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13/01

DRAFT RISK ANALYSIS REPORT

APPLICATION A378

Food derived from glyphosate-tolerant sugarbeet line 77 (GTSB77)

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" in Section 16 of the Act). See under 'Invitation for Public Submissions' for details.

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EXECUTIVE SUMMARY

BACKGROUND

ANZFA received an application from Monsanto on 30 April 1999 to amend the Australian *Food Standards Code* to include food produced from glyphosate-tolerant sugarbeet line 77 in the Table to clause 2 of Standard A18 – Food Produced using Gene Technology. The modified sugarbeet under consideration is known commercially as Roundup Ready® sugarbeet and is tolerant to applications of the herbicide glyphosate. This report describes the scientific assessment of the application.

ISSUES ADDRESSED DURING ASSESSMENT

(i) Safety Evaluation

Nature of the genetic modification

Glyphosate-tolerant sugarbeet line 77 (GTSB77) was generated by the transfer of three new genes: the *cp4-epsps* gene, the *uidA* gene and a modified *gox* gene. Two of the transferred genes - the CP4 EPSPS and *gox* genes confer tolerance to the herbicide glyphosate. Both genes are bacterially-derived and have distinct modes of action. The CP4 EPSPS gene encodes a 5–enolpyruvyl shikimate–3–phosphate synthase enzyme that is not sensitive to applications of glyphosate. The *gox* gene encodes the glyphosate oxidoreductase enzyme that can degrade the herbicide however it was truncated during transformation and 69% of the gene is fused to sugarbeet DNA resulting in a chimeric gene. Although mRNA transcripts from this chimeric gox sequence are present in the sugarbeet, no novel protein is translated and the sugarbeet does not have GOX enzyme activity.

The *uid*A gene encodes β–D-glucuronidase (GUS) which serves as a marker for plant transformation.

Glyphosate-tolerant sugarbeet GTSB77 was generated using *Agrobacterium*-mediated transformation.

Single copies of the CP4 EPSPS, *uid*A and the chimeric *gox* gene were stably integrated at one insertion site in sugarbeet over multiple generations. They were also inherited in a Mendelian manner, and always segregated together.

General safety issues

Sugarbeet has a long history of use as a source of sugar production and accounts for approximately one third of world sugar production. The major food products are pure sucrose and molasses. Sugarbeet pulp may be used as food fibre. By-products from sugarbeet (tops, leaves and post-processing trash) are used as cattle feed.

Two new proteins are present in glyphosate-tolerant sugarbeet GTSB77, namely the CP4 EPSPS and GUS proteins. These proteins were detected in very low levels in root tissue of sugar beet GTSB77 (58ppm and 0.5ppm for CP4 EPSPS and GUS respectively). They were also present at higher levels in leaf and stem tissue (237ppm and 3ppm for CP4-EPSPS and

GUS respectively). Neither protein was detected in the principal food fractions produced from sugar beet, refined sugar and molasses. The novel proteins were also detected at very low levels in sugar pulp which may be used as a source of dietary fibre. However the proteins are not expected to be present due to the extensive refining that pulp undergoes if it is processed into refined dietary fibre. Thus exposure to the novel proteins is likely to be extremely low.

Glyphosate-tolerant sugar beet line GTSB77 contains no antibiotic resistance genes and therefore poses no risk to the development of antibiotic resistant pathogenic bacteria through the horizontal transfer of antibiotic resistance genes. The transfer of novel genetic material from glyphosate-tolerant sugar beet line GTSB77 to human cells via the digestive tract was assessed, but was considered to be extremely unlikely to occur, and unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Toxicological issues

The presence of naturally-occurring toxins and allergens in glyphosate-tolerant sugar beet line GTSB77 was investigated. Saponins are the only known toxicants found in sugar beet and are actively eliminated in sugar processing. However, the levels of saponins were evaluated in glyphosate-tolerant sugar beet GTSB77. In most field trials, no difference between saponin levels in glyphosate-tolerant sugar beet GTSB77 and control sugarbeets were observed. In one trial, small but significant decreases in saponin levels were evident when glyphosate-tolerant sugar beet GTSB77 was treated with glyphosate. However, as the difference was not consistent across all field trials and the differences was a decrease in this toxicant which is regarded as beneficial, it is concluded that no health and safety concerns are raised.

The potential toxicity and allergenicity of the CP4 EPSPS and GUS proteins as well as the potential protein product from the chimeric *gox* gene were assessed. These proteins did not possess characteristics of known toxins and results from acute oral toxicity testing in mice did not indicate any toxic effects.

The potential for the novel proteins to be allergenic was investigated using a number of criteria, including amino acid sequence homology with known allergens, history of use and common physicochemical properties of allergens, including the sensitivity to digestion by digestive enzymes. The novel proteins were found to be rapidly digested in conditions that mimic human digestion. Additionally, they show no amino acid similarity to known allergens and are not detectable in products refined from the glyphosate-tolerant sugarbeet.

Nutritional issues

Detailed compositional analyses conducted over multiple years and geographic regions (USA and Europe) were carried out to establish the nutritional adequacy of glyphosate-tolerant sugar beet GTSB77. The effect of glyphosate use on the composition of sugarbeet was also examined. Analyses included crude ash, crude fibre, crude protein, carbohydrate and dry matter in both tops and roots (both raw and processed into brei powder used in sugar production). Additional quality components were measured in roots including, variously, invert sugar (glucose + fructose) content, polarisation (% sucrose), sodium, potassium and amino nitrogen.

No biologically meaningful differences in any compositional and quality parameters relevant to food were identified between non-transgenic, control sugar beet and sugar beet GTSB77, both untreated or treated with glyphosate at recommended agronomic application rates.

It is concluded that glyphosate-tolerant sugar beet is equivalent to other commercially available sugar beet with respect to composition and nutritional quality. No nutritional risks are posed by consuming food derived from glyphosate-tolerant sugar beet GTSB77.

Conclusion

On the basis of the data submitted in the present application, glyphosate-tolerant sugar beet GTSB77 is equivalent to other commercially available sugarbeet in terms of its safety and nutritional adequacy.

(ii) Labelling

Under the current Standard A18, which remains in effect until 7 December 2001, food derived from glyphosate-tolerant sugar beet GTSB77 does not require labelling as it is regarded as substantially equivalent to food derived from non-genetically modified sugarbeet varieties.

When the amended Standard (A18 in the Australian *Food Standards Code*, 1.5.2 in the *Australia New Zealand Food Standards Code*) comes into effect on 7 December 2001, food products made from glyphosate-tolerant sugar beet GTSB77 will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

(iii) Public Submissions

Forty-five public submissions were received in relation to this application, of which only four 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 carried out by ANZFA, the details of which are in Attachment 2.

CONCLUSIONS

On the basis of the data submitted with the application and evidence obtained from the scientific literature, it is concluded that:

- the introduced gene in glyphosate-tolerant sugarbeet GTSB77 is not considered to produce any increased public health and safety risk;
- food derived from glyphosate-tolerant sugarbeet GTSB77 is equivalent to food derived from other commercial varieties of sugarbeet in terms of its safety and nutritional adequacy.

RECOMMENDATION

Based on the data submitted in the application, ANZFA concludes that food derived from glyphosate-tolerant sugarbeet GTSB77 is as safe for human consumption as food from other

commercial sugarbeet varieties, and therefore recommends that the Australian *Food Standards Code* (Volume 1) and the recently adopted joint *Australia New Zealand Food Standards Code* (Volume 2) be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard A18 and Standard 1.5.2 is provided in Attachment 1.

ANZFA now seeks public comment on the proposed amendment in accordance with the procedures described in Section 16 of the *Australia New Zealand Food Authority Act 1991*.

INVITATION FOR PUBLIC SUBMISSIONS

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 a draft variation to the Australian *Food Standards Code* (Volume 1) and the recently adopted *Australia New Zealand Food Standards Code* (Volume 2). The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the Food Standard *Codes,* and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' in section 16 of the Act).

Written submissions containing technical or other relevant information, which will assist the Authority in preparing the Final Risk Analysis Report for this application, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

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

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 A378** at one of the following addresses:

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Submissions should be received by the Authority by **20 April 2001**.

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.

BACKGROUND TO THE APPLICATION

Glyphosate-tolerant sugarbeet GTSB77 is referred to as Roundup® Ready sugarbeet and is tolerant to applications of the herbicide glyphosate through the transfer of the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) gene. This gene encodes an enzyme that can function under applications of glyphosate unlike plant-derived forms, which are sensitive to glyphosate. The *uid*A gene is also present in the sugarbeet and encodes β–D-glucuronidase (GUS) which is a selectable marker that is used during plant transformation. A chimeric *gox* gene is transferred to the sugarbeet but does not result in a stable protein product in the plant.

Glyphosate-tolerant sugarbeet GTSB77 is not currently grown in either New Zealand or Australia. The two primary products from sugarbeet, pure sucrose and molasses are also not likely to be imported into Australia or New Zealand but may be present as ingredients in imported processed food products.

