



DRAFT RISK ANALYSIS REPORT

APPLICATION A355

Food produced from glyphosate-tolerant cotton line 1445

Note:

This report is the “Full Assessment” as referred to in Section 15 of the *Australia New Zealand Food Authority Act (1991)*.

Public comments are now sought before completion of a Final Risk Analysis Report (referred to as the ‘Inquiry’ under Section 16 of the Act). See under ‘Invitation for Public Submissions’ for details.

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EXECUTIVE SUMMARY

Background

An application was received from Monsanto Australia Ltd on 12 December 1997 for the approval of food derived from genetically modified (GM) cotton line 1445. The cotton has been genetically modified to confer tolerance to the application of the herbicide glyphosate, the active ingredient of Roundup®, and is known commercially as Roundup Ready® cotton. This report describes the scientific assessment of the application.

Issues addressed during assessment

(i) *Safety evaluation*

The glyphosate-tolerant cotton has been evaluated according to ANZFA's safety assessment guidelines. This has involved an extensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with this new GM food. This approach can establish whether foods produced from the glyphosate-tolerant cotton is as safe and nutritious as foods produced from non-GM varieties of cotton ie refined cottonseed oil and processed linters.

The detailed information available on the genetic modification indicate that no unintentional changes have taken place at the molecular level and that the novel genetic material is stably inserted and maintained over several generations.

Data on the potential toxicity and allergenicity of the proteins encoded by the transferred genes have been reviewed and indicate that the new proteins expressed in the glyphosate-tolerant cotton are non-toxic and unlikely to have allergenic potential.

Compositional analyses demonstrated no significant differences between glyphosate-tolerant cotton and its conventional counterpart. This constitutes further evidence that no unintentional effects have occurred as a result of the genetic modification.

The impact on human health from potential transfer of novel genetic material to cells in the human digestive tract has also been considered. The presence of novel genetic material, including a kanamycin resistance gene and a streptomycin resistance gene, in the glyphosate-tolerant cotton is not considered to pose any additional safety concerns.

Cottonseed is not considered to be a human food due to the presence of naturally-occurring toxins. The oil derived from cottonseed is highly refined and does not contain detectable levels of naturally occurring toxins. The processed linters derived from cottonseed are also highly refined and consist of ~99% cellulose and do not contain detectable levels of naturally-occurring toxins. The genetic modification of cotton has not changed this pattern of consumption.

ANZFA has concluded, through assessing all of the above data, that cottonseed oil and linters derived from glyphosate-tolerant cotton do not raise any public health and safety concerns.

(ii) *Labelling*

On the basis of the data considered in the safety evaluation, cottonseed oil and linters derived from glyphosate-tolerant cotton were found to be substantially equivalent to those from non-GM cotton. Therefore no mandatory labelling is required under the current Standard A18.

It should be noted that the labelling provisions in Standard A18 are in the process of being amended. This may result in some changes to the way in which some GM foods, including those derived from glyphosate-tolerant cotton, are labelled.

(iii) Public submissions

Fifty-eight public submissions were received in relation to this application, of which only three were supportive. Those opposing the application did so primarily on the basis that they perceive GM food to be unsafe. The food safety concerns raised in submissions have been addressed by the safety assessment report.

Conclusion

ANZFA considers that refined oil and processed linters derived from glyphosate-tolerant cotton are as safe for human consumption as refined oil and processed linters derived from other commercial cotton varieties. ANZFA is therefore proposing an amendment to the *Australian Food Standards Code* to include refined oil and processed linters derived from glyphosate-tolerant cotton. Based on the data submitted in the present application and in accordance with Standard A18, ANZFA proposes that, as foods derived from glyphosate-tolerant cotton are substantially equivalent to their conventional counterpart, no mandatory labelling is required. Foods derived from glyphosate-tolerant cotton will, however, be required to comply with any new labelling provisions of the standard.

ANZFA will now seek public comment on the proposed amendment to Standard A18 of the *Food Standards Code* (in accordance with the procedures described in section 17 of the *Australia New Zealand Food Authority Act 1991*).

INVITATION FOR PUBLIC SUBMISSTIONS

The Authority has completed a Draft Risk Analysis Report on this application (referred to as the 'Full Assessment' in section 15 of the Act), which includes a draft Safety Assessment Report and draft variation to the Australian *Food Standards Code*. The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the *Food Standards Code*, and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' under section 16 of the Act).

Written submissions containing technical or other relevant information that will assist the Authority in preparing the Final Risk Analysis Report for this application are invited from interested individuals and organizations. 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.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. The *Australia New Zealand Food Authority Act 1991* requires the Authority to treat in confidence trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A355** at one of the following addresses:

Australia New Zealand Food Authority

PO Box 7186
Canberra Mail Centre ACT 2610
AUSTRALIA
Tel (02) 6271 2222 Fax (02) 6271 2278
Email info@anzfa.gov.au

PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942 Fax (04) 473 9855
Email nz.reception@anzfa.gov.au

Submissions should be received by the Authority **by 30 August 2000** .

General queries on this matter and other Authority business can be directed to the Standards Liaison Officer at the above address or by Email on slo@anzfa.gov.au. Submissions should not be sent by email as the Authority cannot guarantee receipt. Requests for more general information on the Authority can be directed to the Information Officer at the above addresses.

INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a bi-national statutory body responsible for making recommendations on food standards which, when approved by the Australia New Zealand Food Standards Council (ANZFSC), are adopted by reference and without amendment into food law. ANZFA is currently working to establish a joint Australia New Zealand Food Standards Code that will apply in both countries. In the interim, a system of dual standards operates for the majority of the food standards. However Standard A18 – Foods Produced using Gene Technology - has been accepted by New Zealand and currently applies in both countries.

Standard A18 was adopted by ANZFSC as a joint Australia/New Zealand standard in July 1998 and came into force on 13 May 1999. Under this Standard, the sale of food produced using gene technology is prohibited unless the food is included in the Table to Clause 2 of the Standard. The Standard requires that a pre-market safety assessment be conducted on all foods produced using gene technology. However, the Standard provides an interim arrangement for those foods currently on the market provided that an application was accepted by ANZFA on or before 30 April 1999, that the food is lawfully permitted in a country other than Australia or New Zealand, and that ANZFSC has not become aware of evidence that the food poses a significant risk to public health and safety.

BACKGROUND TO THE APPLICATION

ANZFA received an application from Monsanto Australia Ltd on 12 December 1997 to amend the Australian *Food Standards Code* to include food produced from glyphosate-tolerant cotton line 1445, known commercially as Roundup Ready® cotton, in the Table to Clause 2 of Standard A18 – Food Produced using Gene Technology.

Commercial cotton line Coker 312 was genetically modified to produce line 1445 in order to provide tolerance to the broad spectrum herbicide glyphosate (Roundup®).

The glyphosate-tolerant cotton is protected against the herbicidal effect of glyphosate through the transfer of the gene encoding 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* strain CP4.

Only refined cottonseed oil and cellulose from processed linters are used in human foods. Therefore the principle food products derived from glyphosate-tolerant cotton are likely to be processed food commodities such as frying oil, mayonnaise, salad dressing, shortening, margarine and packing oil in the case of refined oil or high fibre dietary products, sausage casings and thickeners in ice cream and salad dressings in the case of processed linters. Whole cottonseed is not used as human food, therefore glyphosate-tolerant cotton will not be sold as fresh produce in Australia or New Zealand.

Glyphosate-tolerant cotton is not currently grown in either New Zealand or Australia but has been the subject of research and field trials in Australia by Monsanto Australia Ltd under GMAC planned release guidelines, including proposal PR-83X(2). An application for approval of the commercial release of glyphosate-tolerant cotton in Australia is currently being assessed by the Interim Office of the Gene Technology Regulator (IOGTR).

The principal benefits from glyphosate-tolerant cotton are agronomic in nature, and are therefore likely to accrue mainly to primary producers by allowing a more flexible weed control regime leading to improved crop management, more sustainable agricultural practices and reduced production costs. More general benefits, however, may also flow to the community as a result of reduced primary production costs.

PUBLIC CONSULTATION

The Authority received the first six applications for foods produced using gene technology from Monsanto Australia Ltd. Due to commonalities in these applications, a combined Notice of Application (formally referred to as the Preliminary Assessment Report) was advertised on 28 October 1998, which called for public comment on the applications. A total of 58 submissions were received in response to the combined Notice of Application, of which 53 relate to this application. The submissions were primarily from individuals, consumer organisations and special interest groups from both New Zealand and Australia. The submissions are summarised in Attachment 5.

In the case of foods produced using gene technology, changes to Standard A18 have been notified to the WTO because there is significant international interest in the safety of these foods.

ISSUES ADDRESSED DURING ASSESSMENT

1. Safety assessment

The safety assessment was performed according to the safety assessment guidelines prepared by ANZFA¹ and considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of antibiotic resistance genes to gut microorganisms; (3) toxicological issues; and (4) nutritional issues.

Nature of the genetic modification

Three genes were transferred to the glyphosate-tolerant cotton using *Agrobacterium tumefaciens*-mediated transformation –CP4 EPSPS, *nptII* and *aad*.

The CP4 EPSPS gene is derived from *Agrobacterium* strain CP4, and encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. The EPSPS enzyme of plants is inhibited by glyphosate, however bacterial EPSPSs, such as CP4 EPSPS, have reduced affinity for glyphosate. The expression of the CP4 EPSPS protein confers tolerance to the herbicide glyphosate, known commercially as Roundup®.

The *nptII* gene encodes the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the antibiotics neomycin, kanamycin, and geneticin (G418). It was used as a marker to assist in the selection of transformed plant cells (i.e. cells to which the gene of interest has been transferred).

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

The *aad* gene encodes the enzyme aminoglycoside adenylyltransferase (AAD), which confers resistance to spectinomycin and streptomycin. It was used as a marker to allow selection of bacteria containing the plasmid construct used in the transformation of cotton.

All three genes transferred appear to be stably integrated and maintained in glyphosate-tolerant cotton plants over multiple generations.

General safety issues

The protein expression levels of the CP4 EPSPS and *nptII* genes in cotton line 1445 are low. The CP4 EPSPS protein is expressed in cottonseed at levels ranging from 0.02% to 0.028% of total protein. NPTII is expressed in the cottonseed at 0.0022% of total protein. No AAD expression was detected in line 1445, as predicted from the properties of the *aad* gene construct. Refined cottonseed oil and cellulose from processed linters are considered to be free of protein. No CP4 EPSPS protein could be detected in linters after the first processing step.

The impact on human health from the potential transfer to gut microorganisms of either of the antibiotic resistance genes *nptII* or *aad* introduced into glyphosate-tolerant cotton was evaluated. It was concluded that the probability of antibiotic gene transfer was extremely low and that, even should either gene be transferred and expressed, it would not have any clinical implications as kanamycin and streptomycin resistant bacteria are already commonly found in the human gut and in the environment. In addition, no DNA could be detected in refined cottonseed oil from glyphosate-tolerant cotton line 1445.

Toxicological issues

The levels of naturally-occurring toxins in glyphosate-tolerant cottonseed were assessed as well as the potential toxicity and allergenicity of the two novel proteins — CP4 EPSPS and NPTII.

The only naturally-occurring toxins in cottonseed are the terpenoid gossypol, and the cyclopropanoid fatty acids sterculic acid, dihydrosterculic acid and malvalic acid. The levels of gossypol and the cyclopropanoid fatty acids in seed from glyphosate-tolerant cotton are similar to levels normally found in other commercial varieties of cotton indicating that the genetic modification process has not altered the levels of naturally-occurring toxins. No gossypol was detected in refined cottonseed oil from glyphosate-tolerant cotton.

The toxicity of the CP4 EPSPS and NPTII proteins has been assessed using toxicity testing in mice with no adverse findings. On the basis of this evidence, it can be concluded that the toxicity of the two novel proteins in glyphosate-tolerant cotton is low.

In terms of the potential allergenicity of the two new proteins, it has previously been concluded that both CP4 EPSPS and NPTII are unlikely to be allergenic to humans. Biochemical characterisation of the CP4 EPSPS protein from glyphosate-tolerant cotton supported this conclusion.

Nutritional issues

The only human foods derived from cotton are refined oil and cellulose from processed linters. Detailed compositional analyses were carried out to establish the nutritional adequacy of the glyphosate-tolerant cottonseed and refined oil. Constituents analysed in cottonseed were proximate (total protein, fat, ash, total carbohydrates, calories and moisture), amino acid, and fatty acid content. The fatty acid and α -tocopherol content of refined oil were also determined. These analyses confirmed that the glyphosate-tolerant cotton is compositionally equivalent to other commercial cotton cultivars. Animal feeding studies were not considered essential in this case because sufficient information had been provided about the genetic modification and the composition of the food.

Conclusion

No potential public health and safety concerns have been identified in the assessment of glyphosate-tolerant cotton line 1445. The refined oil and processed linters derived from glyphosate-tolerant cotton can be regarded as equivalent to those derived from commercially available cotton cultivars in respect of their composition, safety and end use.

2. Labelling of the GM food derived from glyphosate-tolerant cotton

Clause 3 of Standard A18 prescribes mandatory labelling of a food produced using gene technology when it contains new or altered genetic material and where it is not substantially equivalent in any characteristic or property of the food. As glyphosate-tolerant cotton has been found to be equivalent to other commercial varieties of cotton there is no requirement for mandatory labelling under the current standard.

It should be noted, however, that the labelling provisions in Standard A18 are in the process of being amended which may result in the mandatory labelling of some glyphosate-tolerant cotton food products.

3. Issues arising from public submissions

3.1 General issues

The general issues raised in the 53 public submissions have been evaluated and are included in Attachment 6. Only those issues raised in submissions that are specific to this application are addressed below.

It was noted that very few of the comments received in relation to the first 6 applications from Monsanto specifically addressed any of the details of the individual applications. The majority of submissions were opposed to the applications as a whole, for a variety of reasons encompassing the broad social, environmental, philosophical or ethical aspects of the use of gene technology in the production of food. Consequently, many of the issues raised were often focussed on matters beyond the scope of the specific safety assessment process conducted by the Authority.

However, a significant number of submissions raised issues concerning the short and long term safety of the food, or provided comment in relation to aspects of the technology in

terms of human safety. In addition, as this group of applications all involved a trait for herbicide tolerance or insect protection, significant comments and information were provided in association with these particular genetic characteristics.

3.2 Specific issues

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

(i) *Use of whole cottonseed in human nutrition*

GeneEthics (Australia) expressed concerns about the ingestion of novel DNA and proteins from cottonseed sprouts.

Evaluation

Whole cottonseed, cottonseed sprouts, cottonseed meal and cottonseed flour are not used as human food, and have a restricted role as animal feed, because of the presence of the natural toxicants gossypol and cyclopropenoid fatty acids. The only foods derived from cottonseed are refined oil and cellulose from processed linters. Protein is removed from these products by processing. No CP4 EPSPS protein was detected in linters after the first processing step. No DNA could be detected in refined oil from cottonseed of line 1445.

(ii) *Exposure to novel proteins through animal feeding*

The National Council of Women of Australia expressed concern that the novel proteins expressed in glyphosate-tolerant cotton will enter the food chain through feeding of cottonseed meal to animals, and that there should be scientific certainty that consumers will not be exposed to the novel proteins.

Evaluation

The CP4 EPSPS and NPTII proteins ingested by domestic animals in feed derived from Glyphosate-tolerant cottonseed do not pose any risk to humans. Both proteins have been shown to be destroyed by digestive conditions. The levels of these proteins are very low, even in whole cottonseed. Proteins are removed from refined cottonseed oil and linters by the chemical and physical treatments employed in processing.

