the *cry*2Ab gene into cotton genomic DNA (Table 1). The presence of the desired trait (i.e. the expression of CRY2Ab) across multiple generations occurred as expected, consistent with a Mendelian pattern of inheritance.

The stability of the insert was also demonstrated using Southern blot analysis where genomic DNA samples from leaf tissues across five generations and control samples were digested with restriction enzymes and probed with the *cry*2Ab coding region. Samples were taken from the R1, R3 and R4 generations and two different varieties that had undergone four generations of back crossing (BC2F3 plants).

There were no differences in the pattern of hybridising fragments among the DNA extracted from any of the five plant generations. As expected, the two control lines showed no detectable hybridisation bands in the Southern blot analysis. These results demonstrate that the DNA insert from plasmid PV-GHBK11 is stable in the plant genome across five breeding generations. Additionally, the expression of CRY2Ab is consistent across the same generations (see Section 4.2).

Table 1: Segregation data and analysis of progeny of cotton event 15985

	Expected		**Observed[1]**		
Generation[2]	**Positive**	**Negative**	**Positive**	**Negative**	**ChiSq**
R1 (3:1)	202.5	67.5	210	60	1.11[ns]
R2 (3:1)	45	15	43	17	0.356[ns]
BC1F1 (1:1)	199	199	213	185	1.970[ns]
BC2F2 (3:1)	568	189	549	208	2.477[ns]

[1] Data expressed as number of plants that are positive or negative for Cry2Ab based on qualitative ELISA.
[2] R1 seed was from the initial R0 transformant in a DP50B background. R2 seed was pooled from heterozygous R1 plants in a DP50B background. BC1F1 and BC2F2 plants were pooled from five different elite cultivar backgrounds.
[ns] not significant at p=0.05 (chi square = 3.84, 1 df)

3.6 Impact on human health of the potential transfer of novel genetic material to cells of the human digestive tract

The human health considerations in relation to the potential for horizontal gene transfer depend on the nature of the novel genes and must be assessed on a case-by case basis.

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

The major concern in relation to the potential transfer of novel genetic material to cells in the human digestive tract is with antibiotic resistance genes. Antibiotic resistance genes can be

[3] Food and Agriculture Organization.

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

In this Application, the transformation process used a specific segment of plasmid DNA for insertion into the plant cells from which cotton containing event 15985 was subsequently generated. The DNA segment corresponded only to the gene of interest and a visual marker gene (*gus*) along with their associated controlling elements. No additional antibiotic resistance marker genes were introduced into this plant line as a result of the second modification. It still retains two antibiotic resistance genes that were introduced in the first modification of the cotton plant.

In cotton containing event 15985, the marker gene that enabled the identification and selection of transformed plant cells is a colour-based trait – the presence of the GUS protein. The presence of the *gus* gene in cotton containing 15985 does not raise any concerns for horizontal gene transfer because it is widespread in nature, including in bacteria that inhabit the human gut and it raises no concerns for human health and safety.

Conclusion

Insect-protected cotton containing event 15985 was produced using the particle acceleration method. A range of molecular techniques were used to fully characterise cotton event containing 15985 including Southern blot hybridisations, PCR and DNA sequence analyses. These analyses indicate that the transformation process has resulted in a single insertion event, comprising one complete copy each of the *cry*2Ab and *uid*A gene cassettes. Both the 5' and 3' ends of the insert have small truncations but these have not affected the coding regions of either of the genes or the functionality of their regulatory elements. The data also support that the inserted DNA is physically stable and is inherited in a predictable manner over multiple generations. No additional antibiotic resistance marker genes were transferred during this round of plant transformation. The line does contain two antibiotic resistance genes from the previous transformation, which were not considered to pose any additional safety concerns, especially since refined oil and linters are essentially free of genetic material.

4. CHARACTERISATION OF NOVEL PROTEIN

4.1 Biochemical function and phenotypic effects

4.1.1 CRY2Ab

The CRY2Ab protein (also known as CRY2Ab2, CryIIAb, CryIIB or CryB2) is a protein of 633 amino acids (with the CTP2 transit peptide) and has a molecular mass of 71 kDa (Widner and Whiteley, 1990; Dankocsik et al, 1990). Although the gene is present in *B. thuringiensis*, CRY2Ab isn't expressed in the bacterium because of an inefficient promoter. Protein characterisation studies to determine its properties and insecticidal activity were therefore done on the protein produced in expression vector *Bt* strain EG7699 and the full protein sequence is available (GenBank Accession No. X55416). With the exception of an additional amino acid introduced to create a restriction enzyme cleavage site for cloning

purposes, the sequence of the *cry*2Ab gene transferred into event 15985 is identical to that found in *B. thuringiensis*.

Both the synthetic CRY2Ab protein produced by the expression vector and the version produced by cotton event 15985 have 88% amino acid sequence identity and 97% amino acid sequence similarity to another *Bt* protein, the CRY2A protein which is naturally present in *B. thuringiensis*[4].

CRY2Ab and CRY2A are in the same class, the proteins of which have selectivity for Lepidopteran and Dipteran insects. The CRY1 class of proteins, of which CRY1Ac belongs, are toxic to only Lepidopterans. Thus, both of the CRY2 proteins have more similar insecticidal activity and selectivity, than for a protein from a different class, such as CRY1Ac. CRY1Ac, which was introduced in the first round modification (event 531), is different to the CRY2 proteins, in that it targets a different receptor in the insect midgut (Dankocsik et al, 1990). Together, the two *Bt* proteins provide a broader range of protection against insect attack as well as potentially delaying the development of resistance in the insect.

4.1.2 GUS

The *uidA* gene inserted into cotton containing event 15985 is derived from *E. coli* and encodes a single β-glucuronidase or GUS protein of 603 amino acids with a molecular weight of 68 kDa. GUS catalyses the hydrolysis of a wide range of glycosides including the synthetic substrate p-nitrophenyl-β-D-glucuronide. Hydrolysis of this chromogenic compound produces a blue colour that has proved a versatile visual marker in a range of plant transformation systems (Jefferson *et al.* 1987).

The history of safe use of GUS is extensive. GUS is naturally present in a wide range of microbes, animals and plants including may human food sources (Schulz and Weissenbock, 1987; Wozniak and Owens, 1994; Hodal et al, 1992). GUS activity has been detected in over 50 plant species in several tissues including embryo, fruit, seed coat and endosperm (Hu et al, 1990). Even when ingested in raw commodities such as apple or shellfish, GUS is not known to cause harmful effects and nor are the metabolites of *E. coli* GUS activity toxic (Gilissen et al, 1998). Human exposure through intestinal epithelial cells and intestinal microflora, bacterial exposure and numerous foods containing the GUS protein, has not been associated with any known harmful effects (Gilissen et al, 1998).

The GUS enzyme expressed in cotton containing event 15985 is 99.7% homologous and functionally equivalent to the GUS enzyme from *E. coli* naturally present in the human gut. The single amino acid difference between the plant-expressed and bacterially-expressed version of the protein accounts for 0.1% of the difference. The remaining difference (0.2%) is due to the addition of a restriction site at the beginning of the sequence for plant transformation purposes. Therefore the GUS produced in cotton containing event 15985 is considered to be equivalent to the protein commonly encountered in the human diet.

[4] Sequence identity refers to identical amino acid residues and sequence similarity refers to residues that are either identical or are conservative substitutions (i.e. those with similar physical and/or chemical properties).

4.2 Protein expression analyses

Studies using western blot (immunoblotting) and ELISA analysis were used to characterise the expressed proteins and to determine their level of expression in cotton containing event 15985.

An additional aspect of this study was to determine if the second modification (i.e. event 15985 - the introduction of the *cry*2Ab and *uid*A genes) affected the mean expression level of the proteins produced by the first round modification (i.e. event 531 – the CRY1Ac and NPTII proteins).

4.2.1 In planta expression of cry2Ab

CRY2Ab

Studies submitted:
D Kolwyck, KA Hamilton and A Reed. 2000. Amended Report for MSL-16081: Protein levels in Insect Protected Cotton Samples Produced in the 1998 US Field Trials. Monsanto Technical Report MSL-16612, Study 98-01-36-05, St Louis, Missouri

D Kolwyck, KA Hamilton, and A Reed. 2001. Amended Report for MSL-16081: Protein levels in Insect Protected Cotton Samples Produced in the 1998 US Field Trials. Monsanto Technical Report MSL-16612, St. Louis, Missouri.

JT Bookout, KA Hamilton, RP Lirette. 2001. Demonstration of the Presence of the Cry2Ab2 protein produced in Multiple Generations of Bollgard II Cotton Event 15985. Monsanto Technical Report MSL-17112, Study 01-01-36-01, St Louis, Missouri.

The expression of CRY2Ab in leaf tissue from five generations - R1, R3, R4 and two BC2F3 varieties (the same generations as discussed in *Section 3.5 Stability of Insert*) was confirmed by western blot analysis. Protein extracts from cotton leaf tissue were tested with an antibody raised in rabbits against *Bt*-produced CRY2Ab protein. Leaf protein extracts as well as the positive control (CRY2Ab protein purified from *Bt* strain EG7699) showed the expected immunoreaction with a protein of molecular weight of approximately 63 kDa (which is smaller than the 71 kDa predicted due to the cleavage of the CTP transit peptide on import to the plastid). The two control lines, DP50B (containing event 531) and the non-transformed DP50 were also assessed in this study and as expected, no immunoreactive proteins were detected in the two control lines

These results confirm that the *Bt*-produced CRY2Ab protein and the CRY2Ab expressed by cotton containing event 15985 are equivalent, based on identical electrophoretic mobility and detection using specific antibodies as well as lacking detectable post-translation modification. The bacterially produced protein is therefore an acceptable substitute for the plant-produced version for safety studies. Both proteins were also shown to have similar functional (i.e. insecticidal) activity.

GUS and GUSE377K

As discussed earlier, DNA sequence analysis of the insert in cotton containing event 15985 has shown that the *uid*A gene in the cotton plant encodes a GUS protein with a single amino acid change compared to the *E. coli* version. This protein is referred to as GUS E377K indicating the substitution of glutamic acid (E) with a lysine (K) at position 377. The

Applicant has submitted a number of studies that assesses the potential impact of this amino acid substitution on the protein.

Amino acid and protein structure comparisons of GUS proteins for more than 100 protein sequences in the NCBI database showed that β-glucuronidases can vary widely in their primary sequence (between 33-99.8% sequence identity), but that individual residues and signature stretches of amino acids are highly conserved. Conserved residues usually play an important role in structure - function properties of the protein. The altered amino acid in cotton containing event 15985 is not near the active site of the protein and nor is it in a conserved region including sections of known secondary structure (i.e. α–helix or β–sheet).

Using 3-D modelling, it can be concluded that the amino acid substitution does not confer any significant structural or functional changes in the GUS protein produced in cotton containing event 15985. The three dimensional structure of the *E. coli* GUS protein has been modelled to the 2.4 Å 3-D X-ray crystal coordinates of the human GUS protein (Jain et al, 1996; Matsumara et al, 1999). When the single amino acid change in the GUS E377K protein is introduced into the model, there is no effect on the active site and no significant impact on overall 3-D structure of the protein.

Given the wide divergence in amino acid sequence of GUS proteins and that structure-function relationships do not appear to be affected by the single amino acid substitution, it can be concluded that the GUS E377K protein is equivalent to the GUS protein in terms of physico-chemical properties and activity. Thus, the range of studies done on the GUS protein to assess for potential toxicity and allergenicity are also relevant to the safety of the variant GUS protein, given that they are 99.7% similar.

4.2.2 Protein expression levels

Levels of the CRY2Ab and GUS proteins were estimated in a range of tissue samples collected from eight field locations in 1998. These locations are representative of the environmental and other site differences under which cotton is grown in the USA. Such studies permit an exploration of the potential differences in protein expression that may arise due to environmental factors. Samples were collected from the test line (15985), the parental control line (DP50B) and the non-transformed control line (DP50) and analysed using ELISA to estimate the levels of the CRY2Ab and GUS protein activity.