The main benefits of glyphosate-tolerant sugarbeet GTSB77 are agronomic in nature, and are therefore likely to accrue mainly to the primary producer. It is envisaged that production of these crops reduce reliance on agricultural chemicals for weed control with potentially higher overall crop yields. More general benefits may flow to the community as a result of reduced primary production costs.

PUBLIC CONSULTATION

ANZFA completed a Notice of Application (formally referred to as the Preliminary Assessment Report) upon receipt of the application and called for public comment on 3 November 1999. A total of 45 submissions were subsequently received. Attachment 5 contains a summary of the submissions.

NOTIFICATION OF THE WORLD TRADE ORGANIZATION

During the ANZFA assessment process, comments are also sought internationally from other Members of the World Trade Organization (WTO). As Members of the WTO, Australia and New Zealand are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreements) (for further details on WTO, see Attachment 4). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

As there is significant international interest in the safety of these foods, the proposed changes to Standard A18 are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters and will therefore be notified to the WTO.

ISSUES ADDRESSED DURING ASSESSMENT

1. Safety assessment (Attachment 2)

Glyphosate-tolerant sugarbeet GTSB77 has been evaluated according to safety assessment

guidelines prepared by ANZFA[1]. The assessment involved an extensive analysis of the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues. On the basis of the data submitted in the present application, ANZFA concludes from the assessment of this data, that glyphosate-tolerant sugarbeet GTSB77 does not raise any public health and safety concerns and is equivalent to other commercially available sugarbeet varieties in terms of its nutritional adequacy. The safety assessment report can be found at Attachment 2 including a summary of findings at the beginning of the document.

2. Labelling of food produced from glyphosate-tolerant sugarbeet GTSB77

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

Until the new labelling requirements take effect, the provisions in the original Standard A18 apply. Under these provisions, food derived from glyphosate-tolerant sugarbeet GTSB77 does not require labelling as it is regarded as substantially equivalent to food derived from non-genetically modified sugarbeet varieties.

3. Issues arising from public submissions

3.1 General issues

Of the 45 submissions received, only a small number addressed issues specific to this application. Rather, the majority of submissions raised issues of a general nature relating to gene technology or issues that had already been addressed in the safety assessment report (see Attachment 2). A discussion of some of the general issues in relation to gene technology that were raised in public submissions can be found in Attachment 6.

3.2 Specific issues

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

(i) Toxicity of glyphosate

GeneEthics and the Genetic Engineering Action Group express the concern that growing a crop with a herbicide-resistant trait inevitably results in higher levels of usage of the herbicide, with concomitant concerns in relation to the potential toxicity of the herbicide in food and its effects on human health.

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

Response

This concern is raised frequently about plants genetically modified to be resistant to a herbicide and in this instance, glyphosate. However, as glyphosate is used commercially on conventional crops, this is not an issue that is peculiar to the transgenic sugarbeet in this application. In Australia, the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is responsible for assessing the toxicity of agricultural chemicals prior to their incorporation into farming practices, especially in the production of food crops. This is a rigorous process that entails investigation into the human and animal toxicity of the chemical, its effects on the environment and the potential effects of occupational exposure to the chemical. Consequently, a wide range of scientific data and technical information is taken into consideration when determining the maximum permissible amount of glyphosate residues in food, referred to as the Maximum Residue Limit (MRL).

The toxicity of glyphosate has been extensively studied in animal testing of a range of different species including rats, dogs, mice, rabbits, guinea pigs and monkeys. The testing of the toxicity of glyphosate also included long term studies in which animals were exposed to varying levels of the herbicide over periods of time in excess of 2 years. An assessment of this toxicological data has been undertaken by the Commonwealth Department of Health and Aged Care to support the establishment of acceptable daily intake levels. The results of the animal studies indicate that glyphosate exhibits a very low degree of toxicity.

Furthermore, in the agricultural environment, when applied to emerged weeds, glyphosate shows no residual activity. This is because it binds strongly to soil particles and is readily broken down by soil microorganisms. Because of the rapid transportation from the leaves of treated plants to the roots, it is effective in destroying perennial weeds which can survive other herbicides which only affect the above-ground parts of the weed plant.

4. Risk management

Under Standard A18 (and Standard 1.5.2 in the Australia New Zealand Food Standards Code), a GM food must undergo a pre-market safety assessment in accordance with ANZFA's safety assessment guidelines. The requirement for the food to be labelled must also be assessed in accordance with the labelling criteria specified in clause 4 of the amended Standard. Labelling according to the original standard A18 must be in accordance with the criteria specified in clause 2 and will be permitted until 7 December 2001. After this date, labelling will be required to comply with Standard 1.5.2 of the Australia New Zealand Food Standards Code.

On the basis of the conclusions from the safety assessment report, together with a consideration of the public submissions, it is proposed that the Table to clause 2 of Standard A18 be amended to include food from glyphosate-tolerant sugarbeet GTSB77. The proposed amendment is provided in Attachment 1.

A public discussion paper on the safety assessment process for GM food[2] is widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

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

5. Regulatory Impact Assessment

The benefits and costs associated with the proposed amendment to Standard A18 to approve food from glyphosate-tolerant sugarbeet GTSB77 have been analysed in a draft Regulatory Impact Statement (Attachment 3). The benefits of the proposed Standard A18 amendment primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

CONCLUSIONS

ANZFA has conducted a comprehensive assessment of the application according to its *Guidelines for the safety assessment of foods to be included in Standard A18 – food produced using gene technology*. These guidelines are based on internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

It is concluded that:

- the introduced gene in glyphosate-tolerant sugarbeet GTSB77 is not considered to produce any increased public health and safety risk;
- on the basis of the data provided in the application, food derived from glyphosate-tolerant sugarbeet GTSB77 is equivalent to food derived from other commercial varieties of sugarbeet in terms of its safety and nutritional adequacy.

RECOMMENDATION

Based on the data submitted in the application, ANZFA concludes that food derived from glyphosate-tolerant sugarbeet GTSB77 is as safe for human consumption as food from other commercial sugarbeet varieties, and therefore recommends that the Australian *Food Standards Code* (Volume 1) and the recently adopted joint *Australia New Zealand Food Standards Code* (Volume 2) be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard A18 and Standard 1.5.2 is provided in Attachment 1.

ATTACHMENTS

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

ATTACHMENT 1

DRAFT VARIATION TO THE FOOD STANDARDS CODE

A378 – FOOD DERIVED FROM GLYPHOSATE-TOLERANT SUGARBEET (GTSB) LINE 77

To commence : On gazettal

The Food Standards Code is varied by:

(1) *inserting into Column 1 of the Table to clause 2 in Standard A18 in Volume 1 –*

Food derived from glyphosate-tolerant sugar beet GTSB77.

(2) *inserting into Column 1 of the Table to clause 2 in Standard 1.5.2 in Volume 2 –*

Food derived from glyphosate-tolerant sugar beet GTSB77.

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT REPORT

A378 – FOOD DERIVED FROM GLYPHOSATE-TOLERANT SUGARBEET (GTSB) LINE 77

SUMMARY AND CONCLUSIONS

Nature of the genetic modification

Genetically modified sugarbeet line GTSB77 was generated using *Agrobacterium* - mediated transformation methods and the gene sequences intended for transfer consisted of:

- The *cp4-epsps* gene, derived from the soil bacterium *Agrobacterium sp.*, expressing the CP4-EPSPS enzyme which confers glyphosate tolerance to the plant;
- The *uidA* gene from the common bacterium *Escherichia coli.* This gene encodes β-D-glucuronidase (GUS) which serves as a marker for plant transformation; and
- A modified *gox* gene from the soil bacterium *Ochromobactrum anthropii* strain LBAA [previously *Achromobacter sp*]. This gene encodes for glyphosate oxidoreductase which metabolises glyphosate to an inactive form.

Molecular genetic analyseis demonstrated that a single insertion event occurred in the generation of sugarbeet GTSB77. The event included a single copy of both the *cp4-epsps* and *uidA* genes. Both the CP4-EPSPS and GUS enzymes were shown to be fully expressed and functional. A truncated copy of the *gox* gene, which represents 69% of the complete *gox* coding sequence, was transferred in the event and was fused to the sugar beet genome. While mRNA transcripts from this chimeric *gox* sequence were identified in sugar beet GTSB77, no novel protein was translated from these transcripts. Sugar beet GTSB77 was also shown to have no GOX enzyme activity.

The molecular and genetic analyses also indicated that the introduced genes were stably integrated into the plant genome and stably inherited for multiple generations.

General safety issues

The novel CP4-EPSPS and GUS proteins expressed by the *cp4-epsps* and *uidA* genes were detected in leaf and stem tissue (collectively referred to as top), and root tissue of sugar beet GTSB77 at relatively low levels particularly for root: CP4-EPSPS 237ppm and 58ppm for mature top and root respectively; GUS 3ppm and 0.5ppm for mature top and root respectively (average values from field trials over two years).