(iii) *Exposure to novel proteins in oil and linters*

The West Australian Food Advisory Committee (Australia) questioned whether the applicant's assertion that refined oil and processed linters are free of DNA or protein.

Evaluation

It is generally accepted that the processing steps degrade and remove DNA and protein from refined oil and processed linters. No CP EPSPS DNA could be detected in refined oil from glyphosate-tolerant cotton. No CP4 EPSPS protein was detected in linters after the first processing step. Additional data presented by Monsanto Australia Ltd in support of Application A341 regarding Ingard cotton (genetically modified to express insecticidal

Bt proteins) showed that no protein could be detected in refined cottonseed oil from either GM or conventional cotton down to a detection limit of 1.3 ppm.

(iv) Potential allergenicity of novel proteins

The Consumers Federation of Australia questioned whether the potential allergenicity of the novel proteins expressed in glyphosate-tolerant cotton had been adequately addressed.

Evaluation

Both the CP4 EPSPS and NPT II proteins have been shown to be readily degraded in simulated mammalian digestive systems. CP4 EPSPS shares significant homology with plant EPSPSs and there are no reports of allergic reaction to plant EPSPSs. Neither the CP4 EPSPS nor the NPTII proteins shows any similarity to any known allergens nor are the genes for these proteins derived from a known source of allergen.

The only foods derived from cottonseed are refined oil and cellulose from processed linters. Neither is considered to contain detectable levels of protein because of the processing steps involved. No CP4 EPSPS protein was detected in linters after the first processing step. In addition data presented by Monsanto Australia Ltd in support of Application A341 regarding Ingard cotton (genetically modified to express insecticidal Bt proteins) showed that no protein could be detected in refined cottonseed oil from either GM or conventional cotton down to a limit of 1.3 ppm. Many refined oils have been shown not to be allergenic even if the source, eg peanuts, can be allergenic.

(v) Animal feeding trials

The Consumers Federation of Australia noted that no animal feeding studies had been submitted as part of the application.

Evaluation

The provision of animal feeding studies is not mandatory under the application guidelines. As the human food products are refined oil and cellulose from processed linters it would be very difficult to design a feeding study which could provide meaningful data on the effect of the GM trait because of the confounding effects of nutritional implications of feeding large amounts of these foods.

The compositional and other data provided in the application is considered adequate to establish the nutritional adequacy and safety of refined cottonseed oil and processed linters from glyphosate-tolerant cotton.

4 Risk management

Under Standard A18, a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines. The requirement for the food to be labelled must also be assessed in accordance with the labelling criteria specified in Clause 3 of the standard.

On the basis of the conclusions of the safety assessment report, together with a consideration of the public submissions, it is proposed that Table 1 to Clause 2 of Standard A18 be amended to include refined oil and processed linters from glyphosate-tolerant cotton line 1445. The proposed amendment is provided in Attachment 1.

In relation to labelling of the food, the safety assessment report found refined oil and processed linters from glyphosate-tolerant cotton line 1445 are substantially equivalent to those derived from commercially available cotton in terms of their safety and nutritional adequacy. Therefore, under the current standard, no mandatory labelling is required.

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

REGULATORY IMPACT ASSESSMENT

The benefits and costs associated with the proposed amendment to Standard A18 have been analysed in a draft Regulatory Impact Statement (Attachment 3). The benefits of the proposed Standard A18 amendment to approve food from glyphosate-tolerant cotton primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

CONCLUSIONS

It is concluded that:

- the introduced genes in glyphosate-tolerant cotton line 1445 do not produce any increased public health and safety risk;
- based on the data submitted in the present application, the human food fractions, refined oil and processed linters, are equivalent to those from other commercial varieties of cotton in terms of their safety and nutritional adequacy;
- refined oil and processed linters derived from glyphosate-tolerant cotton do not require labelling under the current provisions of Standard A18 as they are substantially equivalent to food derived from non-GM cotton. Proposed amendments to the labelling provision of Standard A18 currently under consideration could result in some glyphosate-tolerant cotton food products being labelled in the future; and
- the benefits to government, consumers and industry associated with the proposed amendment outweigh the costs.

ATTACHMENTS

1. Draft variation to the Australian *Food Standards Code*
2. Draft safety assessment report
3. Draft regulatory impact assessment
4. World Trade Organization Agreements
5. Summary of public comments
6. Discussion of general issues

DRAFT VARIATION TO THE AUSTRALIAN FOOD STANDARDS CODE

A355 – OIL AND LINTERS FROM GLYPHOSATE-TOLERANT COTTON

Standard A18 is varied by inserting into Column 1 of the Table to clause 2 -

Oil and linters derived from glyphosate-tolerant cotton line 1445.

SAFETY ASSESSMENT REPORT

**A355 – Food derived from glyphosate-tolerant
cotton line 1445**

SUMMARY AND CONCLUSIONS

The glyphosate-tolerant cotton Line 1445 has been assessed by ANZFA to evaluate its safety as a source of food. Line 1445 is also known commercially as Roundup Ready® (RR) cotton. A number of criteria have been addressed in this assessment including: a characterisation of the genes, their origin and function; the changes at the DNA, protein and whole food levels; stability of the introduced genes in the cotton genome; compositional analyses; evaluation of intended and unintended changes; and the potential of the newly expressed proteins to be allergenic or toxic.

Nature of the genetic modification

Glyphosate-tolerant cotton Line 1445 (Roundup Ready® cotton) was generated by the transfer of the CP4 EPSPS gene which confers glyphosate tolerance to the plant. The CP4 EPSPS protein is an enzyme that is not sensitive to inhibition by glyphosate because of reduced affinity for glyphosate.

Other genes transferred along with the CP4 EPSPS gene were the *nptII* gene and the *aad* gene. The *nptII* gene was used as a marker to select transformed plant cells during the cotton transformation procedure. It codes for the enzyme neomycin phosphotransferase and is derived from Transposon Tn5 from the bacterium *Escherichia coli*. It confers resistance to the aminoglycoside antibiotics neomycin, kanamycin and gentamicin. The *aad* gene is a marker used to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken prior to transformation of the plant cells. It codes for the enzyme aminoglycoside adenyltransferase (AAD) and confers resistance to the antibiotics spectinomycin and streptomycin.

The molecular and genetic analyses indicated that the introduced genes have been stably integrated into the genome of glyphosate-tolerant cotton line 1445 and were stably inherited for multiple generations.

General safety issues

The only human food products derived from cotton are refined oil and processed linters (>99% cellulose) and these have a long history of safe use as human food. Refined oil and linters may be used in processed foods such as frying oil, mayonnaise, salad dressing, margarine, high fibre dietary products, sausage casings and thickeners.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells or bacteria in the human digestive tract. Much of the concern in this regard is with the presence of antibiotic resistance genes in genetically modified foods. In the case of the glyphosate-tolerant cotton, it was concluded that the *nptII* and *aad* genes would be extremely unlikely to transfer to bacteria in the human digestive tract because of the number and complexity of the steps that would need to take place consecutively. More importantly however, in the highly unlikely event that transfer did occur, the human health impacts would be negligible because kanamycin and streptomycin resistant bacteria are already commonly found in the human gut and in the environment. Transfer of novel genetic material from the glyphosate-tolerant cotton to human cells via the digestive tract was also considered to be equally unlikely.

Processing of cottonseed to produce refined oil and cellulose from linters is expected to remove and destroy DNA, further reducing the chances of any transfer. No DNA was detected in refined cottonseed oil of line 1445.

Toxicological issues

The *aad* gene is not expressed in Line 1445. CP4 EPSPS and NPT II proteins are present at very low levels in cottonseed of Line 1445. Refined cottonseed oil and processed linters are considered free of protein. No CP4 EPSPS protein was detected in linters after the first processing step.

Data for the newly expressed CP4 EPSPS and NPT II proteins in glyphosate-tolerant cotton line 1445 have been evaluated for its potential toxicity to humans. Previous studies showed no signs of toxicity among mice following acute oral doses up to 572 mg/kg for CP4 EPSPS and 5000 mg/kg for NPT II. No significant similarity to the amino acid sequences of known toxins was identified for either protein.

Neither of the expressed proteins exhibits characteristics of known allergens. Both proteins have been shown to be rapidly digested in simulated mammalian digestive systems. Amino acid sequence analyses did not reveal any similarities to known allergens.

Therefore, the evidence does not indicate that there is any potential for either protein to be toxic or allergenic to humans.

Nutritional issues

The compositional analyses were comprehensive and indicated that there are no substantial differences in the levels of major constituents, nutrients, anti-nutritional factors or natural toxicants in cottonseed between glyphosate-tolerant cotton Line 1445 and the control line Coker 312, and that there was no change due to the application of glyphosate during cultivation. The components measured were proximate (protein, fat, moisture, fibre, ash, carbohydrates and calories), fatty acids and amino acids.

Analysis of the refined oil demonstrated that the composition is comparable in all respects to the control line C312.

The levels of natural toxicants of cotton, gossypol and the cyclopropenoid fatty acids, were also assessed. Cottonseed of Line 1445 was found to have a slightly higher gossypol content compared to Coker 312 but well within the range for commercial cultivars. No gossypol was detected in refined cottonseed oil of either Line 1445 or the control line C312. There was no difference in the levels of cyclopropenoid fatty acids in either cottonseed or refined oil from line 1445 and C312 indicating that the genetic modification process has not altered the levels of naturally-occurring toxins.

These analyses confirm that insect protected cotton line 1445 is nutritionally and compositionally comparable to other cotton lines and that no health or safety risks are posed by consuming food derived from the genetically modified cotton.

Conclusion

No potential public health and safety concerns have been identified in the assessment of glyphosate-tolerant cotton line 1445 which will be marketed as Roundup Ready® cotton. Based on the data submitted in the present application, refined oil and processed linters derived from glyphosate-tolerant cotton line 1445 can be regarded as equivalent to those foods derived from conventional cotton in terms of their safety and nutritional adequacy.

1 BACKGROUND

Monsanto Australia Ltd have submitted an application to ANZFA to vary Standard A18 (ANZFA 1999) to include foods derived from glyphosate-tolerant cotton, known commercially as Roundup Ready® (RR) cotton, in the Table to the standard.

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

The only human food products obtained from the cotton (*Gossypium hirsutum*) are cottonseed oil and linters. Cottonseed oil is a premium quality oil that may be used in a variety of foods including frying oil, mayonnaise, salad dressing, shortening, margarine and packing oil. Linters are short fibres removed from the cottonseed during processing (delinting). After extensive processing at alkaline pH and high temperatures, the linters may be used as high fibre dietary products, sausage casings and thickeners in ice cream and salad dressings. The linters consist primarily of cellulose (>99%).

The RR cotton described in this application is glyphosate-tolerant cotton Line 1445. The cotton was developed by Monsanto Ltd for cultivation in the United States and was approved for commercial release in the USA in September 1995. Cottonseed harvested from these plants or processed products containing cottonseed oil or linters may have been imported into Australia and New Zealand since 1996.

Glyphosate-tolerant cotton has been the subject of research and field trials by Monsanto Australia Ltd under GMAC planned release guidelines, including proposal PR-83X(2). An application for approval to commercially release glyphosate-tolerant cotton is currently being assessed by the Interim Office of the Gene Technology Regulator (IOGTR).

The data regarding the generation and characterisation of glyphosate-tolerant cotton line 1445 have been published in the scientific literature (Nida *et al* 1996a, 1996b, Sims *et al* 1996).

2 DESCRIPTION OF THE GENETIC MODIFICATION

Monsanto Australia Limited submitted the following reports:

Barry, G.F., Taylor, M.L., Padgett, S.R., Kolacz, K.H., Hallas, L.E., della-Cioppa, G. and Kishore, G.M. 1994. Cloning and expression in *Escherichia coli* of the glyphosate-to-aminomethylphosphonic acid degrading activity from *Achromobacter* sp. strain LBAA. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13245

Padgett, S.R., Barry, G.F., Re, D.B., Eichholtz, D.A., Weldon, M., Kolacz, K., Kishore, G.M. 1993. Purification, cloning and characterisation of a highly glyphosate-tolerant 5-enolpyruvylshikimate-3-

phosphate synthase from *Agrobacterium* sp. strain CP4. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12738

Shaw, M. 1997 Safety, compositional and nutritional aspects of cotton with the Roundup Ready gene, Line 1445: conclusion based on studies and information evaluated according to FDA's policy on foods from new plant varieties. from Kolacz, K., Nida, D.L. and Serdy, F.S. 1995 Monsanto #95-106.

2.1 Methods used in the genetic modification

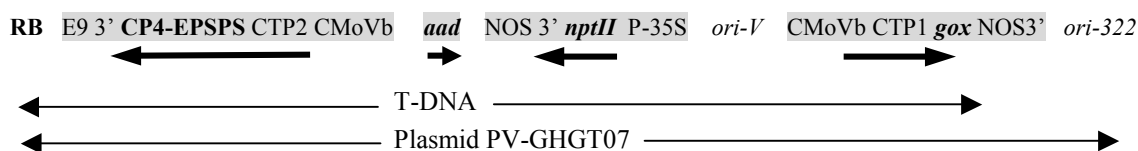
The glyphosate-tolerant (Roundup Ready®) cotton Line 1445 was produced by *Agrobacterium*-mediated transformation of the parental cotton line, *Gossypium hirsutum* L. cv Coker C312, with plasmid PV-GHGT07. The *Agrobacterium*-mediated DNA transformation system is well understood (Zambryski, 1992).

The genes of interest were inserted into the plasmid adjacent to a DNA sequence known as the right border (RB). There is no *Agrobacterium* left border (LB) sequence present in PV-GHGT07 (Nida *et al* 1996a). The LB and RB have been isolated from Ti plasmids from *Agrobacterium* and are 25 base pair repeat sequences that delimit the DNA to transferred (T-DNA) in the transformation event. However it has been shown that the left border is not essential for integration of DNA into the plant genome (Jen and Chilton, 1986).

The plasmid PV-GHGT07 carries the genes encoding EPSPS from *Agrobacterium* strain CP4 (CP4-PESPS, Padgett *et al* 1996a), which is resistant to inhibition by glyphosate and the *nptII* gene which confers resistance to kanamycin (Beck *et al* 1982). Transformed plants were selected on the basis of their ability to grow in the presence of the antibiotic kanamycin conferred by the transfer of the *nptII* gene.

In addition, plasmid PV-GHGT07 also contains: (i) bacterial origin of replication (*ori*) sequences for replication in *Escherichia coli* and *Agrobacterium tumefaciens*; (ii) the bacterial *aad* gene which encodes aminoglycoside adenylyltransferase (AAD), which confers resistance to spectinomycin and streptomycin allowing for selection of bacteria containing the plasmid; and (iii) the *gox* gene from the bacterium *Ochromobacter anthropii* strain LBAA (formerly *Achromobacter* sp.), encoding the glyphosate metabolising enzyme glyphosate oxidoreductase (GOX). The arrangement of the genes in PV-GHGT07 is shown in Figure 1 and their origin and function are listed in Table 1.

Figure 1 Schematic diagram of PV-GHGT07¹



¹ See Table 1 for an explanation of the abbreviations.

2.2 Function and regulation of the introduced gene(s)

5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene

The CP4 EPSPS gene introduced into cotton line 1445 was derived from *Agrobacterium*

strain CP4 and encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. The CP4 EPSPS protein is resistant to inhibition by glyphosate and has previously been transferred to soybeans (Padgett *et al* 1996b) to confer tolerance to glyphosate. The native CP4 EPSPS gene from *Agrobacterium* CP4 contains sequences that might impair its expression in plants. A plant-preferred version of the CP4 EPSPS gene was synthesised in order to substitute plant preferred codons while producing an identical CP4 EPSPS protein (Harrison *et al* 1996). The activity of the resultant CP4 EPSPS enzyme was unaltered and was used for transformation of cotton.