CRY2Ab and GUS levels estimated in leaf and seed tissues are summarised in Table 2. The levels of CRY2Ab are also given for whole plant and pollen tissue. The levels of the novel proteins in the parental control (DP50B) and non-transformed control (DP50) samples were assessed and were at the limit of detection (LOD) for both the CRY2Ab (2.65 µg/g fw in leaf; 2.31 µg/g fw in seed; 1.24 µg/g fw in whole plant; and 0.25 µg/g fw in pollen tissue) and GUS assays (0.91 µg/g fw in leaf tissue and 4.42 µg/g fw in seed tissue).

As expected, CRY2Ab is detected at low levels in various plant tissues (leaf, seed and whole plant), consistent with the use of promoters that drive constitutive gene expression. The mean CRY2Ab and GUS protein levels in cottonseed from event 15985 were comparable across sites indicating that the novel proteins have a similar level of expression across geographically dispersed sites. The levels of CRY2Ab in cottonseed across all locations ranged from 31.8-50.7 µg/g fresh weight with an average of 43.2 ± 5.7 µg/g fresh weight. The levels of GUS in cottonseed across all locations ranged from 37.2-82.3 µg/g fresh weight

with an average of 58.8 ± 13.0µg/g fresh weight. CRY2Ab and GUS are therefore present at low levels, representing less than 0.004% and 0.007% dry weight in the seed, respectively.

Table 2. Mean levels of CRY2Ab and GUS protein in cotton event 15985[1] (µg/g fresh weight).

Cotton Line	CRY2Ab	GUS
Leaf[2]		
15985	23.8 ± 6.3 (10.1-33.3)	106 ± 32 (51.7-176)
DP50B	<2.65	<0.91
DP50	<2.65	<0.91
Seed[2]		
15985	43.2 ± 5.7 (31.8-50.7)	58.8 ± 13.0 (37.2-82.3)
DP50B	<2.31	<4.54
DP50	<2.31	<4.42
Whole plant[3]		
15985	8.80 ± 1.20 (7.28-10.46)	-
DP50B	<1.24	-
DP50	<1.24	-
Pollen[3]		
15985	<0.25	-
DP50B	<0.25	-
DP50	<0.25	-

[1] Mean and standard deviation were calculated from samples, one from each of 8 field sites except for tissues collected from a single site. Range is minimum and maximum value from samples across sites.
[2] leaf tissue n= up to 6 plants/plot from each site, 8 sites, taken at 4-6 weeks post planting. Bulk seed samples were collected for each line from each plot.
[3] The sample of whole plant and pollen was up to 16 plants per line; dashes (-) indicate that these tissues were not tested for the GUS protein.

The mean levels of the two expressed proteins, CRY1Ac and NPTII introduced in the original modification (i.e. event 531) were also assessed in young leaf tissue and seed samples using ELISA analysis. The levels of these proteins were similar in either the presence (i.e. event 15985) or absence (i.e. DP50B) of the second DNA insert (*cry*2Ab and uidA gene cassettes) and their respective gene products consistent with published reports (Greenplate et al, 2000; Penn et al, 2001). This indicates that the second modification (i.e. event 15985) did not appear to affect the level of CRY1Ac or NPTII protein expression (data not shown). Additionally, the AAD protein was not detected in any of the samples analysed, consistent with data presented as part of the assessment of cotton event 531 in Application A341.

As predicted, no novel proteins from either modification, i.e. the CRY1Ac, neomycin phosphotransferase II (NPTII), and aminoglycoside adenylyltransferase (AAD) as well as CRY2Ab and GUS were detected in the non-transgenic control line (DP50) and no novel proteins from the second modification (CRY2Ab and GUS) were detected in the parental control line DP50B.

4.3 Potential toxicity of novel proteins

The protein expression analyses demonstrated that CRY2Ab and GUS are both present in cotton containing event 15985. Although they are present in the seed, exposure to these proteins from oil and linters is not expected since processing removes or denatures protein. However, the results of a series of tests to confirm the safety of these proteins are presented in this section.

(i) History of Bt safety

B. thuringiensis strains have been used commercially to produce microbially-derived products with insecticidal activity. Such products have been assessed by a range of regulatory authorities, including, in Australia, the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) and overseas agencies (US EPA), which concluded that use of registered *Bt* products pose no significant risks to human health and safety (EPA, 2001). Four *Bt* proteins have been considered by ANZFA in eight applications and no concerns with respect to food safety have been raised. These four *Bt* proteins are CRY 2A[5], CRY1Ac[6] , CRY1Ab[7] and CRY3Aa[8]). Food derived from these insect protected varieties of corn, cotton and potatoes are now approved in Australia and New Zealand under Standard A18 – Food Produced Using Gene Technology in Volume 1 (Standard 1.5.2 in Volume 2) of the *Food Standards Code*.

Bt is not pathogenic to humans, animals or non-target organisms. Mammalian species are not susceptible to *Bt* proteins, including the CRY2Ab protein, which has a narrow range of activity against certain lepidopteran (moths and butterflies) insect pests (Bravo 1997). This lack of toxicity to non-target species is in part, due to the lack of appropriate conditions in mammals and most invertebrates required to activate *Bt* proteins (English and Slatin, 1992). No receptors for *Bt* proteins have been identified on intestinal cells of mammals such as rats and rabbits (Sacchi et al, 1986; Hofman et al, 1988).

Bt microbial formulations have been applied to raw agricultural commodities for decades. Humans consume these commodities, often unprocessed, with the potential for exposure to both the *Bt* microbes and their insecticidal *Bt* proteins. As *Bt* is a common soil microbe, exposure to natural populations is likely and has been shown to occur. No adverse effects on human health have been reported when *Bt* is present in either drinking water or food (IPCS, 1999).

The low mammalian toxicity of *Bt* microbial insecticide mixtures has been well demonstrated in numerous safety studies (Sjoblad et al, 1992; EPA, 1998). A range of commercial *Bt* products (Dipel®, Crymax® and Cutlass OF®[9]) have been tested in acute, subchronic and chronic toxicity studies with rats, rabbits, sheep and humans[10]. The highest doses administered to animals in these studies produced no observable effects, consistent with the absence of toxicity of other *Bt* proteins when fed to mammals at high doses. These studies are considered supporting evidence of the safety of CRY2Ab.

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[5] A341 - insect protected cotton, but this line was withdrawn by Applicant
[6] A341 –insect protected cotton
[7] A346 – insect protected corn - Yieldgard, A386 – Bt-11 corn and A385 – Bt-176 corn
[8] A382, A383 and A385 - three different genetically modified potato lines
[9] Dipel contains CRY2A, CRY1Aa, CRY1Ab, CRY1Ac; Cutlass OF contains CRY2A, CRY1Aa, CRY1Ab, CRY1Ac; Crymax contains CRY2A, CRY1Ac, CRY1C.
[10] Carter & Liggett, 1994; EPA, 1996; David, 1989; McClintock et al, 1995; Hadley et al, 1987

4.3.1 *CRY2Ab, GUS and GUS E377K sequence comparison to known toxins*

Studies
RE Hileman and JD Astwood, (1999). Characterisation of Insect Protection Protein (IPP2) protein sequence utilising toxin and public domain genetic databases. Monsanto Technical Report MSL-15742, St. Louis, Missouri.

KS Gustafson, RE Hileman, and JD Astwood, (1999). Characterisation of GUS protein sequence utilising toxin and public domain genetic databases. Monsanto Technical Report MSL-16264, St. Louis, Missouri.

Bannon GA and A Silvanovich. 2002. Summary of bioinformatics analyses for GUS E377K produced in cotton event 15985. Monsanto Technical Report MSL 17618, St. Louis, Missouri.

The amino acid sequences of CRY2Ab, GUS and GUSE377K were compared to all protein sequences in the publicly available genetic databases PIR, SwissProt, EMBL and GenBank (updated weekly), to screen for similarity to other known proteins, including pharmacologically active proteins.

In addition, a subset of 4 677 protein sequences associated with toxicity (not all unique) was assembled from this database. The deduced amino acid sequence of the CRY2Ab protein and the amino acid sequence of both GUS and the variant GUSE377K were compared to protein sequences in the toxin database using the FASTA[11] sequence alignment tool.

Consistent with ANZFA's previous assessments on other *Bt* proteins, similarities were observed only to *Bt* proteins. No significant similarities to known toxins were observed and therefore the analyses indicate that CRY2Ab is not similar to any toxin or other protein relevant to human health. Likewise, a similar result was observed for CRY2Aa, the protein that is naturally present in *Btk* and which shares 97% sequence similarity to CRY2Ab.

As expected, the amino acid sequence of both of the GUS proteins share sequence similarities to homologous *E. coli* and other glucuronidase proteins. These proteins have not been described as toxins relevant to human health. No other structural homology to known toxins was observed.

4.3.2 *Acute oral toxicity studies in mice*

Acute toxicity studies were used to assess the safety of CRY2Ab and GUS, since proteins that are toxic, typically act via acute mechanisms (Jones and Maryanski, 1991).

Studies submitted:
RE Hileman, PD Pyla, TC Lee and JD Astwood, (1999b). Characterisation of Insect Protection Protein (IPP2) produced by fermentation. Monsanto Technical Report MSL-15742, St. Louis, Missouri

CL Bechtel. (2000). Acute Oral Toxicity Study of Insect Protection Protein (IPP2) in Mice. Monsanto Technical Report MSL-16649, St Louis, Missouri.

LA Harrison, NA Biest, R Leimgruber and SR Padgette. (1996). Preparation, Characterisation and confirmation of doses for an acute mouse feeding study with β–Glucuronidase. Laboratory Project ID EHL-92-01-30-10, St Louis, Missouri

MW Naylor. (1992) Acute Oral Toxicity Study of β–Glucuronidase (GUS) protein in albino mice. Laboratory Project ID EHL-92184, St Louis, Missouri.

[11] FASTA is based on the algorithms of Needleman and Wunsch (1970) and of Smith and Waterman (1981), which consider all possible alignments between a query sequence and a database sequence.

(i) CRY2Ab

In addition to published studies on *Bt* sprays, the Applicant has conducted a range of studies to demonstrate the safety of CRY2Ab. The acute oral toxicity study of CRY2Ab was tested in young laboratory mice using purified (65.5%) protein produced by *B. thuringiensis* strain EG7699. The bacterially produced protein was shown to have equivalent molecular weight and immunoreactivity to the protein expressed in cotton and to lack detectable post-translational modification. It was also shown to have similar functional activity.

The study was conducted in general compliance with the EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA 40 CFR Part 160). A total of 100 animals (50 males and 50 females) were used in this study and were either 5 weeks (males) or 8 weeks (females) of age. Mice were assigned to test groups by computer randomisation according to weight. Three groups of ten male and ten female Cr1:CD-1 mice were given an acute oral dose of CRY2Ab protein at concentrations of 67.3, 359 or 1450 mg/kg body weight respectively. One group of ten males and ten females were given the dosing vehicle, purified water, and the final group were given the protein control, bovine serum albumin at a dose of 1200 mg/kg. The protein preparation containing the CRY2Ab protein was administered as two equal doses of approximately 1 ml each at approximately 4 hours apart on the same day. The test substance was not administered in a single dose due to the highly viscous nature of the CRY2Ab protein, which therefore prevented formulation of a single high-concentration dose.

The animals were observed for clinical signs after dosing and daily thereafter. All animals were checked twice daily for mortality and signs of toxicity, except on the day of necropsy. Signs of toxicity can include changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioural changes. Individual animal weights were recorded prior to study start and then again on post-dosing days 7 and 14. Food consumption was measured on post-dosing days 7 and 14.