Neither of these proteins was detected in the two principal human food fractions of glyphosate-tolerant sugar beet GTSB77; refined sugar and molasses. CP4EPSPS and GUS were identified in pulp samples derived from sugar beet GTSB77 at very low levels; 50ppm and 1ppm on average respectively. Sugar beet pulp is occasionally used as a source of dietary fibre for which it undergoes extensive refining. Neither of these proteins would be expected to be present in refined dietary fibre.

Glyphosate-tolerant sugar beet line GTSB77 contains no antibiotic resistance genes and therefore poses no risk to the development of antibiotic resistant pathogenic bacteria through the horizontal transfer of antibiotic resistance genes. Additionally, the horizontal gene transfer of antibiotic resistance genes from plants is not considered to pose any additional risk to public health and safety.

Toxicological issues

CP4-EPSPS and GUS proteins expressed in glyphosate-tolerant sugar beet GTSB77, and the putative protein derived from the chimeric *gox* gene (as assessed in *E. coli* and named Protein 34550), were evaluated for their potential toxicity. None of the proteins possess characteristics of known toxins. No signs of toxicity were observed in mice exposed to more than 1000 fold doses of these proteins as expected to be consumed through eating whole sugarbeet.

In addition, exposure of the proteins to simulated mammalian digestive systems resulted in their rapid degradation. The proteins do not have chemical or physical characteristics that are typical of known food allergens and do not share significant amino acid sequence similarity with known allergens. Finally, the levels of these proteins in refined sugar produced from glyphosate-tolerant sugar beet GTSB77 was determined to be below the level of detection. It is concluded that there is no evidence for any potential toxicity or allergenicity of these proteins in humans

Saponins are the only known toxicants found in sugar beet and are actively eliminated in sugar processing. No difference in the level of saponins was found in glyphosate-tolerant sugar beet GTSB77, when compared to control sugar beets, over three growing seasons conducted over a range of geographical locations in Europe. A similar result was found for a single growing season conducted over a range of geographical locations in the USA. Small but significant decreases in saponin levels were evident when glyphosate-tolerant sugar beet GTSB77 was treated with glyphosate in the European trials but not in the US trial. It is concluded that there is no consistent evidence for any change in the human exposure to saponins in refined sugar products derived from glyphosate-tolerant sugar beet GTSB77 and that no additional human health issues arise with respect to saponin residues.

Nutritional issues

Extensive compositional analyses were conducted over multiple years and geographic regions on glyphosate-tolerant sugar beet GTSB77, both untreated and treated with glyphosate at agronomically recommended levels. Analyses included crude ash, crude fibre, crude protein, carbohydrate and dry matter in both tops and roots (both raw and processed into brei powder used in sugar production). Additional quality components were measured in roots including, variously, invert sugar (glucose + fructose) content, polarisation (% sucrose), sodium, potassium and amino nitrogen.

No biologically meaningful differences in any compositional and quality parameters relevant to food were identified between non-transgenic, control sugar beet and sugar beet GTSB77, both untreated or treated with glyphosate at recommended agronomic application rates.

It is concluded that glyphosate-tolerant sugar beet is equivalent to other commercially available sugar beet with respect to composition and nutritional quality. No nutritional risks are posed by consuming food derived from glyphosate-tolerant sugar beet GTSB77.

Conclusion

No potential public health and safety concerns have been identified in the assessment of glyphosate-tolerant sugar beet GTSB77, marketed as Roundup Ready Sugar Beet. Food derived from glyphosate-tolerant sugar beet GTSB77, principally refined sugar and molasses,

can be regarded as equivalent in terms of its safety and nutritional adequacy to food derived from conventional sugar beet.

1. BACKGROUND

Monsanto Australia Ltd have made an application to ANZFA to vary Standard A18 of the *Food Standards Code* to include food derived from sugarbeet which has been genetically modified to tolerate applications of the herbicide glyphosate. The genetically modified sugarbeet is marketed in the USA under the names Roundup® Ready Sugar Beet or Glyphosate-Tolerant Sugar Beet.

Sugarbeet has been grown for sugar production since the late eighteenth century when 'white Silesian beet' was identified as a source of sugar in Europe. Napoleon encouraged the use and breeding of sugarbeet to provide an alternative to cane sugar which required shipment from the West Indies. Sugarbeet currently accounts for approximately 1/3rd of world sugar production with some 35% being produced in the EU, 20% in Russia and 10% in the USA (Macrae *et al.* 1993). Sugar in Australia is entirely produced from sugar cane.

Sugarbeet is processed into two major food products - pure sucrose and molasses. Sugarbeet pulp is a by-product of processing which has occasionally been purified and sold as food fibre. Waste products from both pre-processing (leaves and tops) and post-processing (trash) are used as cattle feed.

Weed competition in commercial sugarbeet fields constitutes a significant crop production problem. Glyphosate is the active ingredient of the herbicide Roundup® which is used widely as a non-selective pre-emergent weed control agent in primary crops including sugarbeet. Glyphosate acts by specifically binding and blocking the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) that is essential for the biosynthesis of aromatic amino acids in all plants, bacteria and fungi (Steinrucken and Amrhein, 1980). Biochemical studies of the EPSPS enzyme have shown that natural variation in glyphosate-enzyme binding affinity exists across a variety of organisms, particularly across bacterial species (Schulz *et al*. 1985). Tolerance to glyphosate in plants can therefore be achieved by introducing a bacterial version of the *epsps* gene that encodes for a version of the EPSPS protein with a reduced binding affinity for glyphosate, thus allowing plant aromatic amino acid synthesis to function normally in the presence of the herbicide.

The glyphosate-tolerant sugarbeet in this application – referred to as glyphosate-tolerant sugar beet line 77 (GTSB77) – was developed through the introduction of the *cp4-epsps* gene derived from the soil bacterium *Agrobacterium sp.*CP4 (Padgette *et al.*, 1996). The *cp4-epsps* gene has been transferred into a number of other crop plants, including soybean, canola, corn, and cotton, to establish glyphosate tolerance. These plants are also the subjects of applications to ANZFA to vary Standard A18 (ANZFA 1999a).

Glyphosate-tolerant sugar beet GTSB77 was approved for environmental release by the US Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS) in 1998 (US Federal Register 64(5) Jan. 1999). Food and feed use of glyphosate-tolerant sugar beet GTSB77 was also notified to the US Food and Drug Administration (FDA) in 1998.

Glyphosate-tolerant sugar beet GTSB77 has not been submitted for environmental release approval in either Australia or New Zealand.

While refined sugar derived from sugar beet GTSB77 is not specifically imported into Australia and New Zealand, it may occur as an element within ingredients used in locally

produced processed foods or as an ingredient within imported processed foods.

2. DESCRIPTION OF THE MODIFICATION

2.1 Methods used in the genetic modification

Monsanto have submitted the following report:

Kolacz, K.H. and G.F. Barry 1996. Roundup® Ready Sugar Beet: Plant Transformation Vector. Monsanto Technical Report MSL-14678. Monsanto Company, St Louis, USA.

Glyphosate-tolerant sugar beet line GTSB77 was produced by *Agrobacterium*-mediated transformation of the proprietary cytoplasmic male sterile sugarbeet line A1012 with plasmid PV-BVGT03 (see Figure 1). The *Agrobacterium*-mediated DNA transformation system is the basis of natural plasmid-induced crown-gall formation in many plants and is well understood (Zambryski, 1992). The genes of interest were inserted into the plasmid between DNA sequences known as the Left and Right Borders (LB and RB). These border sequences were isolated from the Ti plasmid of *Agrobacterium* and normally delimit the DNA sequence (T-DNA) transferred into the plant.

Plasmid PV-BVGT03 contained four gene cassettes each consisting of the gene of interest plus specific controlling sequences within the Left and Right Borders. The primary genes of interest in each of the cassettes were:

1. The *cp4-EPSPS* gene from *Agrobacterium sp*. strain CP4. This gene encodes for glyphosate-insensitive form of 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSPS) which maintains aromatic amino acid synthesis in the presence of glyphosate;
2. The *uidA* gene from the common bacterium *Escherichia coli.* This gene encodes for β-D-glucuronidase (GUS) which serves as a visible marker during the plant transformation process;
3. A modified *gox* gene from the soil bacterium *Ochromobactrum anthropii* strain LBAA [previously *Achromobacter sp*]. This gene encodes for glyphosate oxidoreductase which metabolises glyphosate to an inactive form; and
4. The *nptII* gene coded by the bacterial transposon Tn5. This gene encodes for neomycin phosphotransferase II that enables selection of transformed plant tissues in the presence of the antibiotic kanamycin.