Expression of the CP4-EPSPS gene in plant cells is under the control of the CMoVb promoter from Modified Figwort virus and the 3' non-translated region of the pea *rbcS* (small subunit ribulose biphosphate carboxylase oxygenase) E9 gene (E9 3').

The endogenous EPSPS enzyme of plants is located within chloroplasts, the site of aromatic amino acid biosynthesis in plant cells. Many proteins with subcellular locations are synthesised as pre-proteins and directed to a particular organelle by a transit peptide at the end of the mature protein. Following delivery to the organelle, the short transit peptide is cleaved from the mature protein and is rapidly degraded. The CP4 EPSPS enzyme is targeted to the plastid by a chloroplast transit peptide sequence derived from the *Arabidopsis thaliana* EPSPS (CTP 2, Klee *et al* 1987). The CTP2 gene sequence was fused to the 5' end of the CP4 EPSPS gene. The CTP2 peptide sequence has been shown to deliver bacterial EPSPSs to the chloroplasts of higher plants where the aromatic amino acid biosynthetic pathway and endogenous EPSPS activity is located (della Ciopa *et al*, 1986).

Neomycin phosphotransferase (nptII) gene

The *nptII* gene is derived from the bacterial Transposon Tn5 and encodes neomycin phosphotransferase II which confers resistance to the aminoglycoside antibiotics kanamycin, neomycin and geneticin, and is widely used as a marker in the initial selection of transformed plant cells. The *nptII* gene is transferred along with the CP4 EPSPS gene, enabling those plant cells successfully transformed with the CP4 EPSPS gene to grow in the presence of kanamycin. Those cells which lack the *nptII* gene, and hence the CP4 EPSPS gene, will not grow and divide in the presence of kanamycin.

The expression of the *nptII* gene in plant tissues requires the presence of a promoter that functions in a plant background. The expression of the *nptII* gene from PV-GHGT07 in cotton is effected by the 35S promoter from cauliflower mosaic virus and the 3' non-translated region of the nopaline synthase gene from the pTiT37 plasmid of *Agrobacterium tumefaciens* strain T37 (NOS 3').

Aminoglycoside adenytransferase (aad) gene

The *aad* gene is derived from bacterial Transposon Tn7 and encodes aminoglycoside adenytransferase (AAD) which confers resistance to the antibiotics spectinomycin and streptomycin. The *aad* gene is under the control of a bacterial promoter and was included in the construct as a marker to allow for selection of bacteria containing PV-GHGT07 prior to transformation of the plant cells. The *aad* gene has no plant regulatory sequences and would not be predicted to be expressed in plant tissues.

Table 1 Genetic Elements contained in PV-GHGT07

Genetic element	Region	Name	Function	Source
Right Border		RB	initiates T-DNA transfer	<i>Agrobacterium tumefaciens</i>
<i>gox</i>	Promoter Chloroplast Transit Peptide <i>gox</i> Terminator	P-CMoVb CTP 2 <i>gox</i> NOS 3'	drives expression in plant cells directs protein to the chloroplast metabolises glyphosate signals termination of transcription	Modified Figwort Virus 35S promoter CTP sequence from <i>Arabidopsis thaliana</i> EPSPS gene <i>Ochromobactrum anthropii</i> NOS 3' gene
CP4 EPSPS	Promoter Chloroplast Transit Peptide CP4 EPSPS Terminator	P-CmoVb CTP 2 CP4 EPSPS E9 3'	drives expression in plant cells directs protein to the chloroplast CP4 EPSPS protein signals termination of transcription	Modified Figwort Virus 35S promoter CTP sequence from <i>Arabidopsis thaliana</i> EPSPS gene from <i>Agrobacterium</i> strain CP4 Pea <i>rbcS</i> E9 gene
<i>aad</i>		<i>aad</i>	Spectinomycin, Streptomycin resistance in bacterial cells	Transposon Tn7
<i>nptII</i>	Promoter <i>nptII</i> Terminator	P-35S <i>nptII</i> NOS 3'	drives expression in plant cells Kanamycin resistance in plant cells signals termination of transcription	Cauliflower Mosaic Virus Transposon Tn5 <i>A. tumefaciens</i> strain T37 pTiT37
<i>oriV</i>		<i>oriV</i>	origin of plasmid replication in <i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>
<i>ori322</i>		<i>ori322</i>	origin of plasmid replication in <i>Escherichia coli</i>	plasmid pBR322

Glyphosate oxidoreductase gene

The glyphosate oxidoreductase (*gox*) gene is derived from the bacterium *O. anthropii* and encodes the enzyme glyphosate oxidoreductase (GOX). GOX catalyses the conversion of glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate thus effectively inactivating the herbicide (Pipke and Amrhein 1988, Jacob *et al* 1988). To ensure expression of the *gox* gene in plant tissues it was fused to the CMOVb promoter from Modified Figwort virus and the 3' non-translated region of the nopaline synthase gene from the pTiT37 plasmid of *A. tumefaciens* strain T37 (NOS 3'). Introduction of both the CP4 EPSPS and *gox* genes would provide an increased likelihood of tolerance to glyphosate, however molecular analysis revealed that the *gox* gene was not integrated into Line 1445 (see 2.3 below).

2.3 Characterisation of the genes in the plant

Molecular characterisation of the integrated DNA present in glyphosate-tolerant cotton Line 1445 was performed using untransformed cotton DNA and plasmid PV-GHGT07 and plasmid PV-GHGT06 as reference material (Nida *et al* 1996a). Plasmid PV-GHGT06 is identical to PV-GHGT07 except that it does not contain the *gox* gene sequence.

To determine the number of sites of insertion and the integrity of the genetic elements contained within the inserted DNA, genomic DNA from the transformed Line 1445 was subjected to Southern blot analysis.

The analyses revealed that the modification in Line 1445 is a single insertion event resulting from the introduction of a segment of DNA of approximately 6.1 Kb, comprised of the region of PV-GHGT07 from the right border to *oriV*, including CP4 EPSPS, *aad* and *nptII*. Table 2 lists the genes integrated into Line 1445. Further analysis demonstrated that that none of the *gox* gene sequence and only a truncated segment of *oriV* was integrated into the genome of Line 1445. The Southern blot data support the conclusion that all of the DNA required for expression of CP4 EPSPS and *nptII* has been integrated into Line 1445. Western blot data (see below) verify that these proteins are expressed.

Table 2 Genes transferred to glyphosate-tolerant Cotton Line 1445

Gene	Function	Source
CP4 EPSPS	gene encoding CP4 EPSPS protein	<i>Agrobacterium</i> strain CP4
<i>aad</i>	Spectinomycin, Streptomycin resistance	Transposon Tn7
<i>nptII</i>	Kanamycin resistance	Transposon Tn5
<i>oriV</i> (incomplete)	origin of replication	<i>Agrobacterium tumefaciens</i>

2.4 Stability of the genetic changes

Progeny from three successive homozygous generations (R₃ through R₅) of the transgenic Line 1445 were tested by Southern blot analysis to determine the stability of the integration of the DNA. The data show that the inserted DNA was present in each generation and is stably integrated into the cotton genome.

2.5 Conclusions regarding the genetic modification

The CP4 EPSPS, *npt II* and *aad* genes were transferred to cotton via an *Agrobacterium*-mediated transformation system resulting in the generation of the glyphosate-tolerant cotton Line 1445. No other genes were transferred as a result of the transformation. The DNA has integrated into the genome of cotton Line 1445 as a single and stable insert.

3 General Safety Issues

Glyphosate-tolerant cotton is grown in the USA for both domestic use and for export. Glyphosate tolerant cotton was approved for environmental release in the USA in 1995 (USDA/APHIS 1995). Refined oil and processed linters from glyphosate-tolerant cotton were approved for use in human food in the USA in 1995 (US FDA 1999) and in Canada in 1996 (Health Canada 1996). Processed foods, including imported processed foods may contain genetically modified cottonseed oil or cellulose from processed linters.

Glyphosate-tolerant cotton has been evaluated against the safety assessment guidelines developed by ANZFA. The data presented has been derived from whole cottonseed, cottonseed meal and flour (used only in animal feed) and refined cottonseed oil and processed linters, the only human food products derived from cottonseed. The safety assessment issues relate to Group B foods – food ingredients - as indicated in the guidelines for safety assessment of food produced using gene technology (ANZFA 1999a).

3.1 History of use of cottonseed products as foods

Only processed elements of cottonseed, ie oil and linters, are used as food in humans and these have a history of safe use. Neither whole cottonseed nor cottonseed meal is used in human food.

Cottonseed contains gossypol which is a biologically active terpenoid aldehyde. Gossypol is toxic *per se* and it also has adverse effects on the protein nutritive value of food by rendering lysine metabolically unavailable (Yannai and Bensai, 1983). The presence of gossypol limits the use of cottonseed as a protein source for humans or in animal feed, except for ruminants where bacteria in the rumen detoxify it (Randel *et al* 1992, Poore and Rogers 1998, Nikokyris *et al* 1991). However, the removal or inactivation of gossypol during processing enables the use of some cottonseed meal in feed for fish, poultry and pigs.

Refined cottonseed oil is free of gossypol (Cherry 1983, Gunstone *et al* 1994). The gossypol that partitions into the oil is eliminated during subsequent refining of the oil

through inactivation by heat and alkali treatment. The reduction of free gossypol in oil is a measure of the food quality and processing efficiency. The refining process also removes protein from the oil.

Cottonseed oil is used as frying oil, and in mayonnaise, salad dressing, shortening, margarine and packing oil. Linters are processed to produce cellulose (99%) using alkaline pH and high temperature and used as high fibre dietary products, sausage casings and thickeners in ice cream and salad dressings.

CP4 EPSPS has previously been introduced in soybeans (Padgett *et al* 1996b) and corn. Products of these transgenic commodities, and of glyphosate-tolerant cotton, are permitted for sale in the USA, Canada and Japan.

3.2 Nature of novel proteins

Two novel proteins are expressed in Line 1445 as the result of the transformation event. These are CP4 EPSPS and neomycin phosphotransferase (NPT II).

CP4 EPSPS

CP4 EPSPS is an essential enzyme involved in the biosynthesis of the aromatic amino acids by the shikimate metabolic pathway. This metabolic pathway is present in all plants, bacteria and fungi. The EPSPS enzyme of plants is inhibited by glyphosate (Steinrücken and Amrhein 1980), however bacterial EPSPSs, such as CP4 EPSPS, have reduced affinity for glyphosate. The CP4 EPSPS protein exhibits approximately 50% amino acid homology with native plant EPSPS proteins.

Plant EPSPSs are localised in the chloroplast. *In vitro* chloroplast uptake assays have shown that the *A. thaliana* EPSPS CTP delivers CP4 EPSPS to the chloroplast and is subsequently cleaved from the pre-protein, yielding mature CP4 EPSPS with no CTP amino acids retained (della Ciopa *et al*, 1986). It has been shown that the chloroplast transit peptides are rapidly degraded after cleavage *in vivo* by cellular proteases. Thus, only mature CP4 EPSPS, without any additional CTP residues at the amino terminus, would be predicted to be expressed in glyphosate resistant cotton.

NPT II

Neomycin phosphotransferase II (also known as aminoglycoside 3'-phosphotransferase II) is an enzyme with a molecular weight of 29 kD that catalyses the transfer of a phosphate group from adenosine 5'-triphosphate (ATP) to a hydroxyl group of aminoglycoside antibiotics, including neomycin, kanamycin and gentamicin A and B, thereby inactivating the antibiotics (Wright and Thompson 1999). The enzyme is encoded by the *nptII* gene, which is derived from transposon Tn5 from the bacterium *E. coli* (Beck *et al* 1982).

3.3 Expression of novel proteins in the plant

Monsanto Australia Limited submitted the following reports:

Berberich, S.A. 1993 Evaluation of protein content in refined cottonseed oil produced from the 1992 insect resistant cotton field trials. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. Study Number 92-01-36-07

Burnette, B.L., Taylor, M.L., Lakemeyer, L.L., Smith, C.E., Bailey, M. and Nida, D.L. 1993 Characterisation of 5-enol-pyruvyl-shikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) produced by glyphosate-tolerant cotton and assessment of equivalence relative to CP4 EPSPS produced by *E. coli*. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13166

Heeren, R.A., Padgett, S.R. and Gustafson, M.E. 1993 The purification of recombinant *Escherichia coli* CP4 5-enolpyruval-shikimate-3-phosphate synthase for equivalence studies. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12574

Nida, D.L., Halsey, M., Jackson, T., Taylor, M., Ebert, C., Taylor, N. and Sims, S. 1994 Evaluation of cotton with Roundup Ready™ genes generated in 1993 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13613

Nida, D.L., Rogan, G.J. and Taylor, M.L. 1995 Evaluation of cotton with Roundup Ready™ genes generated in 1994 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-14046

Sims, S.R., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C., Fuchs, R.L. and Berberich, S.A. 1995 Analysis of expressed proteins in fiber fractions from insect-protected and glyphosate-tolerant cotton varieties. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-14289

CP4 EPSPS

Cotton plants of Line 1445 are tolerant to the normally lethal effects of glyphosate application as a result of CP4 EPSPS expression. Expression of CP4 EPSPS protein in Line 1445 was verified by probing Western blots of protein extracts from cottonseed with a CP4 EPSPS-specific antibody. No expression was detected in the untransformed parental line Coker 312. The reactive protein observed in Line 1445 exhibited the same apparent molecular weight (48 kD) as purified CP4 EPSPS protein isolated from *Escherichia coli* expressing the CP4 EPSPS gene (47.5 kD), also verifying that the CTP2 sequence (8.2 kD) is cleaved upon transport into the chloroplast.

The level of expression of CP4 EPSPS was measured in cottonseed and leaf from Line 1445 by an enzyme linked immunosorbent assay (ELISA, Nida *et al* 1996a). The results of these studies are shown in Table 3. The data presented were obtained from two separate field trial experiments conducted at six separate locations in the USA in 1993 and 1994. The level of CP4 EPSPS protein in cotton line 1445 is low, ranging from 0.02% to 0.028% of total protein in cottonseed (calculated on the basis of protein content determined in the proximate analysis described in Tables 6 and 7).

The effect of application of glyphosate during plant growth on the expression levels of CP4 EPSPS in cottonseed was also investigated in field trials in 1994. There was no significant difference in expression of CP4 EPSPS in cottonseed between plants treated with glyphosate and those that were not treated (Table 3).

Refined cottonseed oil was not tested for the presence of CP4 EPSPS or NPT II protein. However the refining process removes protein, and refined cottonseed oil is considered free of protein (Rogers, 1990). Data submitted as part of an application from Monsanto for Ingard Cotton (A341, cotton expressing the Bt toxin of *Bacillus thuringiensis*) confirmed that no protein could not be detected in refined oil from either untransformed line C312 or Ingard cotton line 531 at a sensitivity of 1.3 ppm.

Combed lint and brown linter stock (ie linters after the first processing step) were tested for the presence of CP4 EPSPS protein by Western blotting. CP4 EPSPS was detected in combed lint but not in brown linter stock, the first product in the sequence of processing linters for cellulose (Sims *et al* 1996).