The animals were sacrificed on day 14 and examined for gross pathology (i.e. macroscopic examination of organs and tissues, opening of internal cavities) and numerous tissues were collected. Tissues retained from the animals included aorta, adrenals, brain, sections of the gastrointestinal tract (oesophagus to rectum), eyes, femur with joint, gall bladder, heart, kidneys, lesions or abnormal masses, liver, lung, lymph nodes, muscle, nerve (sciatic), ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicles, skin (including mammary tissue), spinal cord, spleen, sternum with marrow, testes, thymus, thyroid, trachea, uterus and bladder.

There were two unscheduled deaths in the study: one death in each of the control groups (vehicle and protein control group). Both deaths were associated with gavage dosing injury (i.e. perforations of the oesophagus). There were no clinical observations associated with test article administration. All animals appeared normal throughout the study with the exception of one observation of periorbital wetness and one of eye opacity. Both observations were in 359 mg/kg dose level males and both had a one-day duration. These observations were not considered test related. All animals gained weight throughout the study with no statistical differences and food consumption was also similar for all groups.

In conclusion, there was no evidence of acute toxicity in mice following acute oral doses of up to 1450 mg/kg body weight of CRY2Ab. This dose level represents a highly conservative safety margin (i.e. 8×10^8 based on the average US consumption of cottonseed oil at 4 g/day)

given that the potential exposure to CRY2Ab in oil and linters from the modified cotton is negligible.

(ii) GUS

The Applicant conducted an acute oral toxicity study in young laboratory mice using purified (90%) GUS protein produced by *E. coli*. The bacterially produced version was used because the amount needed for the safety studies could not be extracted from the cotton plants in sufficient quantity. The *E. coli* produced protein was comprehensively characterised using a range of analytical methods including gel electrophoresis, western blot analysis, enzyme activity and ELISA. The bacterially produced version is 99.7% homologous to the version produced by the cotton plant and is considered an appropriate substitute for these studies.

A total of 100 animals (50 males and 50 females) were used in this study and were approximately 9 weeks of age. Mice were assigned to test groups by computer randomisation according to weight. Three groups of ten male and ten female CD-1 mice were given an acute oral dose of GUS protein suspended in Na carbonate buffer solution at doses of 40, 100 and 400 mg/kg of GUS protein (dose concentrations based on the level of purity of the protein and ELISA analyses of the dosing solutions). One group of ten males and ten females were given the dosing vehicle, 50 mM Na carbonate buffer solution and the final group were given the protein control, bovine serum albumin (BSA) at a dose of 100 mg/kg. The protein preparation containing the GUS protein was administered as a single dose by gavage.

The animals were observed for clinical signs after dosing and daily thereafter. All animals were checked twice daily for mortality and signs of toxicity, except on the day of necropsy. Signs of toxicity include such occurrences as changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioural changes. Individual animal weights were recorded prior to study start and then again on day 7 and at termination (day 8 or 9). Food consumption was measured on day 7. The animals were sacrificed on day 8 or 9 and examined for gross pathology as described earlier.

There were three unscheduled deaths in the study. One male in the group administered BSA was sacrificed on day 5. One male in the group administered 100 mg/kg of GUS was also sacrificed on day 5. One male in the group administered 10 mg/kg of GUS was found dead on day 5. No gross abnormalities were observed in any of these animals upon necroscopy. As no consistent pattern emerged in the test group and a death occurred in the control group, these deaths are not considered treatment related.

All other animals appeared normal throughout the study. There were no clinical observations associated with test article administration. All animals gained weight throughout the study (body weight and cumulative body weight) with no statistical differences. Food consumption was also similar for all groups.

In conclusion, there were no treatment related adverse effects in mice administered GUS by oral gavage up to 100 mg/kg. This dose level represents a highly conservative safety margin (i.e. 4×10^8 relative to the average US human consumption of cottonseed oil at 4 g/day) given that the potential exposure to GUS in oil and linters from the modified cotton is negligible.

4.4 Potential allergenicity of new protein

Large quantities of an enormous range of proteins are consumed in human diets each day, and rarely do any of these proteins elicit an allergic response (Taylor, 1992). Although many foods have been reported to cause allergies in some people, the prevalence of food allergy using prospective, population-based studies has been shown to be less than 2% of adults and 2-7% of infants and children, excluding cases of food intolerances such as enzyme deficiencies. Food allergies are primarily due to an individual's immune reaction to a particular protein or glycoprotein component of the food (FAO, 1995).

The potential allergenicity of the new proteins introduced into cotton containing event 15985 has been assessed by considering likely human exposure profiles and comparing certain physio-chemical properties of the new protein to those of known allergens. This may include consideration of the level of exposure and an assessment of the factors that contribute to exposure such as amino acid sequence similarity with known protein allergens, poor digestibility and resistance to processing. Other relevant factors that may increase the likelihood of allergic oral sensitisation to proteins include the level of food consumption, and the relative quantity of the protein in the food.

4.4.1 Source of protein

The CRY2Ab protein is sourced from the soil bacterium *B. thuringiensis* subspecies *kurstaki*. Commercial formulations of *B. thuringiensis* have been used as microbial pesticides for decades and have not been described as sensitising allergens, including through oral exposure (McClintock et al, 1995). Thus, there is no apparent history of allergy associated with crystal proteins from *Bt*.
One *Bt* protein, namely CRY9C protein from *B. thuringiensis* var. *tolworthi* that was introduced into Starlink™ corn was denied human food approval in the USA. Approval was denied because the information on the structural and functional properties of CRY9C could not exclude the potential of causing an allergic reaction in humans. Unlike the other *Bt* proteins, the CRY9C protein is not readily digestible in simulated gastric conditions and has been shown to be stable at 90°C. These two parameters altered the weight of evidence typically used by the EPA for assessing the food allergenicity of an introduced protein. It is important to note however, that there is currently no evidence that the health of any consumer has been affected by exposure to Starlink corn (CDC, 2001) or any other GM food.

In relation to the *uid*A gene, it also is not from a source known to be allergenic. GUS was obtained from *E. coli* (Jefferson, 1986), a bacteria prevalent in the gastrointestinal tract of humans and other animals.

4.4.2 Digestibility of novel proteins

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Kimber et al, 1999; Astwood et al, 1996b; Metcalfe et al, 1996). To address the question of potential allergenicity, the Applicant has investigated the physical and chemical properties of the CRY2Ab and GUS proteins, which are expressed in the cottonseed at low levels, and tested their susceptibility to proteolytic degradation.

(i) CRY2Ab

Study
JN Leach, RE Hileman, JW Martin, MA Nemeth and JD Astwood, (2000). Assessment of the in vitro digestibility of Insect Protection Protein 2 (IPP2) utilising mammalian digestive fate models. Monsanto Technical Report MSL-16640, St. Louis, Missouri

Simulated gastric fluid (SGF) and intestinal fluid (SIF) digestion mammalian models (United States Pharmacopoeia, 1990) were used to assess the susceptibility of CRY2Ab to *in vitro* proteolytic digestion. The protein was incubated at approximately 37°C in SGF and SIF for up to 2 and 24 hours respectively. At defined periods, the digestions were terminated and the level of remaining protein determined by western blot analysis and corroborated by insecticidal activity bio-assays.

The data show that CRY2Ab degraded readily in SGF and that after 15 seconds, greater than 98% of the protein was digested and no fragments ≥2 kDa of the parent protein were resolved. Western blot analysis of SIF incubations showed that a relatively stable CRY2Ab protein fragment (~50 kDa) was produced within 1 minute and observed for at least 24 hours. The ~50 kDa protein product appears to be trypsin resistant and so would be expected to remain in SIF. This is consistent with results from other classes of *Bt* proteins (CRY1, CRY2 and CRY3)[12] which yield stable, tryptic core fragments when incubated in SIF and are known to have trypsin-resistant cores (Hofte & Whitely, 1989).

These observations were corroborated by insect bioassays showing rapid loss of insecticidal activity of samples incubated in SGF and stable activity of samples incubated in SIF. This *in vitro* assessment of CRY2Ab digestibility indicates that this protein will degrade rapidly in the stomach when ingested by mammals.

(ii) GUS

Study
JN Leach, RE Hileman, JW Martin, MA Nemeth and JD Astwood, (2000). Assessment of the in vitro digestibility of Insect Protection Protein 2 (IPP2) utilising mammalian digestive fate models. Monsanto Technical Report MSL-16640, St. Louis, Missouri

Using the same methods described for CRY2Ab, the susceptibility of GUS to *in vitro* proteolytic digestion was assessed using SGF and SIF digestion mammalian models. At defined periods during the incubation in these fluids, the digestion process was terminated and the levels of remaining protein were determined by western blot analysis and enzymatic activity assays. The data show that the GUS protein is readily degraded in SGF fluid as no GUS protein was detected after 15 seconds incubation at 37°C. After 2 hours incubation in SIF, only a very faint protein band was observed in the western blot and the protein had lost approximately 91% of its original enzymatic activity. Based on these results, it is concluded that the GUS protein will readily degrade in the digestive tract when ingested by mammals.

[12] Similar results were found for CRY1Ac and CRY2Aa in A341 - cotton, CRY1Ab in A346, A385 and A386 - corn and CRY3Aa in A382, A383 and A383 - potato.

4.4.3 Sequence comparison to known allergens

CRY2Ab2, GUS and GUS E377K

Study submitted:
RE Hileman, and JD Astwood. 1999. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilising an Allergen Database, Monsanto Technical Report MSL No. 16094, St. Louis, MO.

KS Gustafson, RE Hileman, and JD Astwood, (1999). Characterisation of GUS protein sequence utilising an allergen database. Monsanto Technical Report MSL-16263, St. Louis, Missouri.

CRY2Ab, GUS and GUS E377K were compared to protein sequences associated with allergenicity. A database of 567 protein sequences associated with allergy and coeliac disease was assembled from publicly available genetic databases (GenPept, PIR and SwissProt) and from current literature. The Applicant compared the deduced amino acid sequence of CRY2Ab and the amino acid sequence of both GUS and GUS E337K to these assembled sequences using the FASTA sequence alignment tool. The results of the alignment showed that CRY2Ab, GUS or GUS E377K do not share structurally significant sequence similarities to sequences within the allergen database.

In addition, the amino acid sequences of each protein were compared to the allergen database using an algorithm[13] that scans for a window of eight identical linearly contiguous amino acids. This comparison did not find any stretches of eight immunologically relevant amino acid residues between the introduced proteins and the database sequences. These analyses establish that neither CRY2Ab or either of the GUS proteins share any immunologically significant sequence similarity to proteins known to be allergens or gliadins.

The amino acid sequence of the variant GUS protein was evaluated for the possibility that the single amino acid change would result in the creation of a potentially cross-reacting IgE binding epitope. Using a FASTA algorithm and an eight contiguous amino acid search of the specific amino acid substitution and the surrounding regions, no evidence was found of shared identity between GUS E377K and any known allergen.
Further evaluation of the alignments identified by the FASTA search indicated that the closest alignment resulted in a match of only three contiguous amino acid identities. It is therefore highly unlikely that a high affinity IgE binding epitope was created by this change.

From the bioinformatic search, the amino acid substitution in GUS E377K is not considered to be a significant change and no significant matches to any known allergenic sequences were identified. Therefore, it is concluded that, based on this analysis, the plant expressed GUS protein that contains the single amino acid substitution is unlikely to have any allergenic property.

4.4.4 Presence of CRY2Ab and GUS

A factor that may contribute to the allergenicity of certain food proteins is their high abundance in food (Taylor et al, 1987, 1992; Fuchs and Astwood, 1996). Most allergens are present as major protein components in a specific food, typically ranging between 1% and 80% of total protein (Astwood and Fuchs, 1996). In contrast, the proteins are present at low levels in these plants: the CRY2Ab protein represents 0.02% of the total protein in the cottonseed. On a dry weight basis, CRY2Ab and GUS represent less than 0.004% and

[13] The algorithm is known as IDENTITYSEARCH within the GCG software package.