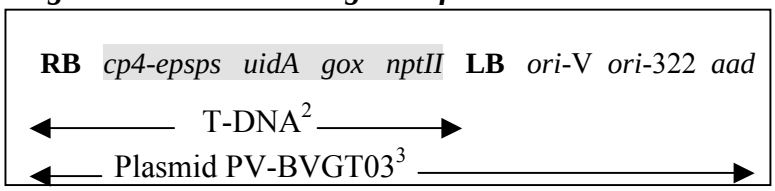
Genes outside the Left and Right Border segments are generally not transferred during the transformation. Three genetic elements are located outside the border sequences in plasmid PV-BVGT03:

1. the vegetative origin of replication (ori-V) that permits plasmid replication in *Agrobacterium* (Rodgers *et al*, 1987).
2. the bacterial origin of replication (*ori-322*) that permits plasmid replication in *Escherichia coli* (Sutcliffe, 1979), and;
3. *aad* – derived from bacterial transposon Tn7 and encodes aminoglycoside adenyltransferase (AAD) which confers resistance to the antibiotics spectinomycin

and streptomycin. This gene was included in the construct as a marker to allow for selection of bacteria containing plasmid PV-BVGT03 prior to transformation of the plant cells.

The gene arrangement is shown in Figure 1.

Figure 1: Schematic diagram of PV-BVGT03[1]



[1]See text or Table 1 for abbreviations.
[2]The shaded region denotes the T-DNA – genes within the LB and RB which are usually transferred via the *Agrobacterium* transformation system.
[3]Genes contained in the entire plasmid. Genes outside the LB and RB are normally not transferred.

2.2 Function and regulation of the novel genes intended for transfer

Monsanto have submitted the following reports:

Mannerlof, M., Tuvesson, S., Steen, P. and P. Tenning. 1997. Transgenic sugar beet tolerance to glyphosate. Euphytica 94: 83-91.

Mannerlof, M. and J. Gielsen. 1996. Molecular analysis of Roundup Ready sugar beet line T9100152 (Note: this line is the same as GTSB77). Novartis Seeds, Technical Report.

Each of the genes of interest, intended for transfer from plasmid PV-BVGT03 to sugar beet requires regulatory sequences that promote and terminate gene transcription into messenger RNA (mRNA) and translation into a protein product targeted to the appropriate cellular compartment. A promoter sequence is the leading control element of a gene that dictates when, where and to what extent, the gene is transcribed into mRNA. A terminator is a DNA sequence that defines the terminal end of a gene by stopping the transcription of mRNA. These sequences can be unique in each organism and thus regulatory elements derived from plants are often used in gene constructs to enable the functioning of novel genes derived from other organisms.

The regulatory and coding regions for each novel gene cassette to be transferred from plasmid PV-BVGT03 are summarised in Table 1 below.

Table 1: Description of gene cassettes for transfer from plasmid PV-BVGT03.

Cassette	Genetic Elements	Source	Function
EPSPS	Modified 35S promoter (35S)	figwort mosaic virus	Promoter of high level constitutive gene expression in plant tissues
	Chloroplast Transit Peptide (CTP2)	*Arabidopsis thaliana* EPSPS gene	Directs the EPSPS protein into the chloroplast where it is active
	CP4-EPSPS coding region (*cp4-epsps*)	*Agrobacterium* sp. Strain CP4	Coding sequence for 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSPS) which maintains aromatic amino acid synthesis through its insensitivity to glyphosate
	Pea E9 3' terminator (E9-3')	*Pisum sativum* *rbcS* gene	Contains signal sequences for termination of transcription and directs polyadenylation
GUS	Modified cauliflower mosaic virus 35S promoter (CaMV)	cauliflower mosaic virus	Promoter for high level constitutive gene expression in plant tissues
	UidA coding region (*uidA*)	Protein coding sequence of the enzyme β-glucuronidase (*uidA* gene) from *Escherichia coli*	Colourimetric marker enzyme used for selection of transformed plant lines
	Pea E9 3' terminator (E9-3')	*Pisum sativum* *rbcS* gene	Contains signal sequences for termination of transcription and directs polyadenylation
GOX	Modified 35S promoter (35S)	figwort mosaic virus	Promoter for high level constitutive gene expression in plant tissues
	Chloroplast Transit Peptide (CTP1)	Chloroplast transit peptide sequence from small subunit 1A of Ribulose bisphosphate carboxylase from *Arabidopsis thaliana*	Directs the GOX protein into the chloroplast which is the site of action
	Gox coding region (*gox*)	Synthetic glyphosate oxidoreductase gene based on sequence from the bacterium *Ochromobactrum anthropii* strain LBAA	Metabolises glyphosate to amino-methyl phosphonic acid (AMPA) and glyoxylate which are not active on EPSPS
	NOS 3' terminator	From nopaline synthase gene from *Agrobacterium* sp.	Contains signal sequences for termination of transcription and directs polyadenylation
NPTII	Modified Cauliflower mosaic virus 35s promoter (CaMV)	cauliflower mosaic virus	Promoter for high level constitutive gene expression in plant tissues
	NptII coding region (*nptII*)	Neomycin phosphotransferase II gene from bacterial transposon Tn5	Confers resistance to aminoglycoside antibiotics used as a plant selectable marker following transformation
	NOS 3' terminator	From nopaline synthase gene from *Agrobacterium* sp.	Contains signal sequences for termination of transcription and directs polyadenylation

The CP4-EPSPS gene cassette

EPSPS is an essential enzyme involved in the biosynthesis of aromatic amino acids via the shikimate metabolic pathway. This metabolic pathway is present in all plants, bacteria and fungi (Haslam, 1993). Plant variants of the EPSPS enzyme are inhibited by the herbicide glyphosate, however, bacterial variants of the EPSPS enzyme are, in general, not inhibited due to reduced binding affinity to the herbicide (Schültz *et al*, 1985). One such low binding-affinity variant is the *cp4-epsps* gene derived from the common soil bacterium *Agrobacterium.* The *cp4-epsps* gene was intended to be transferred to sugar beet to confer tolerance to glyphosate.

In the EPSPS cassette the *cp4-epsps* coding sequence from *Agrobacterium* was fused between a modified version of the 35S promoter from a figwort mosaic virus (P-CMoVb), which promotes constitutive expression of the gene in plant tissues, and the 3' end of the pea rbcS E9 gene (E9 3'), which terminates transcription and contains sequences that will direct the polyadenylation of the mRNA. The bacterial EPSPS gene was modified to create a synthetic gene which allows for higher expression in plants. Bacterial genes have several features that reduce their ability to function efficiently in plants. These features include potential polyadenylation sites that are often rich with A+T nucleotides, a higher G+C nucleotide content than that often found in plant genes and codons that are not frequently used in plants. Some of these features can affect expression or stability of the RNA. These changes to the DNA sequence do not affect the functional activity of the expressed proteins.

The bacterial EPSPS enzyme was targeted to the chloroplast, the active site of the enzyme in higher plants (della Ciopa *et al*, 1986), by the chloroplast transit peptide sequence (CTP2) derived from the *Arabidopsis thaliana epsps* gene. This sequence was fused between the 35S promoter and the *cp4-epsps* coding region.

The GUS gene cassette

The *uidA* gene from the common bacterium *Escherichia coli* (*E. coli*) codes for the enzyme β-glucuronidase (GUS), an acid hydrolase that cleaves β-glucuronides (Jefferson *et al.,* 1987). The *uidA* gene was intended for introduction into sugarbeet line GTSB77 to act as a visible marker in plant transformation. When present, GUS is capable of hydrolysing the chemical p-nitrophenyl-β-D-glucuronide into a colour-forming compound that enables visual scoring of transgenic events. GUS activity also occurs naturally in vertebrates and has been detected in a number of plant species including sugar beet where it can be differentiated from the *uidA* derived GUS due to a different pH activity optimum (Hu *et al.* 1990; Wozniak and Owens 1994).

In the GUS gene cassette the *uidA* coding sequence was fused between an enhanced 35S promoter derived from cauliflower mosaic virus (which promotes high-level constitutive gene expression in plant tissues), and the 3' non-translated region of the *rbcS* E9 gene from pea which directs polyadenylation.

The GOX gene cassette

The *gox* gene from the commonly found soil bacterium *Ochromobactrum anthropii* strain LBAA [formerly *Achromobacter sp*] codes for the enzyme glyphosate oxidoreductase (GOX) which degrades glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate thus

effectively inactivating the herbicide (Pipke and Amrhein, 1988; Barry *et al*, 1992). AMPA is the principal metabolite of glyphosate and is readily degraded by several microorganisms. Glyoxylate is commonly found in plant cells and is broken down by the glyoxylic acid pathway for lipid metabolism.

The *gox* gene was intended for transfer to sugar beet to augment its resistance to glyphosate. In the GOX gene cassette the *gox* coding region was fused, between a modified 35S promoter sequence from a figwort mosaic virus (which promotes constitutive expression in plants) and a terminator sequence derived from the 3' non-translated region of the nopaline synthase gene from *Agrobacterium*. The GOX protein was targeted to the chloroplast, the site of action of glyphosate, by a chloroplast transit peptide (CTP1) sequence fused between the 35S promoter and the *gox* coding region. The CTP1 sequence was derived from the *Arabidopsis thaliana rubisco* gene (Timko *et al*, 1988).