NPT II

The expression of the NPT II protein was determined by ELISA and the results are shown in Table 3. NPT II was detected at low levels in cottonseed and leaf (Nida *et al* 1996a). The NPT II protein content of cottonseed is 0.0022% of total protein (calculated as described for CP4 EPSPS above). The effect of application of glyphosate during plant growth was also investigated. There was no significant difference in expression of NPT II in cottonseed between plants treated with glyphosate and those that were not treated.

Table 3 Protein expression levels in glyphosate-tolerant cotton Line 1445 treated/untreated with glyphosate

Enzyme	Expression levels ($\mu\text{g}/\text{mg}$ fresh weight)	
	- glyphosate	+ glyphosate
cottonseed		
CP4 EPSPS ¹	0.082 \pm 0.017	NA
CP4 EPSPS ²	0.06 \pm 0.012	0.071 \pm 0.015
NPT II ¹	0.0067 \pm 0.001	NA
NPT II ²	0.007 \pm 0.0023	0.007 \pm 0.003
AAD ¹	n.d.	NA
leaf		
CP4 EPSPS ¹	0.052 \pm 0.016	NA
NPT II ¹	0.045 \pm 0.014	NA

n=6, 1: 1993 field trial data, 2: 1994 field trial data, NA: not applicable, n.d.: not detected, +/- standard deviation

AAD

AAD expression was investigated by ELISA. No AAD expression was detected in Line 1445. This result was expected as the *aad* gene is under the control of a bacterial promoter and would not be expected to be expressed in plant tissues.

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

Monsanto Australia Limited submitted the following report:

Jennings, J.C., Doherty, S.C., Hamilton, K.A. and Lirette, R.P. 2000 Assessment of processed oil from Roundup Ready^R and Bollgard^R cottonseed for the presence of transgenic DNA. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-16554

In 1991, the World Health Organization (WHO) issued a report of a Joint FAO²/WHO Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). It was concluded by that consultation that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

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

There are further concerns that, if the antibiotic resistance gene is expressed in the plant, the expressed protein, when ingested, could inactivate oral doses of the antibiotic to which it confers resistance.

This section of the report will evaluate the potential for therapy with antibiotics to be compromised through the presence of antibiotic resistance genes in the glyphosate-tolerant cotton line 1445.

Two antibiotic resistant genes have been transferred to the glyphosate-tolerant cotton. Line 1445 contains the *nptII* gene, under the control of the 35S promoter, meaning it will be expressed in plant cells. Line 1445 also contains a copy of the *aad* gene, which is under the control of a bacterial promoter, meaning it will not be expressed in plant cells (see 3.3 above).

Potential inactivation of an oral dose of kanamycin

A special concern with respect to antibiotic resistance genes is whether inactivation of an oral dose of antibiotics could occur during the treatment of humans. This may

² Food and Agriculture Organization.

happen if the enzyme that confers antibiotic resistance is expressed in the plant and if it remains functionally active in the gastrointestinal tract once ingested.

The *nptII* gene is one of the most widely used selectable marker genes (Kärenlampi 1996). Of the antibiotics that are inactivated by NPT II, both neomycin and kanamycin still have clinical use, although neomycin tends to be used topically due to its oral toxicity (Davis *et al* 1980).

As the *nptII* gene is expressed in the cotton line 1445 the potential for ingested NPT II protein to inactivate an oral dose of kanamycin has been considered.

The potential for ingested NPT II to inactivate oral doses of kanamycin has been addressed in relation to the presence of the *nptII* gene in a delayed ripening tomato (Redenbaugh *et al* 1992, Redenbaugh *et al* 1994). The use of the *nptII* gene has also been reviewed by a WHO Workshop (WHO 1993), the Nordic Council of Ministers (Kärenlampi 1996) and the United States Food and Drug Administration (US FDA 1998). It has been concluded that ingested NPT II, at the low levels likely to be present in transgenic plants, would not interfere with orally administered kanamycin therapy because firstly, NPT II is rapidly degraded in simulated gastric conditions (Fuchs *et al* 1993), secondly, even were NPT II not to be degraded, the enzyme requires ATP as a cofactor in order to inactivate kanamycin and ATP is limiting in the gastrointestinal tract, and finally, NPT II is unlikely to be active in the acidic conditions of the stomach.

The *nptII* gene used in the line 1445 cotton is identical to the gene used in the delayed ripening tomatoes. Therefore, the same conclusion regarding the use of *nptII* in tomatoes can also be applied to the cotton in this application. Furthermore, the only human food products derived from cotton, refined oil and processed linters, do not contain detectable levels of protein so the potential for inactivation of oral doses of kanamycin is negligible.

Potential for horizontal gene transfer

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

The first issue that must be considered in relation to the presence of the *nptII* and *aad* genes present in glyphosate-tolerant cotton is the probability that either of these genes would be successfully transferred to, and expressed in, microorganisms present in the human digestive tract. The following steps are necessary for this to occur:

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

- maintenance and expression of the *aad* gene by the bacteria.

In the case of the *nptII* gene, which does not have a bacterial promoter, an additional step would need to occur before antibiotic resistance could be expressed:

- integration of the DNA fragment containing the *nptII* gene in the appropriate orientation with respect to a functional bacterial promoter.

The transfer of either the *nptII* or *aad* gene to microorganisms in the human digestive tract is therefore highly unlikely because of the number and complexity of the steps that would need to take place consecutively.

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

In the case of transfer of either the *nptII* or the *aad* gene from glyphosate-tolerant cotton to microorganisms of the digestive tract, the human health impacts are considered to be negligible. Of the antibiotics that are inactivated by NPT II, both neomycin and kanamycin still have clinical use, although neomycin tends to be used topically due to its oral toxicity (Davis *et al* 1980). The *nptII* gene already occurs naturally in bacteria inhabiting the human gut (Kärenlampi 1996). Streptomycin resistance genes are common and can be found at high frequencies in natural populations of bacteria and clinical isolates (Shaw *et al* 1993). Streptomycin has mostly been replaced by newer aminoglycosides, however, it is still used for special indications, such as in the treatment of tuberculosis and brucellosis (Kärenlampi 1996).

Therefore, the additive effect of a *nptII* gene or an *aad* gene from the glyphosate-tolerant cotton being taken up and expressed by microorganisms of the human digestive tract would be insignificant compared to the populations of kanamycin- and streptomycin-resistant bacteria already naturally present.

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

Absence of DNA in refined oil

Only refined oil and cellulose from processed linters of cottonseed are consumed by humans. Processed linters are essentially pure cellulose (>99%) and are subjected to heat and solvent treatment that would be expected to remove and destroy DNA. The refining process for cottonseed oil also includes heat, solvent and alkali treatments

that would be expected to remove and destroy DNA, and intact fragments of the *nptII* or *aad* genes are unlikely to survive the processing steps making the chance of horizontal gene transfer even more unlikely. The processing steps can also lead to the release of cellular enzymes (nucleases) which are responsible for degrading DNA into smaller fragments.

Refined cottonseed oil from Line 1445 was analysed to ascertain if any intact DNA could be detected using the Polymerase Chain Reaction (PCR). The primers used were designed to amplify a portion of the CP4 EPSPS gene, the E9 terminator and a portion of cotton genomic DNA. No DNA was detected in refined cottonseed oil. The assay was able to detect as little as one nanogram of purified genomic DNA from Line 1445.

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

3.5 Conclusions regarding general safety issues

The CP4 EPSPS and *npt II* genes are expressed in glyphosate-tolerant cotton Line 1445 and the protein products are expressed at relatively low levels in the seed. There is no significant protein present in the refined oil or processed linters which are the only cotton products used for human consumption. The CP4 EPSPS gene and protein have been well characterised and are considered similar to EPSPS genes that are present in plants and therefore regularly consumed in human foods. The transfer of these genes to cotton is not considered to be a risk public health and safety.

It is extremely unlikely that either the kanamycin or streptomycin resistance genes will transfer from foods derived from glyphosate-tolerant cotton to bacteria in the human digestive tract because of the number and complexity of steps that would need to take place consecutively. In the highly unlikely event that either resistance gene was transferred to bacteria in the human digestive tract the human health impacts would be negligible because kanamycin- and streptomycin-resistant bacteria are already commonly found in the human gut and in the environment.

It is also equally unlikely that novel genetic material from the glyphosate-tolerant cotton will be transferred to human cells via the digestive tract. The novel genetic material comprises only a minute fraction of the total DNA in the glyphosate-tolerant cotton therefore it is unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

The demonstrated absence of DNA in refined cottonseed oil from line 1445, and the probable degradation and removal of DNA through the processing steps for refined oil and processed linters further mitigate against any horizontal transfer of DNA from glyphosate-tolerant cotton to cells in the human digestive tract.

4 Toxicological Issues

4.1 Levels of naturally occurring toxins

Monsanto Australia Limited submitted the following reports:

Nida, D.L., Halsey, M., Jackson, T., Taylor, M., Ebert, C., Taylor, N. and Sims, S. 1994 Evaluation of cotton with Roundup Ready™ genes generated in 1993 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13613

Nida, D.L., Rogan, G.J. and Taylor, M.L. 1995 Evaluation of cotton with Roundup Ready™ genes generated in 1994 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-14046

Gossypol

Gossypol is a biologically active terpenoid aldehyde that is present in discrete glands in various plant tissues, including seed. Gossypol has a number of toxic effects on mammals including: heart damage and heart failure (Poore and Rogers 1998, Randel *et al* 1992); mitochondrial uncoupling (Cuellar and Ramirez 1993); liver damage (Manabe *et al* 1991); disruption of oestrous cycles, pregnancy and embryo development (Randel *et al* 1992); and reduction of fertility because of sperm immotility and depressed sperm counts with specific mitochondrial damage evident (Randel *et al* 1992, Risco *et al* 1993). Whole cottonseed or cottonseed meal is not used in human foods and the presence of gossypol limits its use as in animal feed. As described above (3.1), ruminant animals (eg cattle and sheep) are able to tolerate gossypol more than other animals because of detoxification in the rumen.

The levels of gossypol were determined for control line C312 and Line 1445 in cottonseed and refined oil (Nida *et al* 1996b). The effect of treatment with glyphosate on the gossypol content of cottonseed was also determined. The data are shown in Table 4.

The level of gossypol detected in cottonseed of Line 1445 is statistically higher compared to line C312 (5% level, pairwise T test), but still within the ranges reported in the literature (Beradi & Goldblatt, 1980). The gossypol content observed in cottonseed for both C312 and Line 1445 varied considerably between the six field test sites.

No free gossypol was detected in refined oil, the main food substance produced from cottonseed and consumed by humans.

Table 4 Gossypol levels in cottonseed from glyphosate-tolerant cotton Line 1445 treated/untreated with glyphosate

Component	Control Coker 312	Line 1445 - glyphosate	Line 1445 + glyphosate	Literature Range %
% Total gossypol				
Seed ¹	1.19 (0.99-1.46)	1.32* (1.13-1.63)	ND	0.4 – 1.7
Seed ²	0.902 (0.67-1.02)	1.023* (0.84-1.17)	1.047* (0.88-1.15)	0.4 – 1.7
Refined oil ¹	n.d.	n.d.	ND	<= 0.01
% Free gossypol				
Seed ¹	NA	NA	ND	0.4 – 1.7
Seed ²	0.774 (0.55-0.86)	0.903* (0.75-1.01)	0.947* (0.80-1.06)	0.4 – 1.7
Refined oil ¹	n.d.	n.d.	ND	<= 0.01

n=6, 1: 1993 field trial data, 2: 1994 field trial data, *: statistically significant difference from control line C312 at the 5% level using a pairwise T test, ND=not determined, n.d.= not detected, NA=not applicable, ranges shown in parentheses

Cyclopropenoid fatty acids

The cyclopropenoid fatty acids, sterculic acid (C-17), dihydrosterculic acid (C-19) and malvalic acid (C-18), are unique fatty acids common in cotton and produce undesirable biological effects, including: the inhibition of biodesaturation of stearic to oleic acid affecting phospholipid biosynthesis (Rolph *et al* 1990; Cao *et al* 1993, Gunstone *et al* 1994); and has been reported to induce termination of embryo development in sheep through inhibition of progesterone production in the *corpus luteum* (Tumbelaka *et al* 1994).

The levels of sterculic acid, dihydrosterculic acid and malvalic acid were determined in cottonseed and refined oil. The data are shown in Table 5. No statistical difference was detected in the content of these fatty acids in cottonseed between line C312 and Line 1445 with or without glyphosate treatment (5% level using a pairwise T test).

In refined oil (single samples processed from cottonseed pooled from the six field sites) the levels of dihydrosterculic and malvalic acids were virtually identical in line 1445 and the control line C312. The level of sterculic acid in refined oil from line 1445 was slightly higher than for the control line C312. However each of the cyclopropenoid fatty acids constitutes <1% of the total fatty acids and all the values observed were comparable to ranges observed for commercial cottonseed oil.

Table 5 Cyclopropenoid levels in glyphosate-tolerant cotton Line 1445 treated/untreated with glyphosate

Component	Coker 312 untreated	Line 1445 untreated	Line 1445 + glyphosate	Literature Range (%)
Malvalic acid (C-17)				
Seed ^{1,3}	0.43 (0.25-0.58)	0.41 (0.21-0.58)	NA	
Seed ^{2,3}	0.334 (0.31-0.36)	0.336 (0.29-0.4)	0.357 (0.31-0.38)	
Refined oil ^{1,4}	0.35	0.56	NA	0.08-0.56
Sterculic acid (C-18)				
Seed ^{1,3}	0.71 (0.52-0.92)	0.70 (0.56-0.98)	NA	
Seed ^{2,3}	0.237 (0.12-0.77)	0.183 (0.11-0.37)	0.172 (0.12-0.26)	
Refined oil ^{1,4}	0.44	0.50	NA	0.22-1.44
Dihydrosterculic (C-19)				
Seed ^{1,3}	1.12 (0.34-3.39)	0.58 (0.27-1.07)	NA	
Seed ^{2,3}	0.128 (0.11-0.15)	0.128 (0.10-0.15)	0.122 (0.10-0.14)	
Refined oil ^{2,4}	0.23	0.23	NA	

n=6 for seed values, single values for refined oil, 1: 1993 field trial data, 2: 1994 field trial data, 3: seed values are % of total lipid, 4: oil values are % of total fatty acids, ND=not determined, NA=not applicable, ranges in parentheses

Aflatoxin

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

None of the four primary aflatoxins of cottonseed was detected at a sensitivity of 1 ppb in cottonseed from either line C312 or Line 1445 from any of the experimental sites (Nida *et al* 1996b).

4.2 Potential toxicity of novel proteins

Monsanto Australia Limited submitted the following report:

Ream, J.E., Bailey, M.R., Leach, J.N. and Padgett, S.R. 1993 Assessment of the *in vitro* digestive fate of CP4 EPSP synthase Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12949

CP4 EPSPS

The toxicity of the CP4 EPSPS protein has previously been tested by acute gavage exposure in mice and no deleterious effects were observed (Harrison *et al* 1996). The CP4 EPSPS protein was administered at levels 1000 fold of those in anticipated consumption of food products (high dose: 572 mg/kg body weight).

The CP4 EPSPS protein solution was administered at three dosages up to 572 mg/kg to 10 male and 10 female mice in a single one ml dose at day zero and observations made for possible toxic effects twice daily for seven days. No mortality or moribundity resulted and there were no significant differences in terminal body weights between the treated and control groups. Upon necropsy, body cavities were opened and organs examined *in situ* and removed. No pathological findings attributable to the treatment were observed.