0.007% respectively, of the cottonseed. Although it is difficult to define the level at which a protein can cause an allergic reaction, it is important to note that exposure to protein from oil is highly unlikely. The very low level of novel proteins present in cottonseeds, is reduced even further with the extensive processing cottonseed undergoes to produce oil and linters; products which are not expected to contain any plant protein including the introduced novel proteins.

Summary and conclusion

Two novel proteins (CRY2Ab and a GUS protein) are expressed in cotton containing event 15985 and are present both in the cottonseed, the part of the plant from which food is derived, as well as in the green tissues of the plant which are not used for human consumption.

There is a history of exposure to these proteins through food crops: CRY2Ab is similar to a protein that has been widely used as a biopesticide in agriculture for decades and GUS is naturally present in many plant species as well as present in bacteria that inhabit the human gut.

Neither protein exhibits sequence similarity with known toxins or allergens, and they do not exhibit the biochemical characteristics of known protein allergens. No evidence of acute toxicity was observed when either of the purified proteins was fed to laboratory mice. Additionally, the proteins are readily digested in conditions that mimic human digestion. Thus even in the unlikely event of exposure to these proteins from food (i.e. oil and linters), they are unlikely to elicit a toxic or allergic reaction.

5. COMPARATIVE ANALYSES

Concerns have been raised that genetically modifying a plant may affect the nutritional composition of the food from that plant or may result in an unintended impact that adversely affects the safety of the food. To address these concerns, this safety assessment includes an analysis of the composition of the food part of the plant with a comparison to other commercially available varieties. In this case, both refined cottonseed oil and cottonseed are assessed. The lines used for comparison include the parental line as well as other non-transformed lines.
Generally, for each parameter assessed, if the value for the transformed line is within the normal range for non-transformed lines, it is considered acceptable (Hammond & Fuchs, 1998).

Study submitted:

KA Hamilton, T Olson, R Sorbet, WP Ridley and M Nemeth. 2001. Statistical evaluation of compositional data from 1998 U.S. Field Trial samples comparing cotton cry2Ab2 events 15985 and 15813 to the non-transgenic control. Monsanto Technical Report MSL-17097, St. Louis, MO.

KA Hamilton. 2000. Amended report for compositional analyses of seed, oil and meal from insect protected cotton lines grown in 1998 U.S. Field Trials. Monsanto Technical Report MSL-16975, St. Louis, MO.

Field trial and analytical methodologies

Field trials were conducted at eight US locations within six US states in 1998. These sites were chosen so that the natural variation in composition due to environmental factors could be taken into account. These factors are known to impact on the variability of most constituents and will give an indication of the range that exists in natural environments. At

four of the sites, the plots were planted as a single block without replication and at the other four locations, the plots were planted with four replicates.

Detailed information about the methods used to generate the compositional data was provided. For each parameter tested, as well as appropriate technical references, the limit of detection or quantitation was also stated. Methodologies were predominantly derived from established references such as AOAC (Association of Official Analytical Chemists), AOCS (American Oil Chemists Society) or from published literature methods.

Compositional data have been provided on the seed, oil and meal derived from insect-protected cotton containing event 15985. As humans do not typically consume raw cottonseed (or meal), the assessment focuses on the composition of the oil. However, the data on cottonseed and meal are considered to provide supporting evidence of the similarity in composition between the test line, its parental control line and commercial cotton varieties, including in plant tissues that are not typically used as human food. The lines used for comparison are the non-transformed control line (DP50) and the parental control line (DP50B) as well as data from commercial varieties of cotton.

Cottonseed processing required for food use

Cottonseed is highly processed during the production of oil and meal. After the majority of the fibre is removed at the cotton gin, a significant amount of "fuzzy" fibre remains associated with the seed. These short fibres, known as linters, are removed from the seed during de-linting. After extensive processing at alkaline pH and high temperatures, the linters can be used as a high fibre dietary product. After this processing, the fibre does not normally contain any detectable genetic material or protein.

Once the lint is removed from the seed, the hulls are cut and separated from the seed. After hulling, the cottonseed is flaked by a rolling process to facilitate oil removal. Prior to oil extraction, the flakes are heated to: (i) break down the cell walls; (ii) reduce the viscosity of the oil; (iii) coagulate the protein; (iv) inactivate proteins and kill any microbial contamination; (v) detoxify gossypol by the combination of heat and moisture; and (vi) fix certain phosphatides in the meal to minimise refining losses.
After heating, the oil is typically removed from the meal by direct solvent extraction with hexane. The material left over after the crude oil is extracted, is the cottonseed meal. After this extraction, the gossypol levels in the oil are reduced by about half. Crude cottonseed oil is then further processed, depending on the end use of the product.

A winterisation step is added to produce cooking oil, whereas for solid shortening, a hydrogenation step is added to transform the liquid oil into a solid fat. Further processing (refining) for all the uses of cottonseed oil includes deodorization and bleaching. Deodorization greatly reduces the cyclopropenoid fatty acid content of the oil due to the extreme pH and temperature conditions and the resulting oil generally contains no detectable protein (Jones and King 1990).

5.1 Key nutrients of oil (and meal)

The key nutrients present in refined (bleach and deodorised) cottonseed oil are fatty acids and vitamin E (measured as α-tocopherol). Values for meal from cotton event containing 15985 were also compared to meal values for three non-genetically modified commercial varieties of cotton.

Cottonseed oil and meal samples were generated by pooling cottonseed from each line across the eight field sites to generate enough starting material for processing to a single oil and meal sample per line. As only one oil and meal sample was generated for each line, a statistical analysis of the results is not possible.

Fatty acid profile

The fatty acid values generated for cotton containing event 15985 were consistent with values for the non-transformed control line DP50 and the parental control line DP50B as well as for reference ranges for fatty acids levels in cottonseed oil (Table 3).

Vitamin E (α-tocopherol analysis)

Vitamin E (tocopherol) occurs primarily in wheat seedlings, and has been isolated from wheat seedling oil. It is also present in a range of other vegetable, nut and oilseed crops. Although there are numerous structural isomers of tocopherol, biologically, α- tocopherol is the most important member of the group. The vitamin E level in the refined cottonseed oil sample from cotton containing event 15985 was similar to levels reported from the controls and commercial cottonseed oil samples (Table 3).

The results from the fatty acid and vitamin E analyses of oil from cotton containing event 15985 were consistent with the levels observed in control cotton lines and also in the commercial cotton varieties.

These results are also comparable to published data for oil from a range of GM cotton lines including event 531 (Berberich et al, 1996) from which DP50B has been bred, and to the data evaluated for GM and non-GM cotton lines assessed in Application A341 (refer to Technical Report).

Table 3: Summary of compositional mean values for cottonseed oil samples

	15985	DP50B	DP50	Codex	Commercial range[1]
Vitamin E mg/100g	59.8	45.1	53.4		45.1-58.5
Fatty acid[2]					
Myristic (14:0)	1.32	0.980	1.06	0.4-2.0	0.923-1.45
Pentadecanoic	<0.100	<0.100	<0.100	-	<0.100
Palmitic (16:0)	23.9	25.2	25.3	17.0-31.0	22.7-26.3
Palmitoleic (16:1)	0.832	0.735	0.78	0.5-2.0	0.735-0.954
Heptadecanoic (17:0)	<0.100	<0.100	<0.100	-	<0.100
Stearic (18:0)	2.04	2.34	2.04	1.0-4.0	1.98-2.34
Oleic (18:1)	15.1	15.7	14.7	13.0-44.0	14.7-17.8
Linoleic (18:2)	55.6	53.7	54.9	33.0-59.0	51-54.9
Linolenic and gamma linoleic (18:3)	0.171	0.152	0.145	0.1-2.1	0.120-0.152
Arachidic (20:0)	0.176	0.244	0.178	<0.7	0.178-0.244
Behenic (22:0)	<0.100	0.103	<0.100	<0.5	<0.100-0.103
Lignoceric (24:0)	<0.100	<0.100	<0.100	<0.5	<0.100

[1] Range includes data from three commercially available cotton varieties.
[2] fatty acid values expressed as a percentage total fatty acids.
[3] ranges adopted by the FAO/WHO Codex Alimentarius Committee on fats and oils (Jones & King 1993)

5.2 Key constituents of seed

Values for 48 components of seed from cotton containing event 15985 were compared to those from the parental control line (DP50B) and the non-transformed control line (DP50). Compositional analyses of seed samples include proximates (protein, fat, ash, carbohydrate, moisture and calories), amino acids, fatty acids and minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc). The key toxicants in cotton are gossypol and cyclopropenoid fatty acids. Approximately 20 samples for each analyte per line were collected to evaluate each mean value.

Seed samples were ginned, acid delinted and shipped under ambient conditions to an independent laboratory for compositional analyses[14]. The compositional data for all field sites were combined.

Proximate analysis

A summary of the proximate analyses is shown in Table 4. No statistically significant differences between cotton containing event 15985 and either of the control lines – the non-transgenic control DP50 and transgenic control DP50B were observed in any of the parameters measured ($p<0.05$).

The data assessed for cotton containing event 15985 and its two parental lines were also consistent with compositional data presented for line 531 that were assessed in Application A341. This supports that the first round modification of line 531 (from which DP50B has been bred) is stable and does not appear to have any unintended impact on the nutritional profile of cottonseed over seven years of commercial cotton production.

Table 4: Summary of proximate analysis of seed[1]

Component	15985	DP50B	DP50	Non-transgenic Reference[2]	Commercial Reference[3]
Protein	26.13 21.45-28.82	26.06 21.93-28.15	25.96 21.76-27.79	21.76-27.79	21.76-28.15
Fat	20.52 17.54-27.42	20.37 16.04-23.48	19.74 15.44-23.64	15.44-23.64	15.44-23.83
Ash	4.36 3.93-4.81	4.38 4.06-4.67	4.34 3.76-4.85	3.76-4.85	3.76-4.85
Fibre, crude	16.83 14.93-17.95	17.17 15.42-19.69	17.79 15.38-19.31	15.38-19.31	15.38-20.89
Carbohydrate	49.09 42.97-52.69	49.23 46.85-51.93	49.94 45.64-52.44	45.64-53.62	45.64-53.62
Calories	485.33 468.50-520.01	484.45 463.09-498.71	481.57 457.77-499.84	457.77-499.84	457.77-500.49
Moisture	5.99 4.34-7.59	6.05 4.22-7.28	6.03 3.97-7.26	3.97-7.49	3.97-8.47

[1] All values (average and range) expressed as % dry weight except moisture which is % fresh weight.
[2] Range includes data from four commercially available non-transgenic cotton varieties.
[3] Range includes data from ten commercially available transgenic and non-transgenic cotton varieties.

[14] Covance Laboratories Inc., Wisconsin Facility, 3301 Kinsman Blvd., Madison, WI 53704.

Fatty acid content of seed

Data on the fatty acid content of cottonseed containing event 15985 were compared to the fatty acid profile from the non-transgenic control line DP50 and the parental line DP50B (Table 5). Two fatty acids are not listed in the table because they were measured consistently (i.e. all samples) at the limit of detection of the assay. These are 15:0 pentadecanoic acid and 17:0 heptadecanoic acid. The data were also compared to values determined for commercially available lines.

Statistically significant differences were observed between cotton containing event 15985 and the non-transgenic control line DP50 for myristic, palmitoleic, stearic,

Table 5: Summary of Fatty Acid Profiles (% of total) of seed[1].