The NPTII gene cassette

The *nptII* gene originates from the Tn5 bacterial transposon and is widely used as a selectable marker in the regeneration of transgenic plants (Kärenlampi 1996). The gene functions as a dominant selectable marker in the initial laboratory stages of plant cell selection following transformation (Horsch *et al* 1984, DeBlock *et al* 1984). It codes for the enzyme neomycin phosphotransferase II (NPTII) that confers resistance to the aminoglycoside antibiotics, neomycin, kanamycin, and geneticin (G418). The *nptII* gene was intended for transfer along with the *gox*, *CP4-EPSPS* and *uidA* genes, enabling those plant cells successfully transformed to grow in the presence of kanamycin.

In the *nptII* gene cassette the coding region of the *nptII* gene was fused between the 35S promoter sequence from cauliflower mosaic virus (which drives constitutive expression of the gene in plant tissues) and a terminator sequence derived from the 3' nontranslated region of the nopaline synthase gene from *Agrobacterium*.

2.3 Characterisation of the genes transferred to the plant

Molecular analysis of glyphosate-tolerant sugarbeet line GTSB77 line was used to detect the presence of transferred DNA sequences and to determine the copy number and stability of the inserted DNA.

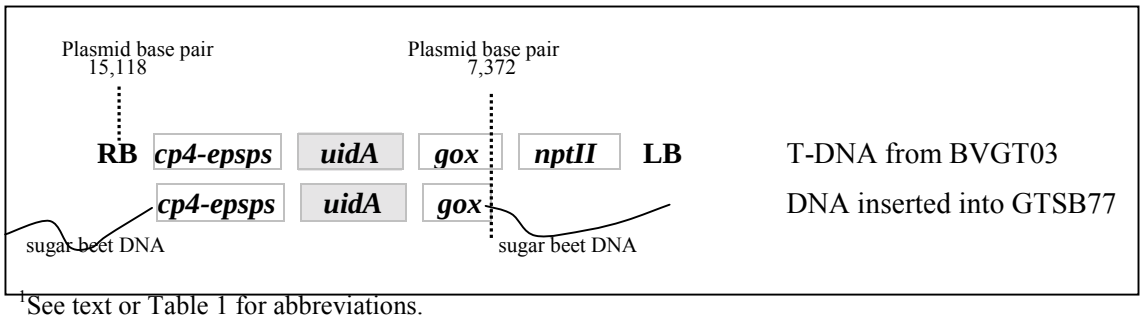
Using DNA hybridisation probes specific to each gene cassette, Southern blot analysis revealed that a single insertion event occurred with complete copies of the *cp4-epsps* and *uidA* genes and a partial copy of the *gox* gene being transferred from the T-DNA of plasmid PV-BVGT03. The analysis also demonstrated that the following genetic elements from PV-BVGT03 had not been transferred into glyphosate-tolerant sugar beet line GTSB77:

- the *nptII* gene;
- the bacterial origins of replication *ori-322* and *ori-V;* and
- the *aad* gene.

A genomic library of sugar beet line GTSB77 was made to characterise the border sequences of the insertion event with the sugar beet genome. Nucleotide sequencing revealed (Fig. 2) that the integrated DNA commenced 25bp downstream of the right border prior to the figwort mosaic virus promoter sequence of the *cp4-epsps* gene cassette and terminated 897 base pairs

downstream of the *gox* gene start codon (at base pair 7372 of the T-DNA) within the coding region of the *gox* gene. The upstream regulatory elements of the *gox* gene, the constitutive promoter 35S and the *A. thaliana* SSU1A gene chloroplast transit peptide (CTP1), were found to be intact. Sequencing downstream of the truncated *gox* gene into the sugarbeet genomic DNA revealed that 897bp of the *gox* gene had been transferred (representing 69% of the complete *gox* coding sequence). Two translational stop codons were identified in the sugar beet genome 130 base pairs and 234 base pairs downstream of the fusion junction in frame with the *gox* gene reading frame. A transcription termination signal was also identified within the sugar beet genomic DNA 650 base pairs downstream of the junction point.

Figure 2: Schematic diagram of the T-DNA from PV-BVGT03 and the DNA inserted into sugar beet GTSB77[1]



[1]See text or Table 1 for abbreviations.

The *nptII* gene and its associated genetic elements were not transferred into sugar beet GTSB77.

As the truncated-*gox* gene had the potential to be transcribed and translated into one or more chimeric GOX-like proteins, expression analysis of these putative proteins was undertaken (see Section 3 – General Safety Issues below).

2.4 Stability of the genetic changes

The stability of the inserted DNA was demonstrated using Southern blot analysis of tissues from the R_2, R_3 and R_4 generations of sugar beet GTSB77. The right junction region was probed using an internal fragment of the *cp4-epsps* gene and the left border region was probed using an internal fragment of the *gox* gene. Segregation analysis based on this molecular analysis showed that a single dominant insertion event had occurred that segregated as a single locus according to Mendelian principles. The analysis further showed that the chimeric *gox* gene was stably maintained through generations.

Phenotypic segregation analysis was also undertaken of the glyphosate-tolerant trait and GUS activity in these generations and confirmed an inheritance pattern consistent with the stable transfer of a single dominant locus for these genes. These phenotypes were also shown to be stable over multiple generations in successive cropping years.

2.5 Conclusions regarding the genetic modification

The data supports the conclusion that an *Agrobacterium*-mediated transformation system was successful in transferring as one insertion event, a single complete copy of the *cp4 epsps* and *uidA* gene cassettes and a truncated version of the *gox* gene cassette from plasmid PV-BVGT03 into glyphosate-tolerant sugarbeet line GTSB77. All three transferred elements were

demonstrated to be stably integrated over several generations. No other genetic elements, including the *nptII* gene cassette, were shown to be transferred from the plasmid in this transformation event. As the gox gene cassette truncation ran into the sugar beet genome, the possibility that novel GOX-like chimeric proteins may be expressed was further analysed (see Section 3.2 – Nature of expressed novel proteins).

3. GENERAL SAFETY ISSUES

Monsanto have submitted the following reports:

Anderson, J.S. 1995. Adaptation of an indirect ELISA to quantitate of CP4-EPSPS in Roundup Ready™ beet leaf and root tissue. Monsanto Technical Report MSL-14332. Monsanto Company, St. Louis USA 63198.

Geis, M.T. 1995. Adaptation of a direct ELISA to detect and quantitated β-glucuronidase (GUS) in Roundup ™ sugar beets. Monsanto Technical Report MSL-14252. . Monsanto Company, USA 63198.

Harrison, L.A., Bailey, M.R., Lleimbruber, R.M., Smith C.E., Nida, D.L., Taylor, M.L., Gustafson, M., Heeren, B. and S.R. Padgette. 1993. Characterisation of microbially-expressed protein: CP4 EPSPS. Monsanto Technical Report MSL-12901. Monsanto Company, USA 63198.

Hontis, A.M. 1997. Protein expression analysis of roots and tops from glyphosate-tolerant beet (RR beet). 1996 European field trials. Monsanto Technical Report MLL-30570. Monsanto Company, USA 63198.

Lee, T.C. and J.D. Astwood. 2000. Determination of CP4-EPSPS, GUS and Protein 34550 in sugar beet pulp, molasses and sugar. Monsanto Technical Report MSL-16285. Monsanto Company, USA 63198.

Nickson, T.E., Mhadeo, D. and G. Go. 1997. Characterization of Protein 34550 produced in *Escherichia coli* containing pMON34550. Monsanto Technical Report MSL-14944. Monsanto Company, USA 63198.

Silvanovich, A., Lee, J.L., Mikols, C.L., and J.D. Astwood. 2000. Characterization of products derived from the *34550* transcriptional unit in Roundup Ready® sugar beet line #77. Monsanto Technical Report MSL 16253. Monsanto Company, USA 63198.

Taylor, M.L., M.T. Geis, P.T., Weston, P. and T.E. Nickson. 1996. Assessment of equivalence of CP4-EPSPS and GUS proteins produced in *Escherichia coli* and Roundup Ready™ sugar beet. Monsanto Technical Report MSL 14560. Monsanto Company, USA 63198.

Glyphosate-tolerant sugar beet GTSB77 was approved for environmental release in the USA in 1998 (USDA/APHIS 1998). Foods derived from sugar beet GTSB77 were approved for use in human food and animal feed in the USA in 1998 (US FDA 1998). While refined sugar derived from sugar beet GTSB77 is not specifically imported into Australia and New Zealand, it may occur as an element within ingredients used in locally produced processed foods or as an ingredient within imported processed foods.

Glyphosate-tolerant sugar beet GTSB77 has been evaluated against the safety assessment guidelines developed by ANZFA. The majority of data presented has been derived from whole sugar beet plants. Refined sugar components and, rarely, refined dietary fibre are the only human food products derived from sugar beet GTSB77. 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 1999b).