NPT II

The toxicity of the NPT II protein has previously been tested by acute gavage exposure in mice and no deleterious effects were observed (Fuchs *et al* 1993). The NPT II protein was administered in three cumulative target dosages of up to 5000 mg/kg body weight. Dissolved NPT II protein was administered to 10 male and 10 female mice in a split dose, in two equal doses over a four hour period. Mice were observed over a seven day period. No mortality or moribundity was observed and there were no significant differences in terminal body weights between the treated and control groups. No abnormalities were observed in internal organs upon post mortem examination.

4.3 Potential allergenicity of existing proteins

Allergic reactions to foods arise from an immune reaction to a particular protein which may be present in the food in very small amounts. Some common foods are known to elicit an allergic response in susceptible individuals. Foods such as cow's milk, soybeans and tree nuts are some of the better known sources of food allergies.

Refined cottonseed oil and cellulose from linters are devoid of protein and, given that most allergens are proteins, their consumption is unlikely to result in an allergic reaction. In addition, many refined oils have been shown not to be allergenic even if the source can be allergenic (eg peanuts, Taylor *et al* 1981). There have been reported incidences of allergic reaction in humans in response to consumption of foods containing cottonseed protein (Atkins *et al* 1988, Malanin and Kalimo 1988). However, whole cottonseed, cottonseed meal and cottonseed flour are not used for human consumption.

Therefore the potential for cottonseed oil or linters from glyphosate-tolerant cotton line 1445 to constitute a source of allergens is extremely low.

4.4 Potential allergenicity of novel proteins

Monsanto Australia Limited submitted the following reports:

Burnette, B.L., Taylor, M.L., Lakemeyer, L.L., Smith, C.E., Bailey, M. and Nida, D.L. 1993 Characterisation of 5-enol-pyruvyl-shikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) produced by glyphosate-tolerant cotton and assessment of equivalence relative to CP4 EPSPS produced by *E. coli*. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13166

Heeren, R.A., Padgett, S.R. and Gustafson, M.E. 1993 The purification of recombinant *Escherichia coli* CP4 5-enolpyruvyl-shikimate-3-phosphate synthase for equivalence studies. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12574

Mitsky, T.A. 1993 Comparative alignment of CP4 EPSPS to known allergenic and toxic proteins using the FASTa algorithm. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12820

Ream, J.E., Bailey, M.R., Leach, J.N. and Padgett, S.R. 1993 Assessment of the *in vitro* digestive fate of CP4 EPSP synthase Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-12949

Sims, S.R., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C., Fuchs, R.L. and Berberich, S.A. 1995 Analysis of expressed proteins in fiber fractions from insect-protected and glyphosate-tolerant cotton varieties. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-14289

Although there are no predictive assays available to definitively assess the allergic potential of proteins, a number of characteristics are common among many of the allergens that have been characterised. Known allergens tend to be glycosylated proteins with a molecular weight of 10–70 kD. Protein allergens also tend to be heat stable as well as resistant to peptic and tryptic digestion and the acidic conditions of the stomach. Consequently, many allergenic factors tend to be resistant to proteolytic digestion.

The CP4 EPSPS and NPT II proteins were evaluated for potential allergenicity against these criteria: size; glycosylation; resistance to heat and digestive degradation; and sequence similarity to known allergens.

CP4 EPSPS

The CP4 EPSPS gene was derived from *Agrobacterium* strain CP4. *Agrobacterium* is not a food substance but is a common soil bacterium found on and around plant produce. The CP4 EPSPS protein also shares 50% homology with plant EPSPS proteins which are normally consumed in plant foods without any reported allergenic effects. The EPSPS proteins are naturally present in foods derived from plants and microbes and have no history of being allergenic.

The molecular weight of the CP4 EPSPS protein is 48 kD, and thus within the size range of typical allergens. However, previous studies have demonstrated that the CP4

EPSPS protein is rapidly degraded in a simulated digestive system (Harrison *et al*, 1996) and is inactivated by heat treatment (Padgette *et al* 1996b).

The CP4 EPSPS protein sequence does not contain typical glycosylation sequences (eg histidine-aspartate-glutamate-leucine, Gomord *et al* 1997). Western blots indicated that the relative mobility of CP4 EPSPS protein in crude extracts from cottonseed of Line 1445 did not differ from purified CP4 EPSPS. This supports the conclusion that the CP4 EPSPS is not glycosylated *in planta*.

Direct testing of CP4 EPSPS protein isolated from genetically modified cotton Line 1698 was negative for glycosylation. Line 1698 is a glyphosate-tolerant line generated by transformation with PV-GHGT06 (see 2.3 above) and the CP4 EPSPS expression cassette is identical to that contained in PV-GHGT07 used to generate line 1445. The glycosylation data obtained for CP4 EPSPS from line 1698 may therefore be considered highly predictive for line 1445.

Amino acid sequence similarity with known allergens is a useful indicator of allergenic potential. The amino acid sequence of the CP4 EPSPS protein was compared to the amino acid sequences of known allergens present in public domain databases (GenBank, EMBL, Swissprot, PIR). No biologically significant homology was found with any of these known allergens.

Comparison of the biochemical profile of the CP4 EPSPS enzyme to known protein allergens also provides a basis for allergenic assessment. It has been shown previously that purified CP4 EPSPS protein is rapidly degraded in simulated mammalian digestive systems (Harrison *et al* 1996). No CP4 EPSPS protein was detectable by Western blot after 15 seconds incubation in simulated gastric fluid and greater than 50% of CP4 EPSPS protein was removed by 15 minutes incubation in simulated intestinal fluid (Harrison *et al* 1996).

It can be concluded that the CP4 EPSPS gene introduced into cotton does not encode a known allergen and that the CP4 EPSPS protein does not share immunologically significant amino acid sequences with known allergens.

NPT II

The *npt II* gene is derived from Transposon Tn5 of *Escherichia coli*, a bacterium commonly found in the human digestive tract. NPT II is a 29 kD protein and thus within the size range of typical allergens. However NPT II has previously been shown to be rapidly degraded under simulated mammalian digestive conditions, being completely removed after 10 seconds in simulated gastric fluid and with 50% digested after five minutes in simulated intestinal fluid (Fuchs *et al* 1993).

The use of the NPT II enzyme as a processing aid has previously been evaluated by the Food and Drug Administration of the USA. The FDA concluded that this enzyme does not have any of the recognised characteristics of food allergens or any attributes that would distinguish it toxicologically from other phosphorylating enzymes in the food supply (US FDA 1994).

4.4 Residues of glyphosate or metabolites

Glyphosate is a herbicide commonly used on crops in the USA and Australia and is also used to desiccate plant tissues prior to harvest of grain. Maximum residue limits (MRLs) for glyphosate in grain crops have been set in the Food Standards Code (used by Australia, Standard A14 – Maximum Residue Limits, ANZFA 1999b) and Codex (used by New Zealand). MRLs are set at levels well below those which would represent a safety concern. Glyphosate has very low acute toxicity to mammals with an oral LD50 of >10,000 mg/kg in mice and >5,000 mg/kg in rats (Smith and Oehme 1992).

The MRL set for glyphosate in crude cottonseed oil is 0.1 mg/kg in the Food Standards Code and 0.05 mg/kg for edible cottonseed oil in Codex (FAO/WHO Codex 2000).

The levels of glyphosate residues in refined cottonseed oil and processed linters would be expected to be very low because of removal in processing. Glyphosate is a very hydrophilic molecule (Malik *et al* 1989) and this would be expected to contribute to its removal in processing. The residue levels in foods derived from glyphosate-tolerant cotton line 1445 would have to comply with either the Australian or CODEX MRL, depending on the jurisdiction.

Reports in the literature concentrate on the fate of glyphosate in weed plants killed by herbicide application, with data indicating that the primary removal of glyphosate is by bacterial activity (Smith and Oehme, 1992). Bacterial degradation results in the production of aminomethylphosphonate (AMPA) and glyoxylate, both non-toxic compounds (Smith and Oehme, 1992). An alternative pathway of degradation exists in many bacteria with glyphosate being converted to sarcosine and then to glycine and inorganic phosphate, all of which are non-toxic (Pipke *et al* 1987, Jacob *et al* 1988, Dick and Quinn 1995).

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

4.5 Conclusions regarding toxicological issues

The data and analyses on the potential for toxicity or allergenicity of the CP4 EPSPS or NPT II proteins support the conclusions that neither protein is derived from an allergenic or toxic food source nor exhibits the characteristics of known protein allergens. CP4 EPSPS does not exhibit sequence similarity with known toxins or allergens. Furthermore, the CP4 EPSPS and NPT II proteins are present in relatively low abundance in cottonseed and both have been demonstrated to be degraded in conditions that mimic human digestion.

Refined cottonseed oil and cellulose from processed linters are the two components of cotton used in human foods. Refined oil is generally accepted as being devoid of protein. CP4 EPSPS protein was detected in raw linters but could not be detected in

linters after the first processing step (brown linter stock, Sims *et al* 1996). From these data it can be concluded that the food products derived from glyphosate-tolerant cotton should pose no greater threat as a source of allergic reaction than food products from conventional cotton.

5 Nutritional Issues

Monsanto Australia Limited submitted the following reports:

Jennings, J.C., Doherty, S.C., Hamilton, K.A. and Lirette, R.P. 2000 Assessment of processed oil from Roundup Ready^R and Bollgard^R cottonseed for the presence of transgenic DNA. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-16554

Nida, D.L., Halsey, M., Jackson, T., Taylor, M., Ebert, C., Taylor, N. and Sims, S. 1994 Evaluation of cotton with Roundup ReadyTM genes generated in 1993 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-13613

Nida, D.L., Rogan, G.J. and Taylor, M.L. 1995 Evaluation of cotton with Roundup ReadyTM genes generated in 1994 US field test locations. Monsanto Company, 700 Chesterfield Parkway North, St Louis, MO, USA 63198. MSL-14046

5.1 Compositional analysis

Cottonseed used for compositional analyses was derived from field trial experiments conducted in 1993 and 1994. Cotton was planted at six sites for each trial. The six sites used in 1994 were different to those used in 1993. In order to assess the impact, if any, of application of glyphosate on the composition of Line 1445, compositional analyses were made from plants subjected to glyphosate treatment or free from glyphosate treatment. These analyses were made at six field sites during trials in 1994. Treated plants were sprayed four times during the growing season: preemergent, postemergent (3-4 leaf stage), post-directed and preharvest (6-8 days prior) with applications of 3.36, 1.26, 1.26 and 1.68 kg/ha respectively.

The compositional analyses of cottonseed included proximate, amino acid, and aflatoxin, and of oil included tocopherols. Toxicant analyses evaluated gossypol and cyclopropenoid fatty acids in cottonseed and refined oil. The components measured included proximates (protein, fat, ash, carbohydrates, moisture, crude fibre), amino acid composition, fatty acids profile, and levels of tocopherols and of the toxicants sterculic and malvalic acid and gossypol. References were provided for all methods used in the analyses and the majority of methods were derived from standard methodology in *Official Methods of Analysis*, Association of Official Analytical Chemists (AOAC) and are validated AOAC International Methods, or American Oil Chemists Society (AOCS) Methods.

Proximate analysis

The proximate analyses of cottonseed from Line 1445 did not vary markedly from the control line C312 (Tables 6 and 7). However some statistically significant differences were observed (5% level using a pairwise T test).

The protein content of cottonseed of Line 1445 obtained from two separate growing seasons (untreated with glyphosate) was be ~6.5% higher than that of C312 (see Tables 6 and 7, 6.1% higher from 1993 and 6.5% higher from 1994). The protein content of cottonseed of line 1445 treated with glyphosate was also higher than that of the C312 control but was not statistically significant. All of the protein content values were within the ranges reported in the literature. The level of carbohydrates was also less in all samples of Line 1445 analysed. It should be noted that the carbohydrate content is calculated by subtraction rather than directly, and the reduction reflects the increased protein level.

Other statistically significant differences observed for Line 1445 (untreated with glyphosate) were reduced ash content and increased fat content, but these differences were not evident in Line 1445 treated with glyphosate (Table 7), or in cottonseed from the previous season (Table 6). However it should also be noted that there was significant overlap in the ranges of individual values for protein, fat, ash and moisture, treated or untreated with glyphosate, and all were well within reported ranges.

The results of the proximate analyses indicate that there are no substantial differences in these components between cottonseed from the control line C312 and Line 1445 either treated or untreated with glyphosate.

Table 6 Compositional analysis of Cottonseed from Line 1445 not treated with glyphosate[#]

Component	Coker 312	Line 1445	Literature Range (%)
Protein %	27.76	29.59*	12-32
Total fat %	23.35	23.79	23.2-25.7
Ash %	4.35	4.70	4.1-4.9
Carbohydrates %	44.35	41.91*	
Calories/100g	498.59	500.17	
Moisture %	11.55	11.05	5.4-10.1

n=6, [#] from plants grown in field trials in 1993, *: statistically significant difference from control line C312 at the 5% level using a pairwise T test

Table 7 Compositional analysis of cottonseed from Line 1445 treated with glyphosate[#]

Component	Coker 312 untreated	Line 1445 untreated	Line 1445 + glyphosate	Literature Range (%)
Protein %	28.80	30.55*	30.03	12-32
Total fat %	24.43	25.57*	25.09	23.2-25.7
Ash %	4.35	4.53*	4.46	4.1-4.9
Crude Fibre %	13.83	13.59	13.66	
Carbohydrates %	42.40	39.62*	40.38*	
Calories/100g	504.7	508.2	507.8	
Moisture %	6.74	7.46	6.06	5.4-10.1

n=6, [#]from plants grown in field trials in 1994, *: statistically significant from control line C312 at the 5% level using a pairwise T test

Amino acid composition of cotton Line 1445

A modified version of an AOAC International method was used to determine the amino acid composition of cottonseed from Line 1445 (Nida *et al* 1996b). Eighteen individual amino acids were quantitated using an automated amino acid analyser. The analysis did not distinguish the related amino acids glutamate and glutamine or aspartate and asparagine. Seed from plants treated and untreated with glyphosate application was compared with the control line 312. The data are shown in Table 8.

No statistically significant differences between cottonseed of control line C312 and untreated or treated Line 1445 were noted in the values for any of the amino acids tested. In particular, it was evident that expression of CP4 EPSPS, an enzyme in the aromatic amino acid biosynthetic pathway, in Line 1445 had no effect on the levels of the aromatic amino acids tryptophan, phenylalanine and tyrosine.

Lipid and fatty acid composition of cotton Line 1445

Cottonseed

Cottonseed from Line 1445, cultivated with or without glyphosate treatment, was compared with control C312 samples for fatty acid composition (Nida *et al* 1996b). Total lipid content and composition of different fatty acid types were determined and the data are shown in Table 9 (1993 field trial data, no glyphosate treatment) and Table 10 (1994 field trial data, including treatment with glyphosate).

No statistically significant differences (5% level in a pairwise T test) were observed between line 1445 and the control line C312 for fatty acid composition of cottonseed grown in the 1993 field trial (Table 9).