Fatty acid (% of total fatty acids)	15985	DP50B	DP50	Non-transgenic Reference[2]	Commercial Reference[3]	Codex ranges	Literature values[6]
Myristic (14:0)[4, 5]	**1.26** 0.88-2.94	**0.92** 0.74-1.91	**1.02** 0.77-2.15	0.77-2.40	0.64-2.40	0.4-2.0	0.68-1.16
Palmitic (16:0)	25.80 24.5-27.90	25.92 24.90-27.60	25.81 24.30-28.10	24.30-28.10	23.40-28.10	17.0-31.0	21.63-26.18
Palmitoleic (16:1)[4]	**0.56** 0.33-0.65	0.58 0.43-0.68	**0.63** 0.43-0.98	0.43-0.98	0.43-0.98	0.5-2.0	0.56-0.82
Stearic (18:0)[4,5]	**2.63** 2.41-3.10	**2.38** 2.24-2.60	**2.30** 2.06-2.71	2.06-3.11	2.06-3.11	1.0-4.0	2.27-2.88
Oleic (18:1)	15.58 13.60-18.10	15.59 13.30-18.10	15.40 12.90-17.40	12.90-20.10	12.90-20.10	13.0-44.0	15.17-19.94
Linoleic (18:2)[4,5]	**52.52** 47.70-55.50	**53.10** 49.00-55.80	**53.31** 49.50-57.10	46.00-57.10	46.00-57.10	33.0-59.0	49.07-57.64
Linolenic & gamma linoleic (18:3)	0.13 0.050-0.29	0.14 0.05-0.55	0.11 0.05-0.31	0.005-0.31	0.05-0.55	0.1-2.1	0.23
Arachidic (20:0)[4]	**0.30** 0.25-0.43	0.29 0.25-0.36	**0.27** 0.24-0.34	0.24-0.34	0.24-0.36	< 0.5	0.41
Behenic (22:0)	0.14 0.12-0.21	0.15 0.11-0.23	0.14 0.12-0.24	0.12-0.24	0.11-0.24	< 0.5	
Lignoceric (24:0)	0.14 0.05-0.26	0.12 0.05-0.26	0.14 0.05-0.29	0.05-0.29	0.05-0.29	< 0.5	

[1] Average and range values given. Values represent samples taken from 8 US field sites . Significant differences indicated in bold.
[2] Range includes data from commercially available cotton varieties: DP50, DP51, DP20 and DP5409.
[3] Range includes data from 10 commercially available transgenic and non-transgenic cotton varieties.
[4] Statistically significant difference to DP50 control (p ≤0.05).
[5] Statistically significant difference to DP50B parent (p ≤0.05).
[6] Cherry and Leffler, 1984; Cherry, 1983; Phelps et al, 1965

linoleic and arachidic acid (Table 5). With the exception of stearic acid, the differences for these fatty acids were observed in only one or two individual sites and were not considered to be of biological relevance or to represent a meaningful difference between the test and control lines. These differences were considered to be indicative of the natural variation that exists for these components and they fall within the ranges observed for other varieties of cottonseed.

The difference noted in stearic acid content occurred at all four replicated sites. However, as with the other differences, it was small and within the values found in previously characterised varieties (literature values listed in Table 5), as well as being within the ranges observed for other commercially available varieties (Table 5). Furthermore, this difference was not noted in the pooled oil samples. Given that stearic acid is a minor fatty acid component in cottonseed oil, typically representing at its highest recorded levels, less than 2.5% of the total lipid content of the oil and less than 3.5% of the lipid content of the seed, this difference is not considered to be nutritionally significant.

Statistically significant differences were also observed between cotton containing event 15985 and the transgenic parent line DP50B for myristic, stearic and linoleic acid. As observed for DP50, these differences are all minor fatty acids and are not expected to have adverse nutritional impacts.

Although significant, all observed differences between 15985 and either of the two control lines were generally small (i.e. within the 95% confidence interval for each true mean difference), were not consistent across sites and were within the range established for non-transgenic lines as well as those published by Codex and in literature. Therefore, they are more likely to reflect variation expected in biological systems. Additionally, most of the differences were noted in minor fatty acids, which would not have a significant nutritional impact or any significance with respect to food safety.

Amino acid analysis of seed

Eighteen essential amino acids were measured in cottonseed containing 15985 and the control lines. The statistical analysis showed significant differences between 15985 and the non-transgenic control DP50 in five amino acids (Table 6).

Table 6: Significantly different amino acids[1]

Amino Acid	15985	DP50	Non-transgenic Reference[2]	Commercial Reference[3]
Alanine	4.32	4.27		
	4.20-4.48	4.15-4.41	4.15-4.41	4.15-4.60
Cysteine	1.79	1.87		
	1.68-2.03	1.67-1.99	1.67-1.99	1.46-2.12
Isoleucine	3.58	3.53		
	3.47-3.79	3.38-3.71	3.38-3.71	3.38-3.78
Leucine	6.58	6.52		
	6.45-6.86	6.43-6.65	6.42-6.65	6.38-6.94
Valine	4.97	4.89		
	4.77-5.34	4.72-5.22	4.72-5.22	4.72-5.34

[1] Significantly different amino acids between 15985 and DP50 ($p \leq 0.05$).
[2] Range includes data from four commercially available non-transgenic cotton varieties.
[3] Range includes data from ten commercially available transgenic and non-transgenic cotton varieties.

As amino acids are not present in the refined oil, only values for the significantly different amino acids are presented. These differences are small and are within the range determined for other non-transgenic cotton lines. They are not considered of biological relevance.

No statistically significant differences were observed between cotton containing 15985 and its parental control DP50B in any amino acids when the data were combined from all sites. Amino acid values for line DP50B are not shown.

Inorganic analysis of seed

Analysis of inorganic components of cottonseed included measurements of nine minerals: calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc. Small but significant differences were observed between the test line and non-transgenic control line DP50 in the iron and phosphorous measurements (Table 7). These differences are not expected to impact on the safety or nutritional adequacy of oil and linters derived from cotton containing event 15985.

No statistically significant differences were observed in any of these mineral components between cotton containing event 15985 and its parent line DP50B (data not shown).

Table 7: Significantly different minerals[1]

Component	15985	DP50	DP50B	Non-transgenic Reference[2]	Commercial Reference[3]
Iron	50.83 43.92-57.56	54.13 42.57-72.15	51.13 41.84-60.76	42.57-72.15	41.84-72.15
Phosphorous	0.70 0.58-0.83	0.73 0.63-0.86	0.71 0.61-0.88	0.63-0.86	0.61-0.88

[1] Statistically significantly different mineral values to DP50 control ($p \leq 0.05$) .
[2] Range includes data from four commercially available non-transgenic cotton varieties.
[3] Range includes data from ten commercially available transgenic and non-transgenic cotton varieties.

Conclusion

Insect-protected cotton containing event 15985 was shown to contain levels of nutrients comparable to those of both the transgenic and non-transgenic control lines as well as to other commercial varieties of cotton. These studies confirm that seed from cotton event 15985 is compositionally and nutritionally similar to seed from the parental and other commercial cotton varieties.

In addition, the results presented here for cotton containing event 15985 and its transgenic parental line DP50B are also comparable to published compositional data from a range of transgenic cotton lines including event 531 (Berberich et al, 1996) from which DP50B has been bred, and to the data evaluated for GM and non-GM cotton lines assessed in Application A341 (refer to Technical Report).

5.3 Key toxicants

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

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.

Processing of cottonseed to reduce gossypol

Unprocessed seed contains gossypol in the 'free' or unbound form, in the pigment glands (Jones, 1991). When cottonseed is flaked and heated during processing to oil and meal, the lysigenous glands are ruptured and gossypol is released. Some of the gossypol binds to seed components, primarily to proteins through the free amino groups of lysine. The binding of gossypol during processing is important because the free form of gossypol is considered toxic, whereas the bound form is unavailable and essentially inactive (Martin, 1990; Berardi and Goldblatt, 1980). Refined cottonseed oil is free of gossypol (Gunstone *et al* 1994). Any gossypol that partitions into the crude oil is essentially completely eliminated during subsequent refining, through inactivation by heat and alkali treatment. The reduction of free gossypol in oil is a measure of the food quality and processing efficiency.

Cyclopropenoid fatty acids

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 (NCPA, 1990).

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).

Two studies of cyclopropenoid fatty acids from several domestic varieties reported ranges of 0.56-1.17% in crude oil (Bailey *et al* 1977), and 0.07-0.32% in refined oil (Lawhon *et al* 1977). In another study cyclopropenoid fatty acids were found at levels up to 2% of crude oil, and 0.64% of refined oil (Jones and King 1990).

Toxicant levels in oil (and meal)

Cottonseed oil (and meal) samples were generated by pooling cottonseed by line across the eight field sites for processing to a single oil sample per line. As only one sample was generated per line, no statistical analysis is possible. Free and total gossypol levels in both oil and meal and the levels of the three cyclopropenoid fatty acids in oil were measured (Table 8).

Table 8: Summary of toxicant analyses of cottonseed oil and meal samples[1].

	15985	DP50B	DP50	Commercial range[2]	Literature[3]
OIL					
Free gossypol	<0.005	<0.005	<0.005	<0.005	≤0.01
Total gossypol	<0.005	<0.005	<0.005	<0.005	≤0.01
Cyclopropenoid fatty acid					
Malvalic C-17	0.378	0.384	0.377	0.294-0.405	0.22-1.44
Sterculic C-18	0.205	0.227	0.217	0.216-0.289	0.08-0.56
Dihydrosterculic C-19	0.165	0.169	0.146	0.146-0.202	-
MEAL					
Free gossypol	0.037	0.042	0.041	0.025-0.068	
Total gossypol	0.986	1.05	1.04	0933-1.43	

[1] Gossypol values expressed as a %of fresh weight; cyclopropenoid fatty acid values as a % total fatty acids.
[2] Range includes data from five commercially available cotton varieties.
[3] Cherry and Leffler, 1984; Phelps et al, 1965.

Gossypol levels in all test, control and reference oil samples were at the limit of detection of the analytical method, indicating that it is essentially removed from refined oil. The mean values observed for sterculic, dihydrosterculic and malvalic acids in oil from 15985 were similar to those observed in oil from the non-transgenic control DP50 and the parent DP50B and were within the ranges determined for oil derived from commercial cotton varieties.

Gossypol levels in meal from cotton containing 15985 were similar to those of meal from the non-transgenic control DP50 and the parent DP50B and were within the ranges determined for meal derived from commercial cotton varieties. Of note is that the levels of gossypol and cyclopropenoid fatty acids in all lines (15985, DP50B and DP50) were lower than those reported for event 531 (as well as the other test and control lines) in the 1993 field trial data assessed as part of Application A341 (refer to Technical Report) and published in 1996 (Berberich et al, 1996). This difference is most likely indicative of breeding efforts that take advantage of plants containing naturally lower levels of the toxicants as well as improved refinement procedures that reduce toxicants from cottonseed.

Toxicant levels in seed

No statistically significantly differences in gossypol levels were detected between seed from cotton containing event 15985 and the non-transgenic control DP50 or the parental control DP50B (Table 9) collected from the eight locations in the 1998 US field trial. All gossypol values were within the non-transgenic reference range and within the ranges reported in the literature (Beradi & Goldblatt, 1980).
The levels of sterculic acid, dihydrosterculic acid and malvalic acid were slightly higher in seed from cotton containing event 15985 than in either the non-transgenic control DP50 or the parental control DP50B (Table 9).