3.1 History of the use of sugar and sugar beet as a food source

Sugar, as the simple carbohydrate sucrose, has multiple uses and functions in food preparation and production. These range from:

- use as a sweetener in, for example, beverages, confectionary and ice cream;
- use as the energy source for yeasts used to leaven bread;
- use as a preservative agent in jams and jellies.

Aside from water, carbohydrates are the single largest energy component in common diets with most being derived from fruit, vegetables and cereal products. Approximately 12% of an individual's daily energy intake is derived from sugar, added in either raw (such as molasses) or refined (such as crystalline sugar) forms to various foods or food components (Silliman and Coulston, 1991). Sugar use patterns in the Australian domestic market in 1990 are shown in Table 2.

Table 2: Sugar use patterns in the Australian domestic market*

Beverages	
non-alcoholic	30%
alcoholic	7%
Retail (table sugar etc)	20%
Confectionary	11%
Other (incl. Wholesale)	11%
Bakery products	8%
Preserved foods	8%
Dairy foods	5%

* from Food Australia, 1995.

Sugar produced in Australia and New Zealand is derived entirely from sugar cane with 3-4 million tonnes being produced annually depending on season and markets. Approximately 20% (600-800,000 tonnes) is used domestically and the remainder exported to markets principally in Sth East Asia and Nth America. Approximately 10,000 tonnes of sugar is imported annually accounting for 1.5% of domestic sugar use. Imported sugars are mainly from sugar cane sourced from Sth. East Asia and are chiefly in the form of refined specialty products.

Sugar beet root has been used as a source of sugar since ancient times being initially cultivated in southern Europe and North Africa, although production was limited. The prominence of sugar beet rose, however, when a practical method for extracting sugar was invented in Germany in the mid 18th century. Sugar beet production in France, Austria, Hungary and Russia grew rapidly thereafter and was boosted in other European countries during the reign of Napoleon who ordered mass plantings following the closure of European ports to ships bearing sugarcane from tropical regions. Sugar beet was brought to the United States in the middle of the 19th century where it now accounts for approximately half of the sugar produced (one third of sugar consumed in the USA is imported).

Sugar beet is currently grown in many climates, from temperate (California, Spain and Italy) to cold climates (Dakota, Finland and Russia) and accounts for approximately 40% of world refined sugar production.

In general, sugar beet in the USA and Europe is converted directly to white refined sugar, through a process involving shredding of the beet root, diffusion of soluble sugars into water,

clarification, concentration (by boiling), crystallisation and centrifugation. Crystalline sugar is further refined from molasses by-products. Some intermediate raw sugar is produced in eastern European areas due to limited processing facilities. Sugar beet root pulp is another by-product that, in recent years, has been purified and sold as food fibre used, for example, in breakfast cereals etc. (CADMOS, 1997).

Sugar and sugar products derived from glyphosate-tolerant sugar beet GTSB77 would be consumed in Australia and New Zealand only as an ingredient within processed foods originating from the USA where it is permitted for use in agriculture and food. Known uses of purified beet sugar include soft drinks, chocolates and confectionery, yoghurts and other milk-based foods, pastries and biscuits, syrups, jams and preserved fruits, wines, breakfast foods, ice-creams and sorbets, liquors and spirits, concentrated and powdered milk, sweets and burnt sugar (used to dye and aromatise).

Some products made from molasses derived from glyphosate-tolerant sugar beet GTSB77 could be used as raw materials by the food industry. These products are handled as bulk materials and are made from various sources thus the use of beet in their production cannot be established because of high purity of these products. The products include e*thanol, citric acid (acidulant and preservative), glutamic acid* (taste enhancer), *and lactic acid* (acidulant).

The main use of sugar beet pulp is in animal feedstuffs. Other products, representing a very small percentage of the total use, are processed from pulp. The food components which could be extracted from pulp include: *L-arabinose* (hemicellulose monomer) and *araban gel* used as a fat substitute, *pectins* (polymer of D-glutamic acid) used for specific food applications (emulsion stabilisation), and *fibre products* used as texturing agents and as a source of fibrin by the bakery and breakfast cereal industry.

As with purified sugar products, citric acid, lactic acid, glutamic acid and pectins are food additives and/or flavourings. All products from these processes are highly purified and thus do not contain viable genetically modified organisms or functional recombinant DNA.

The nutrition and health aspects of sugar consumption have been extensively researched over the last 20 years and other than the contribution to dental caries, there is no conclusive evidence that demonstrates that sugar is a hazard to the general public when consumed at the levels and in the manner currently practised. As a consequence sugar has GRAS (Generally Recognised as Safe) status.

3.2 Nature of the novel proteins

On the basis of the inserted gene cassettes two novel proteins were expected to be expressed in glyphosate-tolerant sugar beet line GTSB77: CP4-EPSPS and GUS. As a truncated version of the *gox* gene had also been transferred, a possibility also existed that chimeric GOX-like proteins may also be expressed.

CP4-EPSPS Protein

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

The CP4-EPSPS protein is 47.6 kD in size and consists of a single polypeptide of 455 amino acids.

Plant EPSPSs are localised in the chloroplast. In sugar beet line GTSB77, the CP4-EPSPS gene has been fused to the *Arabidopsis thaliana* EPSPS chloroplast transit peptide (CTP) that targets the protein to the chloroplast. *In vitro* chloroplast uptake assays have shown that the *A. thaliana* EPSPS CTP delivers CP4-EPSPS to the chloroplast where it is subsequently cleaved from the pre–protein, yielding mature CP4-EPSPS with no CTP amino acids retained (della Ciopa *et al*, 1986). 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 is predicted to be expressed in sugar beet GTSB77.

CP4-EPSPS has previously been introduced in soybeans, potato, canola, corn and cotton (Padgette *et al* 1996b). Products of these transgenic commodities are variously permitted for sale in the EU, USA, Canada and Japan and are the subject of other applications with ANZFA.

GUS protein

The *uidA* gene inserted into sugar beet GTSB77 is derived from *E. coli* and encodes a single β-glucuronidase protein, designated GUS, with an experimentally determined molecular weight of 68.2kD. GUS catalyses the hydrolysis of a wide range of glycosides including synthetic p-nitrophenyl-β-D-glucuronide. Hydrolysis of this chromogenic compound produces a blue colour that has proved a versatile visual marker in a range of plant transformation systems (Jefferson *et al.* 1987). GUS is naturally present in a wide range of microbes, animals and plants including sugar beet (Wozniak and Owens, 1994). The *E. coli* variant of GUS expressed in sugar beet GTSB77 was distinguishable from endogenous sugarbeet GUS due to differential pH specificity for the chromogenic substrate.

Chimeric GOX-like proteins

The *gox* gene intended for insertion into sugar beet GTSB77 is derived from the bacterium *Ochrobactrum anthropii* and encodes a single protein of 431 amino acids with a molecular mass of 46.1 kD. The glyphosate oxidoreductase (GOX) protein breaks glyphosate down to aminomethylphosphonic acid (AMPA) and glyoxylate.

Molecular analysis of the insertion event showed that a truncated version of the *gox* gene was inserted into sugar beet GTSB77. The truncation incorporated 897 bp of the *gox* gene cassette (representing 69% of the complete *gox* coding sequence). The truncation event also had the effect of excluding the incorporation of the *nptII* gene cassette which was intended for transfer. Sequencing into the sugar beet genome identified two translational stop codons in the sugar beet genome 130 base pairs and 234 base pairs downstream of the fusion junction in frame with the *gox* translational frame. A transcription termination signal was also identified within the sugar beet genome 650 base pairs downstream of the junction point.

Because the sequence data revealed the possibility for a functional gene a thorough investigation of *gox*-like expression products was undertaken.

Northern blot analysis of RNA recovered from sugar beet GTSB77 tissue showed that two transcripts of differing abundance hybridised to random primed DNA probe. The high

abundance transcript displayed a molecular weight of approx. 1.5-1.7 kb. The lower abundance transcript had a molecular size of approx. 2.0 kb. The different transcripts were concluded to represent transcription of the chimeric *gox* fusion gene through to the two alternative transcription termination sites. Both transcripts however were concluded to be of sufficient length to code for GOX-like proteins and further studies to identify the expression of these proteins were undertaken (see below).

3.3 Expression of the novel proteins in the plant

As the expression of all three genes; *cp4-epsps*, *uidA*, and chimeric *gox*, are under the control of constitutive promoters it is expected that respectively expressed proteins would be found in all tissues of sugar beet GTSB77.

Expression levels of CP4-EPSP, GUS and putative GOX-like proteins were measured using either ELISA or Western blotting. ELISA is a highly sensitive technique that can detect the presence of a protein generally to a sensitivity of 10-100 pg.