Table 8 Amino acid content of cottonseed from Line 1445 treated with glyphosate[#]

Component	Coker 312 untreated	Line 1445 untreated	Line 1445 + glyphosate	Literature Range ¹
Aspartate (& asparagine)	9.36	9.41	9.478	8.8-9.5
Threonine	3.441	3.442	3.487	2.8-3.2
Serine	4.734	4.705	4.721	3.9-4.4
Glutamate (& glutamine)	19.78	19.28	19.73	20.5-22.4
Proline	3.688	3.640	3.635	3.1-4.0
Glycine	4.362	4.295	4.455	3.8-4.5
Alanine	3.771	3.718	3.733	3.6-4.2
Cysteine	1.548	1.548	1.603	2.3-3.4
Valine	4.018	3.960	4.121	4.3-4.7
Methionine	1.511	1.450	1.484	1.3-1.8
Isoleucine	2.866	2.817	2.920	3-3.4
Leucine	5.899	5.835	5.932	5.5-6.1
Tyrosine	2.722	2.690	2.743	2.8-3.3
Phenylalanine	5.151	5.127	5.190	5-5.6
Lysine	4.573	4.512	4.614	3.9-4.1
Histidine	3.014	2.963	3.017	2.6-2.8
Arginine	10.98	11.10	11.34	10.9-12.3
Tryptophan	1.089	1.087	1.080	1-1.4

n=6, values are g/100g protein, [#]from plants grown in field trials in 1994, 1: Lawhon *et al* 1977, *: statistically significant from control line C312 at the 5% level using a pairwise T test

No statistically significant differences (5% level in a pairwise T test) between cottonseed from Line 1445 (either treated or untreated with glyphosate) and the control line C312 for fatty acid composition of cottonseed grown in the 1994 field trial, with three exceptions (Table 10).

The total lipid content in glyphosate treated Line 1445 was slightly higher (5.6%) than the control. However the lipid contents of cottonseed from the 1994 trial for the control line C312 and line 1445 (+/- glyphosate) were less than those determined for either line in the 1993 trial (Tables 9 and 10). Therefore this small difference is not considered significant because the small variation in lipid content observed for line 1445 treated with glyphosate was less than the interseasonal variation for all lines.

The level of myristic acid (14:0) was lower in cottonseed of Line 1445 treated with glyphosate (21.5%) than the control line C312 (1994 trial, Table 10). The level of arachidic acid (20:0) in untreated Line 1445 is higher (16%) than the C312 control (1994 trial, Table 10). However these differences are not considered significant

because levels of myristic and arachidic acid each constitute less than 1% of the total lipid, and the values were within the limits adopted by FAO/WHO Codex Alimentarius (1993) and values reported in the literature (Lawhon *et al* 1977, Cherry 1983, Gunstone *et al* 1994).

Table 9 Fatty acid content of cottonseed from Line 1445[#]

Component	Coker 312 untreated	Line 1445 untreated	Literature Range ³
Lipid ¹	32.65	32.24	
Myristic (14:0) ²	0.97	0.95	0.64-1.3
Pentadecanoic (15:0) ²	1.00	0.56	
Palmitic (16:0) ²	27.70	26.76	22.18-27.76
Palmitoleic (16:1) ²	0.64	0.65	0.66-1.3
Margaric (17:0) ²	0.16	0.18	
Stearic (18:0) ²	2.68	2.67	2.14-3.23
Oleic (18:1) ²	15.28	15.49	13.95-21.16
Linoleic (18:2) ²	43.18	45.90	45.84-57.83
Linolenic (18:3) ²	0.16	0.21	
Arachidic (20:0) ²	0.24	0.29	
Behenic (22:0) ²	0.15	0.17	

n=6, 1: % of sample weight, 2: % total lipid, 3: Cherry 1983, [#]from plants grown in field trials in 1993, no statistically significant differences from control line C312 at the 5% level using a pairwise T test

Refined oil

Single samples of refined oil from cottonseed of lines C312 and 1445 were assessed for fatty acid composition. The data are shown in Table 11. The oil samples were refined from cottonseed pooled from all six sites from the 1993 field trial. No data was presented for the composition of oil from cottonseed treated with glyphosate. There were no significant differences in the major fatty acid components of refined oil observed between line 1445 and the control line C312 for. The levels of some low percentage fatty acids differed between line 1445 and the control line C312, but all values were within ranges reported in the literature (Lawhon *et al* 1977, Cherry 1983, Rogers 1990, Gunstone *et al* 1994). It can therefore be concluded that the composition of refined oil from glyphosate-tolerant cotton line 1445 is equivalent to oil derived from conventional cotton varieties.

Table 10 Fatty acid content of cottonseed from Line 1445 treated with glyphosate[#]

Component	Coker 312 untreated	Line 1445 untreated	Line 1445 + glyphosate	Literature Range ³
Lipid ¹	26.26	26.74	27.68*	
Myristic (14:0) ²	0.779	0.693	0.611*	0.64-1.3
Pentadecanoic (15:0) ²	0.163	0.147	0.173	
Palmitic (16:0) ²	24.60	24.82	25.30	22.18-27.76
Palmitoleic (16:1) ²	0.388	0.354	0.383	0.66-1.3
Stearic (18:0) ²	2.003	2.256	2.072	
Oleic (18:1) ²	15.33	14.99	14.78	2.14-3.23
Linoleic (18:2) ²	55.33	55.38	55.33	13.95-21.16
Linolenic (18:3) ²	0.138	0.130	0.137	
Arachidic (20:0) ²	0.178	0.212*	0.194	
Behenic (22:0) ²	0.104	0.106	0.105	

n=6, 1: % of sample weight, 2: % total lipid, 3: Cherry 1983, #:from plants grown in field trials in 1994, *: statistically significant from control line C312 at the 5% level using a pairwise T test

Alpha-tocopherols

Tocopherols are naturally present in cottonseed oil and serve as antioxidants and confer good storage properties. Alpha-tocopherols in particular have vitamin E potency. They are affected by processing and lost during refining and deodorising. The levels of α -tocopherol in refined oil from lines C312 and 1445 were 588 mg/kg and 670 mg/kg respectively (Table 11) within the ranges reported in the literature (Dicks 1965, Rogers 1990, Rossell 1991, Gunstone *et al* 1994).

Table 11 Fatty acid content of refined cottonseed oil from Line 1445[#]

Component	Coker 312 untreated	Line 1445 untreated	Literature Range ³
Myristic (14:0) ¹	0.95	0.84	0.5-2.5
Pentadecanoic (15:0) ¹	0.40	0.43	
Palmitic (16:0) ¹	25.54	25.14	17-29
Palmitoleic (16:1) ¹	0.63	0.61	0.5-1.5
Margaric (17:0) ¹	0.16	0.20	
Stearic (18:0) ¹	2.46	2.41	1.0-4.0
Oleic (18:1) ¹	15.03	14.53	13-44
Linoleic (18:2) ¹	50.10	51.27	33-58
Linolenic (18:3) ¹	0.14	0.16	0.1-2.1
Arachidic (20:0) ¹	0.26	0.27	<0.5
Behenic (22:0) ¹	0.12	0.08	<0.5
α -tocopherol ²	580	670	74-660 ⁴

[#]single values for oil of cottonseed refined from pooled samples of plants grown in 6 field sites in 1993, 1: % total lipid, 2: mg/kg, 3: Lawhon *et al* 1977, Cherry 1983, Rogers 1990, Gunstone *et al* 1994, 4: Dicks 1965, Rossell 1991,

Conclusions from compositional analyses

Comprehensive data from a range of compositional analyses conducted on cottonseed from both untreated and treated cotton Line 1445 and the control line C312 were presented for assessment. The compositional components measured included proximates (protein, fat, ash, carbohydrates, moisture, and crude fibre), amino acid composition, fatty acids profile, gossypol and α -tocopherol levels.

The results of the compositional data do not indicate that there are any substantial differences between glyphosate-tolerant cotton Line 1445, either untreated or following treatment, and the non-transgenic control line Coker C312 for any of the parameters measured. Some small statistically significant differences were observed in protein, fat, ash and carbohydrate content for Line 1445, but the various values were within ranges previously reported for cotton and were not considered to be of either biological relevance for commercially grown cotton varieties or of significance in terms of food safety.

5.2 Levels of anti-nutrients

In addition to its toxic effects the terpenoid gossypol, naturally occurring in cottonseed, has antinutritive characteristics through reducing the availability of lysine (Yannai and Bensai, 1983). The level of gossypol in Line 1445 is described above (4.1 and Table 4). No gossypol was detected in refined oil.

5.3 Ability to support typical growth and well-being

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

In the case of Line 1445, no data was presented on feeding studies to animals. The compositional and other data provided in the application is considered adequate to establish the nutritional adequacy and safety of refined cottonseed oil and processed linters from glyphosate-tolerant cotton. Cellulose derived from linters would not be expected to support typical growth and well-being from either the modified or unmodified cotton line.

The nutritional profile of cotton line 1445 was determined by compositional analysis of the major components of cottonseed and refined oil and these were found to be comparable to the conventional control line Coker C312. Refined oil and cellulose from processed linters are the only human food products derived from cottonseed. The composition of refined cottonseed oil from Line 1445 was equivalent to, and would be expected to be equally nutritious as, the control parent line C312.

5.4 Conclusions regarding nutritional issues

The nutritional qualities of glyphosate-tolerant cotton Line 1445 were determined by compositional analyses of the major components of the seed and processed fractions and these were found to be comparable in all respects to the conventional control line Coker 312. Genetically modified cotton plants that were treated with the herbicide glyphosate (Roundup®) during cultivation were also analysed and found to be comparable to the parent line.

The applicant has demonstrated that the levels of the antinutrients sterculic acid, malvalic acid and dihydrosterculic acid in cottonseed and refined oil from glyphosate-tolerant cotton Line 1445 are not substantially different from the parent line and are within normal ranges. Gossypol was not detected in refined oil.

There is a long history of safe use of cottonseed oil and cellulose derived from processed linters. Based on the data submitted in the present application, refined oil and processed linters derived from glyphosate-tolerant cotton Line 1445 are nutritionally and compositionally comparable to that from conventional cotton and are not considered to pose a risk to human health and safety.

Acknowledgement

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DRAFT REGULATORY IMPACT ASSESSMENT

Regulatory Impact Assessment

The Authority is required, in the course of developing regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Identification of affected parties

1. Governments in Australia and New Zealand
2. Consumers in Australia and New Zealand
3. Manufacturers, producers and importers of food products

Options

Option 1–To prohibit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	Benefits <ul style="list-style-type: none"> • no benefits were identified. 	Costs <ul style="list-style-type: none"> • the governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • a prohibition on food produced using gene technology in Australia and New Zealand could result in retaliatory trade measures from other countries. • there may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	Benefits <ul style="list-style-type: none"> • Some companies may benefit from being able to exploit niche markets for non-GM products overseas. 	Costs <ul style="list-style-type: none"> • food manufacturers and producers will be unable to use the processed food fractions from foods produced using gene technology thus requiring the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be \$A 207m in Australia and \$NZ 37m in New Zealand³. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

³ Report on the costs of labelling genetically modified foods (2000)

CONSUMERS	Benefits <ul style="list-style-type: none"> • no benefits were identified, however as some consumers perceive GM food to be unsafe, they may perceive prohibition of GM food to provide a public health and safety benefit. 	Costs <ul style="list-style-type: none"> • could lead to decreased availability of certain food products. • increased costs to consumers because manufacturers and producers may have to source non-GM ingredients.
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Option 2– to permit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	Benefits <ul style="list-style-type: none"> • increased innovation and competitiveness in the food industry will benefit the economy. 	Costs <ul style="list-style-type: none"> • minor costs associated with amending the <i>Food Standards Code</i>.
INDUSTRY Manufacturers, producers and importers of food products	Benefits <ul style="list-style-type: none"> • food producers and manufacturers will be able to capitalise on the latest technology. • food importers will continue to be able to import manufactured products from overseas markets including the USA and Canada where there is no restriction on the use of food produced using gene technology. 	Costs <ul style="list-style-type: none"> • there may be some discrimination against Australian and New Zealand food products in overseas markets that have a preference for non-GM foods (e.g. Japan and the European Union).
CONSUMERS	Benefits <ul style="list-style-type: none"> • consumers may have access to a greater range of food products. 	Costs <ul style="list-style-type: none"> • those consumers who wish to avoid GM food may experience restricted choice in food products. • those consumers who wish to avoid GM food may have to pay more for non-GM food.

Conclusion of the regulatory impact assessment

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

WORLD TRADE ORGANIZATION

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

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

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

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

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

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

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

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

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

SPS Notifications

These are primarily health related, and refer to any measure applied:

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;
- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

- to protect human life or health from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; and
- to prevent or limit other damage from the entry, establishment or spread of pests.

The Agreement on the Application of Sanitary or Phytosanitary Measures relates to any measure applied to protect animal, plant or human life or health which may directly or indirectly affect international trade. Whether the SPS measure is in the form of a law or mandatory regulation, an advisory guideline, a code of practice or a requirement, it is the purpose of the measure that is important - not its regulatory status. Each WTO member country is entitled to apply SPS measures that are more stringent than the international standards in order to protect the health of its population. In the interests of transparency, each instance of such non-alignment which could result in an impediment to trade must be identified and justified and the documentation of that justification must be readily available

Each member country is also required to apply its methods of risk assessment and management consistently so arrangements under the SPS Agreement do not generate what may really be technical barriers to trade

Under the SPS Agreement, an exporting country can have resort to the WTO's dispute settlement procedures with respect to such a non-alignment. These arrangements mean there is potential for a code of practice to introduce an SPS measure that may bring about non-alignment with international requirements. Such non-alignment would need to be justified scientifically on the grounds that it is necessary to protect human, animal or plant life or health.

TBT Notifications

A technical barrier to trade arises when a mandatory requirement in a country's food regulatory system does not align with the international standard and it is more trade restrictive than is necessary to fulfil a legitimate objective. However, it can be acceptable for a country to have a more stringent requirement than that set internationally for reasons including:

- Maintaining national security;
- Preventing deceptive practices; and
- Protecting human health or safety.

Instances of non-alignment with international standards which could result in trade barriers must be identified and, if questioned, justified. Voluntary codes of practice are not expected to generate technical barriers to trade except where compliance with a code of practice or some aspect of a code of practice is expected. Consequently, it is possible for a voluntary code of practice to be viewed by the WTO as mandatory and subject to all the notification and other provisions applying to mandatory regulations.

The Agreement on Technical Barrier to Trade relates to requirements covering product characteristics or their related processes and production methods. TBT covers measures that are not SPS, such as requirements relating to terminology, symbols, packaging, marking, labelling, food composition and processing methods.

It is considered that these Full Assessments do constitute a potential Technical Barrier to Trade or a Sanitary/Phytosanitary matter. Matters raised in these Full Assessments therefore will be notified to the WTO.

SUMMARY OF PUBLIC COMMENTS

The Authority received the first six applications for foods produced using gene technology from Monsanto Australia Ltd. Due to commonalities in these applications, a combined Notice of Application (formally referred to as the Preliminary Assessment Report) was advertised on 28 October 1998, which called for public comment on the applications. A total of 58 submissions were received in response to the combined Notice of Application, of which 53 relate to this application.

Jean Adams (Aust)

- does not want these experimental foods in the common food supply until they have been long-term tested for undesirable side-effects related to public health or to environmental damage;
- questions the legality of forcing such genetically modified foods onto the public and the intention to remove labelling of such foods.

Robert Anderson (member of Physicians and Scientists for Responsible Application of Science and Technology)

- knowledge about the nature of the promoter, genes and the type of antibiotic resistance genes is crucial to a proper assessment;
- the applications should be rejected because most of the New Zealand population does not want to eat genetically engineered food. There are real dangers of allergic reactions, the Maori people are opposed to genetic engineering and these products are all an unknown risk to human health because they have not been tested.

Aoraki Greens and the Organic Garden City Trust (NZ)

- opposed to the amendment to the *Food Standards Code* to permit the foods in the applications;
- claim there is no alternative but to decline the acceptance of these products until they are clearly labelled and can be differentiated from their conventional counterparts;
- believe consumer choice is being violated;
- consider that because the science is new, potential problems or long term implications are yet to be made apparent.