Table 9: Summary of toxicant analyses[1]

Component	15985	DP50	DP50B	Reference range[2]	Commercial range[3]
Total gossypol					
Average	1.00	0.96	0.97		
Range	0.79-1.29	0.72-1.23	0.78-1.24	0.72-1.23	0.71-1.24
Cyclopropenoid fatty acids					
Malvalic (C-17) [4,5]					
Average	0.45	0.39	0.39		
Range	0.26-0.71	0.17-0.61	0.22-0.51	0.17-0.61	0.17-0.61
Sterculic (C-18) [4,5]					
Average	0.30	0.24	0.25		
Range	0.21-0.58	0.13-0.43	0.16-0.44	0.13-0.56	0.13-0.66
Dihydrosterculic (C-19) [4,5]					
Average	0.18	0.16	0.15		
Range	0.12-0.22	0.12-0.19	0.11-0.17	0.12-0.22	0.11-0.22

[1] Gossypol measured as a % dry weight; cyclopropenoid fatty acids measured as a % of total fatty acids
[2] Range includes data from four commercially available non-transgenic cotton varieties.
[3] Range includes data from ten commercially available transgenic and non-transgenic cotton varieties
[4] These values from cotton event 15985 are statistically significantly different to DP50 ($p \leq 0.005$).
[5] These values from cotton event 15985 are statistically significantly different to DP50B ($p \leq 0.005$).

Increased levels of dihydrosterculic acid were observed at three of the replicated field trial locations. In each instance, these differences are small (i.e. within the 95% confidence interval for each true mean difference) and they are all within the range observed for the commercially available lines. These small differences are considered to be indicative of the variation that exists in nature and the impact of environmental conditions.

Of further significance is that during processing to refined oil, such acids are largely deactivated or removed by hydrogenation or during deodorisation at 230-235˚C (Gunstone et al, 1994). Therefore, the small difference in the cyclopropenoid fatty acid levels do not affect oil safety (Section 4.1.2).

Conclusion of comparative analysis of seed

Most crops, including oilseed crops, exhibit considerable variability in their nutrient composition. Environmental factors and the genotype of the plant have an enormous impact on composition. Thus, variation in these nutrient parameters is a natural phenomenon and is considered to be normal. The Applicant submitted a comparison of nutrient data of cotton containing 15985 to its two control lines, with particular attention to the fatty acid profile given that the primary food fraction is refined oil. These data support that there are no differences of biological significance between cotton containing event 15985 and the parental control in any of the components tested. The small but statistically significant differences in cottonseed that were observed either in the key constituents or toxicants between the test and control lines are not considered to raise food safety concerns, as the levels were within published ranges that are normally expected of and observed in commercial cotton varieties.

It is therefore concluded that there are no concerns regarding the safety of cottonseed oil for human consumption arising from these differences between insect-protected cotton event 15985 and its control lines.

The comparative data presented for cotton containing event 15985 as well as its parental GM line DP50B are also comparable to data presented for the assessment of cotton line 531 (i.e. from which DP50B has been bred) in Application A341. This is further evidence that the inserted DNA from the first round modification is physically and phenotypically stable over seven years of commercial cotton production.

6. OTHER INFORMATION

6.1 Ability to support typical growth and well being

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

In the case of insect-protected cotton containing event 15985, animal feeding studies were not considered necessary given the comprehensive analysis on the composition of the cottonseed. The nutritional profile of 15985 including fractions that are not considered a human food indicates that the parameters were essentially comparable to the parental and control lines, as well as to published ranges for other non-transgenic commercial cotton varieties. In addition, studies on the specific novel protein sequences found that no significant similarities to known allergens or toxins exist for the CRY2Ab or GUS proteins. An acute oral toxicity study in mice using variable amounts of both novel proteins, found no evidence to indicate that the protein produces toxic effects in animals. Furthermore, the novel proteins have been shown to be rapidly degraded in model digestive systems. Finally, the level of dietary exposure to the novel protein is expected to be nil, given that the major food from cottonseed is refined oil.

The Applicant did provide however, two animal feeding studies using cottonseed meal derived from insect-protected cotton event 15985 as a feed ingredient for channel catfish (*Ictalurus punctatus*) and lactating cows.

Study submitted:

Li, MH and EH Robinson. 2000. Evaluation of cottonseed meal derived from insect protected cotton lines 15813 and 15985 as a Feed Ingredient for catfish. Monsanto Report MSL-16179, St. Louis, MO.

Hartnell, GF, C Gianni, GW Videla, AR Castillo, M Maciel and M Gallardo. 2001. Sponsor summary Report: Effect of feeding cottonseed produced from cotton containing Bollgard, Bollgard II or Roundup Ready on feed intake, milk production and composition in lactating dairy cows in Argentina. MSL 17294.

Channel catfish

This study fed a diet containing approximately 20% processed cottonseed meal to catfish and assessed the growth and survival of fish fed meal from cotton containing event 15985. This diet was based on the typical amount of cottonseed meal added to commercial catfish diets. The growth of these fish was compared to fish fed meal from the parental line (DP50B), non-transformed control (DP50) and two non-transformed commercial cotton lines (ST474 and DP1266).

The diet was pelleted and fed twice daily to 100 catfish per treatment group (i.e. 5 replicates with 20 catfish each) for 8 weeks. Fish in each aquarium were counted and weighed at initiation, 4 weeks and at 8 weeks. Mortality and behaviour were recorded daily. At the end of the study, five fish fillets from each aquarium (25 fish per treatment group) were collected, pooled and processed to determine % moisture, % crude protein, % crude fat and % ash. The data were statistically analysed to determine if significant differences were noted between fish fed the test and control meal.

There were no significant differences in survival, weight gain, feed conversion ratio or fillet composition in the catfish fed diets containing the test substance compared to control or commercial substances. There were no mortalities during the study. No abnormal fish behaviour was observed during the study.

These data indicate that processed cottonseed meal from cotton containing event 15985 can be used as a feed ingredient in catfish diets at levels of up to 20% without adverse effects on fish growth, feed conversion efficiency, survival, behaviour or body composition.

Dairy cow

As ginned cottonseed (linted) is utilised extensively in dairy cattle rations as an energy, fibre and protein source, the effects of feeding dairy cows with cottonseeds containing various inserted genes were evaluated. The genes present in the various cotton varieties are *cry*1Ac (line DP50B known as Ingard®), *cry*1Ac and *cry*2Ab (line DP50BII known as Bollgard II®) and/or CP4 EPSPS (line DP50RR known as Roundup Ready®) genes. Cottonseed from the non-GM parental line DP50 was used as a control line.

Twelve lactating multiparous Argentinean Holstein cows weighing approximately 570 kg and 42-64 days in milk (DIM) at the beginning of the study were used. A 4x4 Latin Square design with three squares each containing four cows was used. Cows in each square were assigned to treatments for the first period (and three following periods), each period lasting 28 days in duration. All cows received the same diet of corn silage, alfalfa hay, ground corn, soybean meal, minerals and vitamins. The individual cottonseed treatments (2.75 kg/cow/d) or about 10% of the total dry matter intake were hand mixed in with the diet.

There were no significant differences ($P<0.05$) in total dry matter intake (DMI) or cottonseed intakes, ranging from 23.4-23.9 and 2.25-2.29 kg DM/cow/d, respectively among treatment groups. Milk yield, milk composition and body condition score (BCS) were comparable ($P>0.05$) among treatments.

These results indicate that genetically modified cottonseed varieties support similar performance as non-transgenic control cottonseed and did not affect dry matter intake, milk yield, milk composition and body condition score of dairy cows under controlled feeding conditions. It is concluded that cottonseed from the transgenic lines were as wholesome and nutritious as the parental line.

6.2 Dietary exposure assessment

Although exposure to CRY2Ab and GUS is considered to be nil, based on their very low presence in cottonseed and the processing that essentially removes any protein from refined oil, the Applicant estimated the dietary exposure possible in a worst-case scenario for CRY2Ab. This estimate assumes that protein could survive processing and be present at very low levels in cottonseed oil.

If it is assumed that cottonseed protein is present in refined oil at the limit of detection of the assay (1.3 µg protein/ml oil) and that CRY2Ab represents 0.02% of the total protein in the cottonseed, then there is 2.6×10^{-4} µg CRY2Ab present in one ml (which is equivalent to one gram) of oil.

Using US estimates of cottonseed oil consumption of 4.1 g/per day, this equates to 0.07 grams cottonseed oil /kg bodyweight for a 60 kg person. Therefore, the exposure to CRY2Ab from consumption of cottonseed oil is 1.8×10^{-8}. The NOEL from the CRY2Ab mouse gavage study is 1450 mg/kg (Section 4.2.2). Thus, there is a very conservative safety exposure margin of 8×10^{10}.

Conclusions

The animal feeding studies confirm that the introduced genes have not resulted in adverse effects on the nutritional adequacy of seed and meal derived from the test cottonseed line. No significant differences in fish growth, development or behaviour were observed between Channel catfish fed meal from cotton event 15985, control or commercially available lines. Similarly, cottonseeds from three genetically modified cotton lines fed to dairy cows supported typical performance in several criteria such as milk yield and composition and body score. These studies do not raise any public health or safety concerns with respect to the overall nutritional characteristics of the seed or meal from this transformed cottonseed. The compositional data of the oil and seed are supported by the two feeding studies.

Additionally, a dietary exposure assessment demonstrates that exposure to the CRY2Ab protein from cottonseed oil is highly improbable.

On the basis of the data provided for the comparative analysis, refined oil and cottonseed from cotton containing event 15985 are both compositionally equivalent to oil and cottonseed from other commercially available varieties of cotton.

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ATTACHMENT 3

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

A total of 57 submissions were received during the first round public consultation.

1. Oraina Jones (NZ)

- Opposes the approval of insect-protected cotton containing event 15985.
- Is concerned about the safety of GE foods for the following reasons:
  - There have been no long terms feeding or peer reviewed studies on these products.
  - That the DNA construct is unstable, as demonstrated by the report of unknown DNA in RoundUp Ready Soybean.
  - The way GM foods have entered the food chain makes it now impossible to prove the foods safe or unsafe.
  - There have been no long terms tests or independent, peer reviewed research done.
- Comment that plants sprayed with herbicide that normally they couldn't tolerate, look sick. They are therefore likely to be unable to uptake nutrients and are nutritionally inferior. Eating sick plants may have adverse effects on human health as did eating sick animals suffering BSE.
- Believes that:
  - ANZFA has again failed the consumer with current inadequate labelling requirements.
  - The legislation that allows ANZFA to make decisions on one aspect of GE and other agencies to have responsibility for others without any connection is flawed.

2. Environment and Conservation Organisation of New Zealand (Inc)

- Opposes the approval of insect-protected cotton containing event 15985.
- Considers that approval should not be given for the following safety reasons:
  - There have been no independent, peer reviewed, long terms tests done on these products or any feeding trials.
  - Approval should be delayed until the foods are independently verified and until then, all such foods withdrawn from the market.
  - ANZFA considers products in isolation without taking into account the range of GE foods available.
- Considers that the approval process is not equipped to adequately protect consumer as illustrated by:
  - The minute differences resulting in the contaminated tryptophan batch that had a detrimental effect.
  - The presence of unknown DNA in RoundUp Ready Soybeans that was also published by Belgian scientists.
- Comments that:
  - ANZFA would be foolish to be the first country in the world to approve this cotton variety for food.
  - Consumer choice has been undermined in the current labelling laws.
  - Approving GM foods is tantamount to approving the release of GE organisms into the environment and the dangers are still unknown.

- It would be precipitous of ANZFA to approve such foods prior to the completion of the NZ Royal Commission on Genetic Modification.

- Economic or trade issues such as promoting new technologies or products, should not be of primary concern to ANZFA, but food safety should be.

3. Mary Thompson (NZ)

- Opposes the approval of insect-protected cotton containing event 15985.
- Is concerned that these products, if permitted, will not be labelled thereby denying consumer choice.
- Is concerned about the safety of the food:
  - That GM introduces unnatural aspects into known food.
  - The potential for food products crossing the blood-brain barrier and the creation of new viruses.
  - That each new food product should be assessed as if a new drug.
  - That there could be long term consequences worse than those associated with BSE.
- Labelling according to the current standard does not require labelling of the food derived this cotton variety.