ELISAs for CP4-EPSPS and GUS protein were conducted using antibodies raised to each protein expressed in *E. coli* cultures into which the *cp4-epsps* and *uidA* genes were cloned. The *E. coli*-derived proteins were determined to be equivalent to the plant-expressed forms through a number of analyses (see 4.2 - Potential toxicity of newly expressed protein(s)). Analyses were performed on early and mature leaf tissue (referred to as top) and processed root (referred to as brei), from segregating populations of sugar beet GTSB77 in separate field trials. Segregating plants not expressing the glyphosate-tolerant phenotype were used as controls. Trials were conducted in six different geographic sites in Europe in 1995 and 1996, and in five different geographic sites in the USA in 1996. Results are presented in Table 3.

The results outlined in Table 3 show that expression of CP4-EPSPS and GUS proteins are generally at low levels in sugarbeet with highest expression occurring in young leaves and mature tops and the lowest in brei. The level of CP4-EPSPS protein is near two orders of magnitude higher than the level of GUS in nearly all tissues. Expression levels for both proteins in mature tops and brei from GTSB77 plants treated with glyphosate levels representative of commercial conditions were comparable to levels in untreated GTSB77 plants. Similar results were obtained in studies run over two successive years in a range of geographical locations. These data indicate that expression of *cp4-epsps* and *uidA* genes is consistent over generations and reflects that both genes are stably inherited. They also indicate that spraying with glyphosate does not influence the expression of these two genes.

Table 3. Summary of expression levels of CP4-EPSPS and GUS in sugar beet GTSB77*

Tissue Type	CP4-EPSPS protein[1] (ng/mg tissue fresh wt)			GUS protein[1] (ng/mg tissue fresh wt)		
	EU 1995[2]	EU 1996[3]	US 1996[4]	EU 1995[2]	EU 1996[3]	US 1996[4]
Early Leaf						
Mean	145	n.a.	n.a.	2	n.a.	n.a.
Range	130 - 179			0.8 - 3.6		
Top Untreated						
Mean	285	190	172	3.0	3.4	2.78
Range	249 - 370	134 - 273	126 - 193	2.4 - 3.6	2.4 - 3.6	2.35 - 3.35
Treated						
Mean	n.a.	n.a.	151	n.a.	n.a.	2.69
Range			130 - 167			2.29 - 3.18
Brei						
Untreated						
Mean	54	63	47	0.6	0.5	0.39
Range	46 - 64	50 - 76	32 - 60	0.4 - 0.8	0.08 - 0.6	0.28 - 0.55
Treated						
Mean	n.a.	n.a.	50	n.a.	n.a.	0.48
Range			32 - 60			0.41- 0.64

n.a. not available
* Treated values represent plants sprayed with glyphosate at the recommended agronomic rate: 3 applications at 0.75 lb (active equivalent) per acre.
[1] No expression products were detected in untransformed negative control plants for either protein.
2 Mean and range were calculated using n=6 with one sample being provided from 6 different geographic sites.
[3] Mean and range were calculated using n=12 with two samples being provided from 6 different geographic sites.
[4] Mean and range were calculated using n=10 with two samples being provided from 5 different geographic sites.

The level of GOX-like proteins potentially expressed in sugar beet GTSB77 tissue was analysed by Western blot analysis using antibodies raised to protein expressed in *E. coli* cultures into which the chimeric *gox*-fusion gene had been cloned. Cloning was undertaken using a PCR-based strategy to amplify the *gox*-fusion sequence from sugar beet GTSB77. The *gox*-fusion cloned into *E. coli* included the CTP1 sequence, the truncated *gox* sequence and 130bp of fused sugar beet genomic DNA ending at an identified translational stop codon. The plasmid transformation vector containing the *gox*-fusion sequence was named pMON34550.

Protein extracted from refractile bodies in cultures of *E. coli* transformed with pMON34550 were purified and run on SDS-PAGE against GOX protein standards (from a transgenic sugar beet line expressing GOX). A protein – designated Protein 34550 – ran concurrent with the GOX standard at approximately 46kD. Amino acid sequencing of Protein 34550 indicated it was composed of the 89 amino acids of the CTP1 sequence, 299 amino acids from the N-terminus of the GOX protein, and 43 amino acids encoded by sugar beet genomic DNA.

Western blots using antibodies raised and affinity purified to Protein 34550 were undertaken on plant tissue extracts from three different sugar beet GTSB77 varietal lines grown at three different locations in the USA. Tissues analysed included seeds, whole plants (at the two leaf stage and at three months of age), and extracts from leaves and roots from plants at the four,

six and eight leaf stage. For all samples, no Protein 34550 was shown to be present at a limit of detection in plant tissue of 180ppb.

Further, analysis of mRNA transcripts from the truncated *gox* gene in sugar beet GTSB77 tissues showed that the mRNA transcribed contain a C-terminal coding region and a 3' untranslated region that is rich in AU nucleotide sequence. Such AU motifs are associated with mRNA degradation (Di Noia *et al.,* 2000). Translation from such transcripts may be inhibited or the translation products are highly unstable polypeptides, which results in their rapid degradation and thus a lack of detectable Protein 34550 in GTSB77 tissues.

To confirm that Protein 34550 does not exhibit GOX-like activity an ancillary study was undertaken by monitoring the production of glyoxylate, the breakdown product of glyphosate associated with GOX activity. No detectable GOX-like activity could be demonstrated for Protein 34550 purified from *E. coli*. A similar study confirmed that sugar beet GTSB77 does not display GOX activity.

3.4 Dietary intake of expressed proteins

Sugar beet is generally converted directly to refined white sugar (which is composed almost entirely of sucrose) through extensive purification processes described previously. Sugar beet pulp is a by-product that, in recent years, has been purified and sold in limited amounts as food fibre for breakfast cereals etc. (Macrae *et al*. 1993).

Analysis of highly refined sugar liquor and crystalline sugar from sugarbeet shows that total protein within derivative food products range from 0.004 to 1.2 μg per g (Potter *et al.* 1990). This compares with an average figure of 56 mg total protein per g of brei. On this basis sugar refining reduces protein content in refined sugar by a minimum factor of 1.7×10^5.

Using Western blot methodology no immunoreactive protein bands were detected for CP4-EPSPS, GUS or Protein 34550 in either sugar or molasses samples derived from sugar beet GTSB77 that had been treated with agronomically recommended levels of glyphosate or left untreated. The limits of detection were 2ppb, 4ppb and 100 ppb for each protein, respectively, in these processed components. These results indicate that consumers would not be exposed to any of the three novel proteins from consuming sugar or molasses derived from sugar beet GTSB77.

In the case of pulp derived from the same sources of GTSB77, CP4-EPSPS was detected in a range of 11.5 - 71.8ppm across five samples analysed (mean approximately 50ppm). Levels of GUS ranged from 0.3 – 1.2ppm (mean approximately 1ppm). No Protein 34550 was detected in any pulp sample. Limits of detection were the same as those described above for sugar and molasses.

Human consumption of CP4-EPSPS and GUS proteins through consumption of dietary fibre of fibre components derived from sugar beet GTSB77 are considered to be negligible as:

- sugar beet pulp is used on a limited scale to produce dietary fibre or fibre components,
- dietary fibre additives are refined to accentuate only soluble carbohydrate fractions, and
- dietary fibre additives are used at concentrations of 1% v/v in the final food (Southgate, 1986).

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

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

The major concern in relation to the transfer of novel genetic material to gut microorganisms is with antibiotic resistance genes. 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 are concerns, however, that there could be horizontal gene transfer of the antibiotic resistance gene from ingested food to gut microorganisms and that if the microorganisms are able to express the transferred resistance gene this could lead to an increase, in the gastrointestinal tract, of microorganisms resistant to a specific antibiotic. This, in turn, might lead to an increased potential for the transfer of the antibiotic resistance gene to pathogenic microorganisms, thus compromising the therapeutic use of such 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.

While the T-DNA intended for transfer from plasmid PV-BVGT03 contained the antibiotic resistance gene *nptII* (Table 1), Southern blot and sequencing analysis showed this gene was not integrated into sugar beet GTSB77 as a consequence of the *gox* gene truncation event which occurred at the Left Border of the T-DNA.

As discussed above, 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.

Given the information above, the horizontal gene transfer of any genetic material from the glyphosate tolerant sugar beet GTSB77 is not considered to pose any risk to public health and safety, including the development of antibiotic resistant pathogenic bacteria.

3.6 Conclusions

The CP4-EPSPS gene and protein have been sourced from the common soil bacterium *Agrobacterium tumefaciens* and have been well characterised. CP4-EPSPS is considered

[3] Food and Agriculture Organization.

similar to plant EPSPS genes which are consumed in the normal diet.

The GUS gene and protein have been sourced from the common bacterium *E. coli* which is commensal in many animals, including humans, and are also well characterised.

The GOX gene and protein has been sourced from a common soil bacterium *Ochrobactrum anthropii,* which has no history of food-borne pathogenicity. The chimeric truncated GOX gene found in sugar beet GTSB77 was cloned into *E. coli* and a protein – Protein 34550 - was expressed which has no known history of consumption in food.