Elaine Attwood (Aust)

- supports Option 1 in the combined Preliminary Assessment - that is, to maintain the status quo and not approve any of the six applications;
- re: A338 - considers 4 weeks of laboratory animal testing inadequate and doubts the applicant's claim that the need for herbicide will be reduced. Comments on proposed increase in the MRL for glyphosate;
- re: A355, A362 and A346 - genetically modified material will enter the food chain via cotton seed meal and hulls and corn waste being fed to animals;
- re: A363 - canola free of genetic modification would be marketable overseas;
- re: A341 - the results of laboratory feeding studies in rats are of concern. Long term safety is uncertain and therefore the genetically modified cotton should not

- be permitted;
- trade considerations should not prevail over consumer rights to have all genetically modified foods labelled as such.

Australian GeneEthics Network

- Monsanto's proposals should all be rejected as inadequate;
- there should be pre-market human testing to provide data for a precautionary approach on safety and nutritional efficacy;
- there should be full labelling of all approved foods in keeping with the Ministerial decision;
- there should be public review of the MRLs for Roundup in these foods;
- there should be public review of the toxicity of the quantities of Bt toxins likely to enter the human and animal food supplies, taking cultural, social, ethnic and age diversity into account;
- an adverse reactions register should be established to enable systematic monitoring of any impacts of these foods;
- all proposals should be submitted for GMAC assessment and recommendation including an updated and public review of Bt cotton and Roundup Ready soy for environmental and health impacts;
- GMAC's assumption that AQIS regulations would keep imported soy out of the Australian environment does not apply to the other commodities, and the geographical limits and performance of Bt cotton need public review;
- Monsanto has not studied the dietary implications of these products and presents no evidence that the company considered the diversity of diets among different cultures, social or ethnic groups;
- RR soy and corn crops are very different in containing novel DNA, proteins at elevated levels, and new levels of synthetic chemical residue not in food before;
- RR canola and cotton seed oils are so extensively processed before human consumption that no DNA or proteins will remain. This ignores, for example, the use of whole seeds for sprouting, the inclusion of whole seeds in uncooked foods, and the cold pressing of oils;
- Bt cotton and corn are substantially equivalent to parental lines in composition, safety and wholesomeness, yet Bt has never been in the food supply in such quantities before;
- the toxicological studies of RR foods are brief and insufficient as no chemical residue studies are cited, proteins created by inserted genes have only been checked against known protein toxins and allergens, no human, and very few animal testing of the products has been done, whole genetically engineered soybean, corn, canola or cotton were not checked in simulated gastric and intestinal fluids;
- no toxicological studies were carried out on the Bt crops as Monsanto asserts that "regulatory agencies world-wide have determined that the use of registered B.t.k products pose no significant risks to human health, non-target organisms or the environment." Believes this is grossly misleading as it refers to the topical use of a whole organism which quickly disappears from the environment following spraying, whereas Bt crops express large amounts of toxin throughout their systems.

Berylla (NZ)

- these foods will be in 60–80% of all processed foods therefore freedom to choose will be compromised;
- as these foods will also be fed to animals, choices will be restricted even further and if the animals were eaten then the degree of risk will increase;
- support the submissions of the Natural Law Party and Clive Elwell.

Willi Borst (NZ)

- wants all genetically modified foods to be labelled and if not they should all be banned;
- concerned about antibiotic resistance, viral recombination and environmental pollution;
- all genetically modified food should be deemed unsafe until proven otherwise;
- submits that ANZFA not amend the *Food Standards Code* to permit foods derived from genetically modified crops.

Jim Chapple (NZ)

- strongly opposed to all six applications on the grounds that approval of these foods may create a market monopoly for the applicant in the supply of agrochemicals and that gene technology is potentially unsafe;
- very strongly objects to the term "substantially equivalent" as a play on words;
- genetically modified foods are not identical to their conventional counterpart and therefore all such products must carry labelling.

Commerce Commission (NZ)

- no issues raised by the applications on which the Commission has any comments.

Consumers' Association of South Australia Inc. (Aust)

- supports comments made by Elaine Attwood.

Clive Elwell (NZ)

- the applications should be rejected because Maori people find genetic engineering in conflict with their beliefs and values, the overwhelming majority of people in Australia and New Zealand do not want to eat genetically modified food, there is a danger of allergic reactions, and genetically modified food is insufficiently tested and so cannot be regarded as safe for human consumption;
- the foods cannot be sufficiently tested because it is impossible to carry out appropriate tests; the tests that are carried out are limited and inappropriate.

Consumers' Federation of Australia Inc.

- not supportive of these applications being approved at this stage;
- questions the safety of soya milk as infant food because of the presence of trypsin inhibitor and other anti-nutrients after heat processing, and also the presence of isoflavones;
- refers to a reference (no details supplied) which has shown that the isoflavone levels may differ from the levels in conventional soybeans;
- application A338 does not provide sufficient evidence of anti-nutrients to prove that the soybeans are safe for processing into infant formula. In light of this, interprets ANZFA's safety assessment guidelines as requiring a full toxicological and nutritional assessment of the soybeans. Believes these

concerns are serious enough to warrant a recall of foods containing Roundup Ready soy ingredients;

- no evidence is presented by the applicant on glyphosate residues in A338, A362, and A363, despite a specific requirement to do so in ANZFA's safety assessment guidelines;
- does not accept the assertion by the applicant that there is only one novel protein in the Roundup Ready soybeans;
- does not believe that testing for homology of protein structure is a sufficient test for allergenicity. At the very least these foods should be fed to human volunteers in closely monitored trials before they are released generally;
- traces of the introduced proteins could be present in cold-pressed oils at levels sufficient to precipitate allergic reactions, if there is an allergic potential. At the very least, such oils should carry precautionary labels warning of the possibility of allergic reactions;
- the approval of Roundup Ready maize will facilitate even greater use of high fructose corn syrups in Australian processed foods. The end result of this could well be that consumption of high energy products by Australians will rise and that the current excessive levels of nutritional diseases such as obesity, diabetes and heart disease will increase further;
- ANZFA needs to be satisfied that anti-nutrient levels in canola are safe and that they will not rise over time;
- expresses concern about the decreased weight gain by laboratory rats in the first week of a 4 week feeding trial with INGARD cotton seed. Believes that further feeding trials on a range of animals should be performed before this product is released;
- approval of foods produced using gene technology should be deferred until a national coordinating system for regulatory approvals is in place so that a global assessment of their likely impacts can be made;
- a system for monitoring adverse reactions to these foods should be established before they are released into the diet of Australians;
- approval and release of these foods should not occur until the system of labelling agreed to by Health Ministers is established;
- Australia should not be bullied by other countries to accept their exports of unsegregated mixtures of genetically modified and non-modified foods.

Francela Davies (NZ)

- concerned about the addition of food additives in the form of genetically engineered foods that have not been given adequate testing of their benefits or side effects to human health;
- wants ANZFA to address the long term effects of the consumption of foreign proteins, antibiotic resistant marker genes and viruses;
- the applications should be rejected because there is no evidence that these foods are contributing anything positive to the food supply or the environment.

Food Technology Association (FTA) Victoria Inc.

- the risk assessment must be completed and reported to ANZFA stakeholders prior to any decision on the Applications;
- it is unclear from Standard A18 as to the labelling that would apply to these products;
- wants to know what special conditions might apply to these products;

- the option to not permit the sale of these foods is the preferred option;
- the application needs more detail and background information such as a Full Assessment report, details on special conditions and labelling and a complete risk assessment.

Friends of the Earth (NZ)

- share the same concerns as expressed in the submission of the Natural Law Party and Clive Elwell;
- glyphosate has not been included among the residues tested, and there is no awareness of any program that monitors for glyphosate residues in food;
- Treaty of Waitangi obligations have not been considered in ANZFA processes;
- the New Zealand Bill of Rights provides that no New Zealand may be subjected to experimentation without providing informed consent, therefore full disclosure of all transgenic foods and ingredients via labelling is the only way this can begin to be achieved;
- Monsanto has not done any long term studies on health effects;
- submit that ANZFA should approve these foods for a period of 6 months only conditional on a requirement for immediate, prominent labelling of all food products and a warning logo. This should be followed by a moratorium on any further approval of genetically engineered foods.

Noeline Gannaway (NZ)

- supports labelling of all food containing genetically engineered products;
- there may be risks of toxic or allergic reactions;
- oppose the transfer of genetic material between different species as unethical and potentially unsafe.

Goodman Fielder (Aust)

- is fully supportive of developments in the agri–food industry through the application of gene technologies provided that consumer benefits are clearly defined and communicated;
- urges ANZFA to undertake wide consultation with all affected parties, including growers, crushers (in the case of oilseeds), food industry users and consumers before these modified plants are introduced.

Nathan Green (NZ)

- objects vehemently to the further introduction of genetically modified foods, specifically the applications by Monsanto;
- there have not been sufficient tests to prove safety;
- NZ should exploit the GMO free market opportunities;
- there has not been adequate public debate on the introduction of genetically modified foods;
- does not agree with the concept and use of substantial equivalence.

Mike and Jeanne Gregory (NZ)

- the public has not been properly consulted or informed by Government or ANZFA on the introduction of genetically modified foods;
- strongly opposed to genetically modified foods on grounds that these are not adequately tested;

- there is significant and growing scientific concern worldwide about the technology and the processes undertaken to evaluate the safety of genetically modified foods;
- NZ would have a market advantage if genetically engineered foods were prohibited altogether.

Martin Hartman and Cornelia Baumgartner (NZ)

- object to genetically modified foods;
- call for mandatory labelling of all genetically modified foods.

Karen Hunt (NZ)

- demands that all genetically modified foods be labelled;
- states that consumer rights are violated if products are deemed substantially equivalent and consequently are not subject to mandatory labelling.

InforMed Systems Ltd (NZ)

- the transfer of EPSPS genes to soybean, maize, cotton and canola is acceptable;
- the transfer of the gox gene to canola and the use of the cry1Ac gene are also acceptable;
- noted that no mention was made of any marker genes in the applications for soybeans, corn or canola;
- noted that the nptII gene is used in cotton and one insect resistant corn variety. Considers that there are remaining questions with regard to the use of antibiotic resistance genes. It would be reassuring if independent biomedical advice were available to reassure us that this does not pose a risk to the future use of these or related antibiotics in the management of human disease;
- notes that none of the modified plants provides any nutritional or functional benefit for the consumer. It is unfortunate that the first applications should not demonstrate benefits to the consumer, who may thus feel that failure to permit the use of such foods will have no measurable effect on them.

Oraina Jones (NZ)

- genetically engineered foods have not been adequately tested for their benefits or side effects to human health;
- what are the long term effects of the consumption of foreign proteins, antibiotic resistant marker genes and viruses;
- questions whether Monsanto supplied any evidence of long term trials;
- requests that the application be declined as the foods are not contributing in any way to the food supply or environment.

Michael Karas (Aust)

- is opposed to applications A338, A355, A362 and A363 because they are for herbicide resistant crops;
- is concerned about the potential for Roundup residues to be increased in human food supply;
- is concerned about the out-crossing of herbicide resistant crops to create 'super-weeds'.

Colin Kell (NZ)

- criticises some of the wording used in the preliminary assessment report;

- claims that genetically altering food decreases the nutritional value;
- the application provides no proof that glyphosate does not cause long term effects;
- there has been insufficient testing of these genetically modified foods;
- balanced information on genetic modification needs to be made available and the rights of everyone taken into consideration;
- imported commodities should be segregated at source;
- the applications do not indicate the source of the genes being used - believes that genes from fish and animals are being used which is unethical and against nature.

Janine Kelly (NZ)

- concerned about the depth of investigation into the safety of genetically modified foods and the apparent lack of concern by regulatory authorities for the opinions of informed members of the general public and some scientists;
- ANZFA puts too much faith in the integrity of companies who are producing genetically modified foods;
- urges ANZFA to consider the long-term implications of allowing the sale of genetically modified foods;
- if they are allowed, they should all be labelled.

Kristen Khaine (NZ)

- consumer rights include the choice not to eat any genetically modified foods, therefore labelling is of paramount importance;
- trade barrier issues are secondary to public health and safety.

Hilde and Kristin Knorr (Aust)

- advocate a prohibition on genetically modified foods altogether, but otherwise strongly demand mandatory labelling.

Susie Lees (NZ)

- not enough information has been provided in these applications;
- the public do not want to eat these products;
- if the products are approved, we will be at risk of unknown toxins and allergens.

Margaret and Leonard Krohn (Aust)

- opposed to genetically modified foods on the grounds that insufficient scientific testing has been done and the effects on public health are unknown.

C. Lamprecht (Aust)

- concerned about the possible detrimental health effects of genetically modified foods;
- concerned about increased pesticide residues in food;
- advocates full mandatory labelling of all genetically modified foods.

Hannah Levy (Aust)

- strongly opposed to genetically modified foods because of the limited knowledge concerning the risks associated with the technology;
- demands full labelling.

Mahikari Australia

- strongly advocates the mandatory labelling of all foods or food ingredients produced using gene technology to allow consumer choice;
- disagrees with validity of "substantial equivalence" as a basis for labelling because of a lack of scientific rigor;
- completely opposed to all six applications because of the potential long term risks;
- concerned about increased levels of glyphosate in food;
- considers gene technology unethical;
- considers the outcomes of gene technology scientifically unpredictable because of the possibility that DNA can readily transfer between species.

Nadine McRae and others (NZ)

- opposes all of the six applications on the grounds that gene technology is unpredictable, unsafe and harmful to the environment;
- demands that all food with some genetically modified food content be labelled.

National Council of Women of Australia

- requests that ANZFA maintain the status quo and not amend Standard A18 to permit the sale of the indicated foods;
- no deliberations on applications should be made under this Standard until the situation with labelling is resolved;
- there is no mention of monitoring pesticide residue increase in the final product as a result of a greater tolerance to what is an obvious need to increase the pesticide used;
- for the soybean applications there should be absolutely no doubt about the safety of the source of the soybean if it is to be used in the Australian food supply;
- only two out of the six foods have been tested by feeding to laboratory animals and then only for 6 weeks;
- no evidence was provided about herbicide residue levels in any of the soybean foods despite there being an application to increase the MRL for glyphosate in soybeans;
- although the CP4 EPSPS protein may be inactivated on processing, the application does not take into account the use of raw soybeans to grow sprouts. This could represent an allergy problem and therefore the foods should be labelled;
- ANZFA has not taken into consideration the considerable consumer backlash that is occurring;
- there must be scientific certainty that humans are not exposed to any newly expressed proteins;
- objects to the commercial-in-confidence aspects of A362;
- concerned about the feeding of genetically modified seeds to animals as this is another source for these products entering the human food supply;
- there is no justification for using glyphosate-tolerant canola;
- Australia should be able to prohibit the import of genetically modified foods if it wishes;
- if ANZFA allows genetically engineered foods to be imported into Australia unlabelled, consumers will be affected by a lack of choice.

Natural Law Party (NZ)

- in the absence of a moratorium on genetically modified food, demands labelling of all genetically modified foods on the grounds that there has been no long term pre-market testing or screening for risk factors associated with this technology and that unlabelled products deprive individuals of their basic freedom of choice;
- rejects the notion of substantial equivalence on the grounds that differences at the DNA level make them substantially different;
- concerned about the potential for increased glyphosate levels;
- the effects of glyphosate on health and on phytoestrogens in genetically engineered soy has not been addressed;
- genetically engineered soy contains genes from a virus, a soil bacterium and from petunia, none of which has been in our food before;
- the technology is being introduced in the total absence of an informed public debate about the general acceptance of GMO technology;
- believe that there is significant potential for environmental or health disasters associated with the current introduction of this technology. Believes that serious liability implications exist and need to be explored;
- recommends that, until long term independent safety and risk assessment studies on genetic technology in food production have been completed and their safety to human health and the ecosystems that support human life is established, approvals for these foods should be declined;
- no further applications should be considered until proper public debate has occurred.