4. Laurence Melton, Shane Lal

- Reviewed the assessment as part of a Masters of Food Science course and concludes that:
  - The report is well documented and is based on published and/or international guidelines.
  - The one weakness in this comprehensive report is the lack of data on the long-term risks. This problem is not restricted to the ANZFA process but is a matter of worldwide concern.
  - The report represents a realistic compromise from a food standards and world trade perspective.
- Reviewed the impact analysis and comment that the considerable public opposition to GM foods for ethical, environmental or religious reasons cannot be ignored and neither can the diminishing public trust in food safety or food manufacturers. They therefore suggest that ANZFA:
  - Encourage debate and comment from all sectors, particularly IBAC.
  - Demonstrate a commitment to achieving its objectives.
  - Encourage ministers to ensure enforcement of GM regulations.
- Recommend that ANZFA:
  - participate in and contribute to the feasibility study on an international post-market health surveillance system proposed by the UK and OECD, when it is actioned.
  - establish an internal monitoring system to ensure that all councils/committees set up to perform a particular role in the development of new food regulations are held accountable.
  - Review progress being made by both Australian and New Zealand governments in the implementation of appropriate legislation and ministerial office to ensure new GM legislation is effective.
  - Enforcement and review of the draft GM food regulations to the ministerial council.

5. Consumers' Association of South Australia Inc.

- Supports the submission made by the National Council of Women of Australia.

6. Food Technology Association of Victoria

- Endorses the approval of insect-protected cotton containing event 15985.

7. New Zealand Ministry of Health

- Did not make a submission at this stage.

8. J. Scott, (NZ)

- Believes that:
  - Comprehensive and mandatory labelling is a basic human right
  - Agricultural experiments on GE foods have not resulted in higher yields and have been flops.
  - Allergy, autism and diabetes relate to adulterated food chains

9. Odile Balas; Tremane Barr; Ninian Blackburn; Chris Bone; Jill Bradley & Keith Aswood (Ocean Organics Ltd); Cheree Corbin; Walter Dendl-Jaud; Sabine Drueckler; Carmen Faulkner and Dave Hancox; Kristine Ford; Barry Foster; Irmgard Habl; Corey Hallett; Elora Hallett; Rowan Hallett; Stephen Hallett; Wolfgang Hiepe; Bonny 'Faulkner' Hollis; Claire Insley; Jenny Laycock; Baerbel Leeker; Heidelind Luschberger; Arana Horney; Wendy McGuiness; Michelle Jones; Marissa Oakley-Browne; Ruth Ordish-Benner; Rick, Sharon, Joe & Hamish Palmer; Marc Pawley; Michael Quinlan; Mark Rapley; Mark & Tanea Ray; Janet Redmond; Marion & Martyn Rix; Jenny Stewart; Brian Stoddard; Jackie Stoddard; Andrea Thomas; Sharyn van Heerden; Sam Vallings; Tim Vallings; John & Jeanne van Kuyk; Tanya Whitford; (NZ)

- Does not want GE foods in the national food supplies.
- Opposes the approval of any more GE foods entering the food supply.
- Comments include that:
  - Comprehensive and mandatory labelling is essential and a basic right.
  - Labelling of GM foods should be implemented immediately.
  - The precautionary principle should be used.
  - ANZFA should implement traceability of the food supply, long term monitoring and toxicology testing.
  - Approval should be delayed until they are proven safe.
  - Some scientists do not think it is safe and includes quotes.
  - All antibiotic resistance marker genes should be removed.

10. Irmgard Habl (see 9 above also)

- In addition to above, also comments that:
  - Organic growers will lose a valuable tool, i.e. use of *Bt,* as resistance to it develops.

11. Greenpeace

- Opposes the approval of insect-protected cotton containing event 15985.
- Comment that:

- There are grave environmental and human health concerns about genetically engineered organisms and a lack of supporting research about their impact in the food chain.
- The precautionary principle should be adopted.

▪ Provided information about the growing of Bt crops.
  - Indicated a section of the Biosafety Protocol that negates concerns about not meeting WTO requirements under the TBT agreement.

12. Betsey Kettle & David Kettle

▪ Comment that
  - Labelling of GM foods should be implemented immediately.
  - There is probably insufficient research on the impacts of decomposition of GE cotton on the soils and on the impacts of consuming GE cotton on human health.

13. Alex Lautensach (NZ)

▪ Believes that public safety issues are ignored by caving in to the lobbying efforts of industry.

14. Australian Food and Grocery Council

▪ Supports the approval of insect-protected cotton containing event 15985 contingent upon satisfactory safety assessment by ANZFA.
▪ Supports the commercialisation of this cotton if grown in Australia, subject to approval through the OGTR process.
▪ Considers that:
  - food manufacturers should make their own choice with regard to use of GM crops or products derived from them.
  - ANZFA must undertake its own safety assessment to fulfil its responsibility of protecting public health and safety.
  - An appropriate information and labelling scheme would enable consumers to make an informed choice.

15. National Council of Women of Australia

▪ Opposes the approval of insect-protected containing cotton event 15985.
▪ Comment that:
  - They are concerned about engineering the cotton a second time to try and achieve the original objective of insect protection and that he original modification did not therefore achieve its objective.
  - Cotton plants containing two *Bt* genes result in more *Bt* being used will increase the likelihood of the development of insect resistance and at a more rapid rate.
  - Labelling according to the current standard does not require labelling of the food derived this cotton variety and this is unacceptable as it denies consumer choice.
  - Safety has been compromised to allow innovation in the food industry.
  - The Application should be rejected until it approved by the OGTR.
  - The impact analysis of costs and benefits to government, industry and consumers is flawed.

- Seek clarification as to whether the cotton line will be compared to the GM parental line or a non-GM parental line.
- Consider the use of substantial equivalence outmoded and unscientific.

16. GE Free New Zealand

- Believe that the assessment process is inadequate and unacceptable.
- Regard GM foods as having unproven safety and that they do not possess the claimed benefits but instead have:
  - Lower yields with high pesticide/herbicide input.
  - Unstable inserts as demonstrated by the presence of unknown DNA in RoundUp Ready Soybeans
  - All the benefits going to the intellectual property owner.
- Comments that:
  - The current approval process should have more strict and consistent standards.
  - The labelling requirements still denies consumers choice about GM foods
- Deny that the NZ Ministry of Health are monitoring and enforcing Food Standards.
- Considers that New Zealand submissions have been ignored and raised issues that were considered by the New Zealand Royal Commission on Genetic Modification.

ATTACHMENT 4

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received in response to Gazette Notices in relation to GM foods express general views opposed to the use of gene technology and assert that food produced using this technology is unsafe for human consumption. The general issues, which are not necessarily specific to the Application, are addressed below.

1. ANZFA's processes

ANZFA's general processes for the risk assessment of GM foods have been criticised by several submitters from Australia and New Zealand.

Response

The processes used by ANZFA for safety assessment and labelling of GM foods were subject to an independent assessment by the New Zealand Royal Commission on Genetic Modification which was conducted during the first quarter of 2001. In its deliberations, the Royal Commission considered that both the New Zealand Environmental Risk Management Authority (ERMA) and ANZFA provided a robust regulatory environment and stated that the authorities acted conscientiously and soundly in carrying out their duties. The Commission expressed confidence in the ANZFA safety assessment process, stating that it considered it unlikely that foods that have satisfied the food standard will have harmful effects. The Commission also considered that ANZFA carries out its functions with an appropriate degree of independence not only from political influence but also from the influence of commercial interests. In reaching this view, it should be noted that the Commission examined the criticisms levelled at ANZFA's processes and the detailed rebuttal of those criticisms supplied to the Commission by ANZFA, including issues such as adequacy of the toxicological studies, use of substantial equivalence, sources and independence of data, and the use of antibiotic resistance marker genes.

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

2. Sources of data

The use of company data from the Applicant during the assessment is seen by some submitters to compromise the independence and validity of the safety evaluation.

Response

It is a requirement of the ANZFA assessment process that raw data from experiments supporting the safety of a GM food are submitted to ANZFA for assessment. These data are assessed in detail by ANZFA scientists and then the assessment report undergoes a robust process of internal review by ANZFA's own scientific experts and external review by ANZFA's expert panel and senior health officials from State and Territory and New Zealand Health Departments.

The quality and sources of the data supplied to ANZFA in support of applications for approval of GM foods was the subject of particularly intense scrutiny during ANZFA's evidence at the New Zealand Royal Commission on Genetic Modification. ANZFA submitted a full data package (15 volumes of raw data on Roundup Ready Soybeans) to the Commission for inspection. The Commission states that it looked closely at the quality of these data and came to the view that ANZFA did receive and assess raw data and that the processes were valid in this regard.

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

3. Imported GM foods versus GM crops

Some submitters have argued that approvals for GM foods or commodities as imports to Australia and New Zealand is a tacit approval for the GM crop to be grown in either country.

Response

The regulatory framework for approval by ANZFA of safety of GM foods (imported foods and derived from GM crops grown in Australia) is separate from that of the Office of the Gene Technology Regulator (OGTR) and the Environmental Risk Management Authority (ERMA), which have responsibility for approving the environmental release of GM crops in Australia and New Zealand respectively. ANZFA's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard A18 of the *Food Standards Code* (Standard 1.5.2 in Volume 2) cannot be regarded as tacit approval for the environmental release of the crop in Australia since the environmental issues are completely separate and entirely different to food safety issues.

4. Compositional studies

The compositional analysis occasionally reveals that some of the components of the genetically modified plant line under assessment are statistically different to the control line. Some submitters therefore claim that the GM line is not comparable to the control line.

Response

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

The use of published ranges and historical control data in safety assessment studies is standard procedure in the interpretation of biological and analytical components of variation. Although the most appropriate control group for interpretative purposes is always the concurrent control, there are instances in which the use of historical control information can aid an investigator in the overall evaluation of safety data. Studies (Carokostas and Banerjee (1990), *Interpreting Rodent Clinical Laboratory Data in Safety Assessment Studies: Biological and Analytical Components of Variation*, Fundamental and Applied Toxicology) suggest that statistically significant laboratory findings that are not biologically or toxicologically important will be present in many safety assessment studies with a standard design. An over-reliance on the result of standard prepackaged statistical analyses for determining the presence of toxicologically significant findings can lead to misinterpretation of laboratory data. It is well recognized that sound judgement must be applied to laboratory findings using appropriate statistical analyses as a tool for pattern recognition.

5. The safety of genetically modified foods for human consumption

Many submitters raise the issue of public health and safety in relation to food produced using gene technology. In particular, it is often stated that there has been inadequate testing of genetically modified foods, that there is limited knowledge concerning the risks associated with the technology and that there may be potential long–term risks associated with the consumption of such foods.

<u>Response</u>

It is a reasonable expectation of the community that foods offered for sale are safe and wholesome. In this context, *safe* means that there is a reasonable certainty of no harm. As with other aspects of human activity, the absolute safety of food consumption cannot be guaranteed. Conventionally produced foods, while having a long history of safe use, are associated with human disease and carry a level of risk which must be balanced against the health benefits of a nutritious and varied diet.

Because the use of gene technology in food production is relatively new, and a long history of safe use of these foods has yet to be established, it is appropriate that a cautious approach is taken to the introduction of these foods onto the market. The purpose of the pre–market assessment of a food produced using gene technology under Standard A18/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

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

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD).

The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

6. The need for long-term feeding studies

Concerns are often expressed in relation to the lack of long-term toxicity studies on genetically modified foods.

Response

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to laboratory animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

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

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

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

7. Substantial equivalence

Some submitters express concern regarding the use of the concept of substantial equivalence as part of the assessment process and reject the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

Response

Substantial equivalence embodies the concept that, as part of the safety assessment of a genetically modified food, a comparison can be made in relation to the characteristics and properties between the new food and traditionally produced food. This can include physical characteristics and compositional factors, as well as an examination of the levels of naturally occurring allergens, toxins and anti-nutrients.