New Zealand Nutrition Foundation

- submission identical to InforMed Systems Ltd

Office of Regulation Review (Aust)

- comments on the preparation of the RIS for the full assessment report;
- ANZFA should discuss, in the background section of the report, why products such as the Roundup Ready soybeans, which previously entered the commercial markets without segregation from the non-transgenic counterpart, now require an approval process. Questions whether the regulation is to address health and safety and/or consumer information concerns;
- the problem section of the RIS should outline the characteristics of food produced using gene technology and why these characteristics might give rise to the need to list special conditions. The RIS should specifically canvass the possible special conditions which could apply and fully discuss the varying costs and benefits that each set of conditions entails;
- the material present in the sections on potential regulatory impacts and identification of affected parties should be summarised in the RIS in matrix form;
- when the RIS is fully developed it will need to include a conclusion section which summarises the views elicited from the main affected parties, a conclusion and recommendation option section which states what the preferred option is and why this option was accepted and the others rejected, and an implementation and review section which outlines how the proposal will be administered, implemented and enforced.

Martin Oliver (Aust)

- opposes all six applications on the grounds that the long term safety of eating foods from herbicide tolerant or insect resistant crops has not been adequately established;
- all genetically modified foods should be labelled in order for consumers to choose;
- claims that the foods have not been tested for any health impact on humans.

The Pacific Institute of Resource Management/Revolt Against Genetic Engineering (NZ)

- all genetically modified food should be labelled so that there can be post-market monitoring for new allergens or toxic effects in consumers;
- strongly opposed to the technology because of a range of concerns about public health and safety;
- raised a number of concerns in relation to Application A338 specifically:
 - the bacterial EPSPS is unlike any protein that humans have ever eaten and there is no reliable method for predicting its allergenic potential;
 - a major allergen, trypsin inhibitor was found to be 26.7% higher in transgenic soybeans;
 - the compositional analyses of the soybeans were not done on soybeans that had been treated with the herbicide;
 - there were significant increases compared to controls in the milk fat of cows fed transgenic soybeans; and
 - the applicant did not submit any data on glyphosate residues in the transgenic soybeans.

Sara Parsons (NZ)

- objects to the applications because she is a vegetarian;
- it is harmful to be introducing genetically modified soybeans, corn, canola oil and cottonseed into the NZ food chain;
- these products are a threat to the safety and well being of animals and humans and are of no benefit to society;
- the testing of genetically modified foods on animals and the harm that may be caused to animals in the wider environment is unacceptable;
- the lack of labelling of genetically modified foods means that NZ consumers have no way of making appropriate choices if they wish to avoid eating such foods which may cause allergic reactions and offend ethical beliefs.

Eric Phimister (NZ)

- is concerned about the importation of unlabelled genetically modified food;
- does not wish to consume soybeans with a higher pesticide level than the previously allowed maximum. This alone should make it not substantially equivalent.

Marja Rouse (Aust)

- opposes all six applications on the grounds that the genetically engineered crops pose a major environmental hazard and human health hazard;
- claims that the technology promotes unsustainable farming practices;
- believes consumers have the fundamental right to be informed about all the ingredients in foods and therefore demands mandatory labelling;

- the safety assessment for the applications should not be based on information provided by the applicant in these cases, as the company has a vested interest in having the applications approved.

Dean Scahill (NZ)

- is opposed to the foods which are the subject of Monsanto's applications on the grounds that the costs in terms of potential risk to health, risk to organic crop contamination, and current inability of consumers to identify such foods, greatly outweighs the benefits;
- if NZ remains GMO-free is represents an opportunity to create a niche market;
- a labelling system should be developed which would provide consumers with a choice so that they can retain the right to not eat genetically modified food should they choose;
- ANZFA should address the large public concern associated with the introduction of genetically modified foods onto the market.

Emma Subue-Timson (Aust)

- opposed to foods produced using gene technology on the grounds that the technology contravenes nature.

Christine Taylor (Aust)

- opposes all applications because of the presence of new genes, new proteins and increased herbicide residues in genetically modified foods;
- concerned about the potential for herbicide resistance genes to transfer to other plant species, creating undesirable effects.

Bridget Thrussell (NZ)

- supports regulatory option 1- to not permit the sale of any of the foods in the applications;
- no long term safety tests have been done;
- worried about antibiotic resistance increasing because of the antibiotic resistance marker genes in A355;
- concerned about gene transfer between Roundup Ready canola and other Brassicas.

E.M. Trevelyan (NZ)

- does not believe that genetically modified foods can be assessed as safe because of the possibility of "gene flow";
- crops containing the Bt gene will inevitably lead to resistant insect populations;
- envisages an enormous marketing advantage to NZ if it maintains a clean, green image by not allowing genetically modified food onto the market;
- all genetically modified food products should be labelled.

Richard van Wegen (Aust)

- supports the restricted use of genetically modified plants for food production;
- strongly supports mandatory labelling as a democratic right to make informed decisions about food purchases.

Arnold Ward (Aust)

- opposed to all applications on the grounds that long term safety has not been

- established;
- ANZFA only concerns itself with public safety rather than adopting a 'holistic' approach which takes into consideration the broader issues to do with genetic engineering
 - Roundup herbicide contains other chemicals which are harmful. Considers that the acceptable daily intake of glyphosate does not take into account the higher toxicity of the surfactant POEA in Roundup, on individuals with increased susceptibility such as children, immune compromised individuals or the elderly;
 - notes examples of scientific evidence which show glyphosate can increase levels of plant oestrogens, which are known to affect humans. Very concerned about the potential health effects, particularly in children, of higher levels of oestrogens;
 - feeding experiments in cows indicate a change in the milk fat production in animals fed on Roundup Ready soybeans versus non-transgenic soybeans;
 - where resistance to Bt toxin occurs because of a widespread use of insect resistant crops, this would mean that organic farmers, who now rely on Bt formulations, could lose an important pest control agent;
 - expresses concern about the possibility of recombination and horizontal gene transfer resulting in environmental catastrophies;
 - glyphosate does not degrade in soils as efficiently as claimed by the applicant;
 - all transgene products should be given the same testing applicable to pharmaceuticals;
 - the seeds from genetically engineered crops could spread due to natural disasters;
 - plant viruses can acquire viral DNA from a transgenic plant;
 - Bt cotton is not very effective in controlling bollworm infestations;
 - calls for a moratorium of 10 years on the introduction of genetically modified foods.

Joyce Weatherhead (NZ)

- opposes approval for the applications on the grounds that genetically modified foods have not undergone an independent scientific testing;
- calls for a moratorium on genetically modified foods in NZ for ethical and religious reasons;
- demands mandatory labelling of all genetically modified foods;
- believes that approval for herbicide resistant soybeans will result in a huge increase in the level of contaminating herbicides in foods derived from these crops.

Western Australian Food Advisory Committee

- a safety assessment of the foods is lacking along with the absence of any supporting scientific evidence;
- post-market monitoring to confirm the results of risk assessment and establish evidence of a safe history of use is an unacceptable alternative to a full scientific evaluation, with the results being available for public scrutiny;
- the claim that CP4 EPSPS is destroyed in heat processing requires independent scientific validation and it is unclear from ANZFA's papers whether this evidence has been provided and reviewed;
- insufficient evidence has been provided in the discussion document to support claims that these products are safe or that the Authority has undertaken a

- rigorous analysis or comprehensive scientific evaluation of these products;
- the issue of decreased availability of food choices in the marketplace listed under both Options 1 and 2 is not nearly as important as the safety issue;
- given the heightened public concern about genetically modified foods it is essential that scientific information relating to compositional variance due to novel gene expression, toxicology, potential for allergenicity, nutritional and dietary properties for each of the foods proposed by Monsanto, is publicly available;
- the Committee recommends the adoption of Option 1 at this time.

S. and L. Wintergraas

- ANZFA should stop all genetically engineered foods from entering into any food products in NZ, as it will destroy the clean green image;
- ANZFA is not able to guarantee safety of these foods - cites DDT, nuclear power and antibiotics as examples;
- ANZFA should protect the consumer, not big business.

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, expressed general views against the use of gene technology and asserted that food produced using this technology is unsafe for human. A number of general issues were raised in these submissions and are addressed below.

1. *The safety of genetically modified foods for human consumption*

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

Evaluation

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

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

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

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD). The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are consistent with international protocols developed by these bodies.

2. The need for long-term feeding studies

A number of submissions were concerned about the lack of long-term toxicity studies on genetically modified foods.

Evaluation

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to 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 studies on foods is the need to maintain the nutritional value and balance of the diet. A diet that is poorly balanced will compromise the interpretation of any feeding study, since the effects observed will confound and usually override any small adverse effect which may be related to a component or components of the food. 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 re-assurance that the proteins will have no adverse effects in humans when consumed as part of a food. Such experiments can provide more meaningful information than experiments on the whole food. Additional re-assurance regarding the safety of newly-expressed protein can be obtained by examining the digestibility of the new protein in *in vitro* assays using conditions which simulate the human gastric system.

3. Substantial equivalence

A number of submitters expressed concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some rejected the premise

of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

Evaluation

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

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

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

The concept of substantial equivalence was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment.*' The concept has been internationally recognised and embraced as a valuable tool in the safety assessment of foods produced using gene technology. The OECD also advocates an approach to safety assessment based on substantial equivalence as being '*the most practical to address the safety of foods and food components derived through modern biotechnology.*'

4. The nutritional value of food produced using gene technology

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

Evaluation

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

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

5. Potential toxins and allergens

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

Evaluation

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

6. Antibiotic resistance

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

Evaluation

The human health considerations in relation to the potential for the development of antibiotic resistance depend on the nature of the novel genes and must be assessed on a case-by case basis. This issue arises because of the use of antibiotic resistance marker genes in the generation of genetically modified plants. In some circumstances, antibiotic resistance genes are linked to the gene of interest, to enable the initial selection of the engineered cells in the laboratory. Those cells that contain the antibiotic resistance marker gene, and hence the gene of interest, will be able to grow in the presence of the antibiotic. Those cells that failed the transformation process are eliminated during the selection procedure.

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

In 1993, the World Health Organisation Food Safety Unit considered this issue at a Workshop on the health aspects of marker genes in genetically modified plants. It was concluded at that Workshop that the potential for such gene transfers is effectively

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

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

7. Transfer of novel genes

Some submitters have expressed concern that the transfer of any novel gene may be a health concern.

Evaluation

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

8. Viral recombination

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

Evaluation

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

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

9. Labelling of foods produced using gene technology

A majority of submissions focussed on this issue. Specifically, the submissions called for the labelling of all foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters based their demands for full labelling on the presumption that all foods produced using gene technology are unsafe and on consumer “right to know” arguments. It was stated that full labelling was the only means of identification of foods produced using gene technology available to consumers.

Evaluation

The existing Standard A18 already makes provision for mandatory labelling of genetically modified foods that are substantially different from their conventional counterparts. However, ANZFA is committed to implementing the in-principle decision of ANZFSC Health Ministers of August 1999 to require labelling of all genetically modified foods, including those that are substantially equivalent in composition to the unmodified form. In conjunction with a task force of officials from State and Territory Health Departments and the New Zealand Ministry of Health, ANZFA developed draft revision to Standard A18 in October 1999 that requires labelling of other categories of genetically modified foods. At the Ministers request this draft was circulated for public review and a cost-benefit analysis of full labelling was commissioned. The task force considered both public comments and the cost-benefit analysis in finalising their recommendations to Ministers, which were delivered in May 2000. Ministers are to meet to resolve the issue in July 2000 following whole-of-government consideration of the issue. It is therefore expected that, following a decision and legal amendments to the standard, labelling requirements will be implemented that will apply to all current and subsequent applications.

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

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

Evaluation

Surveillance of potential adverse or beneficial effects of GM foods is seen by many as a logical follow-up to the initial scientific risk assessment. Nevertheless, it is recognised that there are limitations to the application of epidemiology studies, particularly in relation to food components. A key requirement for post-market surveillance systems is that a clear hypothesis be identified for testing. Establishing a system for the surveillance of potential health effects of exposure to novel foods requires monitoring of the consumption patterns of novel foods in the population, and health effects in both “exposed” and “non-exposed” individuals/populations, so that risk estimates can be derived. For any such monitoring system to be useful, there needs to be a range of exposures, otherwise, any variation in health outcome would be unexplainable by that exposure. Variations in exposure could be apparent over time (temporal trends), space (geographical trends) or both.

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

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

11. Public consultation and information about gene technology

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

Evaluation

The issue of gene technology and its use in food has been under consideration in Australia since 1992. The Agreement between the Governments of Australia and New Zealand for a joint food standard setting system, however, did not occur until 1995, and the New Zealand community therefore had not been consulted on this matter by the Authority until after that time. Consequently, the proposed standard (the current Standard A18) underwent only one round of public comment in New Zealand at which time significant objections were raised by the New Zealand community to the use of gene technology in food production. Many New Zealand consumers, both in these submissions, and in previous submissions to the Authority, have expressed the view that there has been insufficient consultation and a consistent lack of information about gene technology.

Although Standard A18 came into force in May 1999, the public have a continuous and ongoing opportunity to provide comment in relation to applications under the standard. ANZFA's statutory process for all applications to amend the *Food Standards Code* normally involves two rounds of public comment. Furthermore, all the documentation (except for commercial in confidence information) relating to these applications is available in the public domain, including the safety assessment reports. There is ample evidence that the provision of such information by ANZFA has already significantly stimulated public debate on this matter.

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

In response to the concerns raised in public submissions with regard to gene technology and GM foods, ANZFA is in the process of preparing a public discussion paper on the safety assessment process for GM foods. This will be widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

12. *Maori beliefs and values*

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

Evaluation

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

13. Environmental concerns and the broader regulatory framework

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

Evaluation

These issues are considered in the assessment processes of GMAC in Australia and the Environmental Risk Management Authority (ERMA) in New Zealand. The Authority does not have the mandate to assess matters relating to environmental risks resulting from the release of food produced using gene technology into the environment. However, links exist between ANZFA and other regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs. ANZFA would not recommend the approval of a food produced using gene technology if the genetically modified organism from which it was derived did not have the appropriate clearance for general release from either GMAC (or its successor) or ERMA, as appropriate.

The regulatory system in Australia will comprise the existing regulators with a legal remit to cover some aspects of GM products (such as imports, food, agricultural and veterinary chemicals):

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

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

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

In Australia a new Office of the Gene Technology Regulator (OGTR) will complement the existing arrangements. OGTR will supersede the existing

arrangements under the Genetic Manipulation Advisory Committee (GMAC), which advises on research and environmental release of GMOs. OGTR will regulate all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

All GM food is assessed and regulated by the Australia New Zealand Food Authority (ANZFA) under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as Australia New Zealand Food Standards Council (ANZFSC).

There will be an interface between ANZFA and OGTR. Consequential amendments proposed to the ANZFA Act arising from the draft Gene Technology Bill 2000 will establish a statutory interface between OGTR and ANZFA. This will involve amendments to the ANZFA Act requiring the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (currently Standard A 18).

14. Maximum residue levels of agriculture/veterinary chemicals

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

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 Food Regulations (1984) in New Zealand.