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

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

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for over ten years and has been an integral part of the safety assessment of some 50 products.

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

8. The nutritional value of food produced using gene technology

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

Response

The assessment of food produced using gene technology by ANZFA entails an exhaustive evaluation of analytical data on any intentional or unintentional compositional changes to the food. This assessment encompasses the major constituents of the food (fat, protein, carbohydrate, fibre, ash and moisture) as well as the key nutrients (amino acids, vitamins, fatty acids). There is no evidence to suggest that genetic modification *per se* reduces the nutritional value of food.

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

9. Potential toxins and allergens

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

Response

This issue is considered in detail as part of the safety assessment conducted on each new genetic modification applied to a food or commodity crop. New toxins or allergens may be introduced into food by either gene technology or by traditional breeding techniques, or by altered production processes. It is also possible to use these techniques to develop foods specifically where such compounds are significantly reduced or eliminated. One advantage of gene technology, in comparison with these other methods, is that any transferred genes are well characterised and defined, thus the possibility of developing a food with a new toxic or allergenic compound is likely to be reduced.

10. Antibiotic resistance

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

Response

The human health considerations in relation to the potential for the development of antibiotic resistance depend on the nature of the novel genes and must be assessed on a case-by case basis. This issue arises because of the use of antibiotic resistance marker genes in the generation of genetically modified plants. In some circumstances, antibiotic resistance genes are linked to the gene of interest, to enable the initial selection of the engineered cells in the laboratory.

Those cells that contain the antibiotic resistance marker gene, and hence the gene of interest, will be able to grow in the presence of the antibiotic. Those cells that failed the transformation process are eliminated during the selection procedure.

Concern has arisen that ingestion of food containing copies of antibiotic resistance genes could facilitate the transfer of the gene to bacteria inhabiting the gut of animals and humans. It is argued that these genes may then be transferred to disease causing bacteria and that this would compromise the therapeutic use of these antibiotics.

In 1993, the World Health Organisation Food Safety Unit considered this issue at a Workshop on the health aspects of marker genes in genetically modified plants. It was concluded at that Workshop that the potential for such gene transfers is effectively zero, given the complexity of the steps required. Since this time, several separate expert panels (Report to the Nordic Council, Copenhagen 1996; Advisory Committee on Novel Foods and Processes, UK 1994, 1996; The Royal Society, UK 1998) and numerous scientific papers published in peer reviewed journals have also considered the available evidence on this issue. It is generally agreed that the presence and subsequent transfer of an intact functional gene from transgenic food to micro-organisms in the human intestine is an extremely unlikely event. Furthermore, if this were to occur, bacteria would not normally retain the resistance genes unless there was an environment for positive selection. The majority of these genes provide for resistance to antibiotics whose use is confined to the laboratory and are not considered to be of major therapeutic use in humans.

Antibiotic resistant bacteria are naturally occurring, ubiquitous and normally inhabit the gut of animals and humans. There is a general consensus that the transfer of antibiotic resistance genes is much more likely to arise from this source and from associated medical practices, rather than from ingested genetically modified food. Even so, at the OECD Conference (GM Food Safety: Facts, Uncertainties, and Assessment) held in Edinburgh on 28 February – 1 March 2000, there was general consensus that the continued use of antibiotic marker genes in GM food crops is potentially unnecessary given the existence of adequate alternatives, and therefore should be phased out.

The recent JETACAR (Joint Expert Technical Advisory Committee on Antibiotic Resistance) Report states (page 117, referring to a specific gene, *nptII*) that the use of antibiotic resistance genes in GM foods is unlikely to contribute in any significant way to the spread of antibiotic resistance in human pathogens. The issue of the use of antibiotic resistance marker genes in GM foods was discussed at the Ministerial Council meeting held in late July 2000. At that meeting, Professor John Turnidge, former Chair of JETACAR and now Chair of the NHMRC Expert Advisory Group on Antibiotic Resistance (EAGAR), appeared at the Council meeting as expert adviser on this matter in support of ANZFA's assessment on this issue.

11. Transfer of novel genes to humans

Some submitters have expressed the view that the transfer of any novel gene within the human digestive tract may be a health concern.

Response

It is extremely unlikely that novel genetic material will transfer from GM foods to bacteria in the human digestive tract because of the number of complex and unlikely steps that would need to take place consecutively. It is equally unlikely that novel genetic material will transfer from GM foods to human cells via the digestive tract. In considering the potential impact on human health, it is important to note that humans have always consumed large amounts of DNA as a normal component of food and there is no evidence that this consumption has had any adverse effect on human health. Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in GM foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

12. Viral recombination

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

Response

This is an issue that is commonly raised because some of the genes that are transferred to plants use a plant virus promoter. Promoters are controlling DNA sequences which act like a switch and enable the transferred genes to be expressed (i.e. to give rise to a protein product) in a plant cell. The routine use of these viral promoters is often confused with research which has shown that plant virus genes, which have been transferred into plants to render them virus–resistant, may recombine with related plant viruses that subsequently infect the plant, creating new viral variants. This research demonstrates that there may be a greater risk to the environment if viral genes are transferred to plants because it may lead to the generation of new plant virus variants capable of infecting a broader range of plants. This is a matter that is considered by the scientific technical committee of the Office of the Gene Technology Regulator (OGTR) on a case–by–case basis when assessing such projects.

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

13. Labelling of foods produced using gene technology

Submissions generally call for comprehensive labelling of foods produced using gene technology, based on perceptions that the foods are potentially not as safe as conventional foods, even where no novel genes are present. Based on consumer “right to know” arguments, it is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

Response

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard A18 (Volume 1) / 1.5.2 (Volume 2) in the *Food Standards Code* came into effect on 7 December 2001, allowing 12 months implementation period for compliance to the new provisions.

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

Exempt from these requirements are:

- highly refined food, where the effect of the refining process is to remove novel genetic material and/or protein;
- processing aids and food additives, except where novel genetic material and/or protein is present in the final food;
- flavours which are present in a concentration less than or equal to 0.1 per cent in the final food; and
- food intended for immediate consumption which is prepared and sold from food premises and vending vehicles, including restaurants, takeaway outlets, caterers or self-catering institutions.

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

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

14. The need for post marketing surveillance of genetically modified foods

A number of submitters have commented on the need for post-market surveillance of genetically modified food consumption.

Response

Surveillance of potential adverse or beneficial effects of GM foods is seen by many as a logical follow-up to the initial scientific risk assessment. Nevertheless, it is recognised that there are limitations to the application of epidemiology studies, particularly in relation to food components. A key requirement for post-market surveillance systems is that a clear hypothesis be identified for testing. Establishing a system for the surveillance of potential health effects of exposure to novel foods requires monitoring of the consumption patterns of novel foods in the population, and health effects in both “exposed” and “non-exposed” individuals/populations, so that risk estimates can be derived.

For any such monitoring system to be useful, there needs to be a range of exposures, otherwise, any variation in health outcome would be unexplainable by that exposure. Variations in exposure could be apparent over time (temporal trends), space (geographical trends) or both.

Availability of robust data on consumption of the foods in question is vital in order to establish a surveillance system. The other side of the equation is the need for access to data on population health outcomes. Such a system could also be used to identify potential positive health outcomes, such as improved nutritional status or lower cholesterol levels. The availability of linked basic data (e.g. date of birth, sex, geographical location), and the ability to correlate with demographic data, could potentially offer the means of establishing links with food consumption.

The possibility of setting up a post-market health surveillance system for novel foods, including GM foods, has been examined by the UK's Advisory Committee on Novel Foods and Processes (ACNFP). Recognising the many difficulties involved in developing such a system, an initial feasibility study to look at the available data and its usefulness has been proposed. Work is currently being commissioned; when completed in 18 months, it will be subject to peer review. If such a feasibility study suggests that post-market surveillance is practical, methods and details concerning data collection will be determined in the UK, but common strategies might be able to be harmonised internationally in order to minimise the use of resources while maximising the reliability of the final results. This is an area that ANZFA will be monitoring closely, along with international regulatory bodies such as the OECD Taskforce for the Safety of Novel Foods and Feeds.

15. Public consultation and information about gene technology

A number of submitters were concerned that the public has not been properly consulted or informed by government or ANZFA on the introduction of foods produced using gene technology. Some submitters urged to undertake wider consultation with all affected parties including growers, the food industry and consumers before these food commodities are introduced, and to ensure that adequate consultation is undertaken as part of its assessment process.

Response

The issue of gene technology and its use in food has been under consideration in Australia since 1992. The Agreement between the Governments of Australia and New Zealand for a joint food standard setting system, however, did not occur until 1995, and the New Zealand community therefore had not been consulted on this matter by the Authority until after that time. Consequently, the proposed standard for GM foods underwent only one round of public comment in New Zealand at which time significant objections were raised by the New Zealand community to the use of gene technology in food production. Many New Zealand consumers, in previous submissions to the Authority, have expressed the view that there has been insufficient consultation and a consistent lack of information about gene technology.

Although Standard A18 came into force in May 1999, the public have a continuous and ongoing opportunity to provide comment in relation to applications under the standard. ANZFA's statutory process for all applications to amend the *Food Standards Code* normally involves two rounds of public comment.

Furthermore, all the documentation (except for commercial in confidence information) relating to these applications is available in the public domain, including the safety assessment reports. There is ample evidence that the provision of such information by ANZFA has already significantly stimulated public debate on this matter.

In addition, other government departments including the Environmental Risk Management Authority (ERMA) are potential sources of information about gene technology available to consumers in New Zealand. ERMA is a statutory authority set up by the New Zealand Government to administer the *Hazardous Substances and New Organisms (HSNO) Act 1996*, and has responsibility for assessing the risks to the environment from genetically modified organisms. This body has been assessing applications for the approval of genetically modified organisms since July 1998 and this has involved a number of public meetings.

In response to the concerns raised in public submissions with regard to gene technology and GM foods, ANZFA has prepared a public discussion paper on the safety assessment process for GM foods[15], available at no charge on request. Since completion, this document has been widely distributed and may assist in addressing some of the safety concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

16. Maori beliefs and values

Some New Zealand submitters stated that Maori people find genetic engineering in conflict with their beliefs and values and that, out of respect to Maori, no genetically modified foods should be allowed into New Zealand until a wider discussion, both within Maori and non–Maori, is held.

Response

This issue was also raised during consideration of the proposal for the establishment of Standard A18. At that time, it was stated that the likely implications for Maori regarding genetically modified organisms surround the issues of the rights of Maori to the genetic material from flora and fauna indigenous to New Zealand and the release into the environment of genetically modified organisms. The *HSNO Act 1996* requires that these matters be considered by ERMA.

17. Environmental concerns and the broader regulatory framework

A number of submitters have raised concerns that genetically modified crops may pose a risk to the environment.

Response

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

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

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

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

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

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

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

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

18. Maximum residue levels of agricultural/veterinary chemicals

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

Response

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

It is important to note that MRLs are not direct public health and safety limits but rather, are primarily indicators of appropriate chemical usage. MRLs are always set at levels lower than, and normally very much lower than, the health and safety limits.

The MRL is determined following a comprehensive evaluation of scientific studies on chemistry, metabolism, analytical methods and residue levels. In Australia, the National Registration Authority (NRA) applies to ANZFA to amend the MRLs in the *Food Standards Code* and the application is considered by ANZFA through its legislated decision making processes. In New Zealand MRLs are set by the Ministry of Health, generally following a request from, and in collaboration with, the Ministry of Agriculture and Forestry. Only following demonstration that the use of agricultural and veterinary chemicals will not result in unsafe residues will the MRL enter into food law, through its inclusion in either the *Food Standards Code* in Australia, or the *New Zealand Mandatory Food Standard 1999 (Maximum Residue Limits of Agricultural Compounds)*.