From Western and ELISA analyses both CP4-EPSPS and GUS are expressed at very low levels in the tops and brei of glyphosate-tolerant sugar beet GTSB77. In contrast, Protein 34550 was not detected in any tissue of sugar beet GTSB77 tested.

The products intended for human consumption from sugar beet GTSB77 include refined and semi refined sugars, and potentially, refined dietary fibre to a limited extent. No CP4-EPSPS, GUS or Protein 34550 were detected in sugar and molasses derived from sugar beet GTSB77. CP4-EPSPS and GUS were found at very low levels in the case of beet pulp (average of 50 ppm and 1 ppm respectively) and their levels are expected to be negligible in refined dietary fibre derived from sugar beet pulp. No Protein 34550 was found in beet pulp (limit of detection 100ppb).

The risk of transfer of the novel genetic material to cells of the human digestive tract is considered negligible. Additionally, there is no risk of horizontal transfer of antibiotic resistance genes to gut bacteria as no such genes were transferred into glyphosate-tolerant sugar beet GTSB77

4. TOXICOLOGICAL ISSUES

Monsanto Australia Limited submitted the following related reports with their application:

Astwood, J.A. 1995. CP4-EPSPS synthase shares no significant sequence similarity with proteins associated with allergy and coeliac disease. Monsanto Technical Report MSL-14174 Monsanto Company, St. Louis, USA, 63198.

Astwood, J.A. 1996. β-D-glucuronidase (GUS) shares no significant sequence similarity with protein associated with allergy or coeliac disease. Monsanto Technical Report MSL-14632 Monsanto Company, St. Louis, USA, 63198.

Astwood, J.A. 1996. β-D-glucuronidase (GUS) shares no significant sequence similarity with protein toxins found in the public domain databases. Monsanto Technical Report MSL-14633. Monsanto Company, St. Louis, USA, 63198.

Astwood, J.D. 1997. Protein 34550 has no significant sequece similarity to known allergens and toxins. Monsanto Technical Report MSL-14988. Monsanto Company, St. Louis ,USA, 63198.

Harrison, L.A., Bailey, M.R., Nida, D.L., Taylor, M.L., Holden, L.R. and S.R. Padgette. 1993. Preparation and confirmation of doses for an acute mouse feeding study with CP4-EPSPS. Monsanto Technical Report MSL-12900. Monsanto Company, St. Louis, USA, 63198.

Harrison, L.A., Biest, N.A., Leimburger, R. and S.R. Padgette. 1996. Equivalence of plant- and microbially-expressed proteins: β-D-glucuronidase from glyphosate-tolerant soybean and *E. coli*. Monsanto Technical Report MSL-12881. Monsanto Company, St. Louis, USA, 63198.

Harrison, L.A., Biest, N.A., Leimburger, R. and S.R. Padgette. 1996. Preparation, characterisation and confirmation of doses for an acute mouse feeding study with β-D-glucuronidase. Monsanto Technical Report MSL-12979. Monsanto Company, St. Louis, USA, 63198.

Leach, J.N. and J.D. Astwood. 1997. Assessment of the digestibility and fate of purified Protein 34550 *in vitro* using mammalian digestive fate models. Monsanto Technical Report MSL-14973. Monsanto Company, St. Louis, USA, 63198.

Lee, T.C. and G. Go. 1997. Preparation and confirmation of doses for an acute mouse toxicity study (EHL-96210) with Protein 34550. Monsanto Technical Report MSL-14982. Monsanto Company, St. Louis, USA, 63198.

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

Naylor, M.W. 1992. Acute oral toxicity study of β-D-glucuronidase (GUS) protein in albino mice. Monsanto Technical Report MSL-12485. Monsanto Company, St. Louis, USA, 63198.

Naylor, M.W. and F. Ruecker. 1997. Acute oral toxicity study of Protein 34550 in albino mice. Monsanto Technical Report MSL-15042. Monsanto Company, St. Louis ,USA, 63198.

Ream, J.E., Bailey, M.R., Leach, J.N. and N. Biset. 1993. Assessment of the *in vitro* digestive fate of CP4-EPSPS synthase. Monsanto Technical Report MSL-12949. Monsanto Company, St. Louis, USA, 63198.
Ream, J.E. 1996. Assessment of the in vitro digestive fate of β-glucuronidase. Monsanto Technical Report MSL-14607. Monsanto Company, St. Louis, USA, 63198.

Taylor, M.L., Go,G., Mahadeo, D.A. Rochester, D.E. and T.E. Nickson. 1997. Assessment of equivalence of Protein 34550 expressed in Roundup Ready® sugar beet (Line #77) and *E. coli*. Monsanto Technical Report MSL-14870. Monsanto Company, St. Louis, USA, 63198.

4.1 Levels of naturally-occurring toxins

Sugar beet varieties naturally contain low levels of toxic saponins which, as their name implies, are a group compounds with properties resembling soap and detergents. Saponins are a complex and chemically diverse group incorporating both triterpenes and steroids linked to one or more sugar groups. Saponins are found naturally, and in significant amounts, in commonly used food and forage plants such as clover, alfalfa, soybeans, chickpeas, eggplant silver beet and spinach (Oakenfull and Sidhu, 1989) and are characterised by having a bitter and astringent taste. Saponins have been generally well characterised with the predominant sapogenic form in sugar beet being oleanolic acid. Due to their surface-active properties saponins have been implicated in foaming and turbidity problems during sugar production from sugar beet and efforts are made to limit saponin levels through processing.

The wide range of chemical and physical properties of saponins is matched by the extent and range of their physiological and pharmacological properties. In general they have been shown to interact with biological membranes, due to their detergent qualities, and to both inhibit and stimulate enzymes and metabolic activity (Oakenfull and Sidhu, 1989). Whilst there has been a tendency to treat saponins exclusively as antinutritional or toxic constituents, more recent work has shown several beneficial dietary effects of saponins, including an enhancement of nutrient absorption in digestion and an ability to lower blood cholesterol levels (Oakenfull and Sidhu, 1989).

Levels of saponin were analysed in the roots and tops of non-transgenic control and GTSB77 sugar beet plants, both untreated and treated with glyphosate at agronomically recommended rates, in European field trials conducted in 1995, 1996 and 1997 and in European and US field trials conducted in 1996. The results of the saponin analysis are presented for root tissue (Table 4), as this is the only tissue used in food production.

Table 4. Mean values and ranges of saponin levels in roots for control and sugar beet GTSB77 (both untreated and treated with Roundup) from all field trials*.

Trial/Year	Control		GTSB77 - untreated		GTSB77 – treated[1]		Literature Range[2]
	Mean	Range	Mean	Range	Mean	Range	
EU 1995	151	72-233	137	60-261	134	91-197	75-965
EU 1996	529	304-999	484	293-846	365[3]	215-609	
USA 1996	215	111-304	208	128-260	180	116-255	
EU 1997	446	290-720	422	305-689	338[3]	217-496	

* Values are given on a mg/kg fresh weight basis. Values are means of samples analysed from 6 (Europe 1995, Europe and USA; 1996) or 8 (Europe 1997) sites. Analysis determined according to published methods.
[1] Treated with three applications 0.75lb active equivalents per acre Roundup.
[2] Reference Lüdecke *et al.*, 1958.
[3] Significantly less than control at the 5% significance level.

Two-sided pooled-variance statistical *t*-tests were undertaken in the European trials to determine whether significant differences occur in saponin level between conditions and treatment. No statistical differences, at the 5% level of significance, were observed for saponin levels between untreated GTSB77 and the non-transgenic control in trials covering multiple growth seasons and geographic regions. No significant difference was found for saponin levels between glyphosate-treated GTSB77 and control sugar beet except for the 1996 and 1997 European field trials were saponin level was reduced in treated GTSB77. These differences are, however, not biologically or nutritionally significant as they were inconsistent across seasons and sites (suggesting that it is not an influence of the genetic modification *per se*). Furthermore, all mean saponin values are within the range established for conventional sugar beet in the literature. On this basis, saponin level in the root of sugar beet GTSB77, both untreated and treated with glyphosate at recommended agronomic application rates, is similar in terms of saponin levels to commercially available sugar beet.

It is concluded that saponin levels in sugar beet GTSB77, both treated and untreated with glyphosate, are not considered to pose a risk to human health since:

- saponins are, in general, at very low levels in sugar beet tissues;
- sugar beet processing aims to eliminate saponins and other extraneous material from refined sugar products; and
- saponin levels in both glyphosate-treated and untreated GTSB77 do not differ significantly across seasons and sites, and fall within the range described for traditional sugar beet varieties in the literature.

4.2 Potential toxicity of newly expressed protein(s)

The potential toxicity and dietary exposure of the CP4-EPSPS and GUS proteins, and Protein 34550 were assessed through four different approaches;

1. potential human exposure;
2. homology to known protein toxins;
3. digestive fate in simulated gastric and intestinal fluids; and
4. acute mouse toxicity studies.

The toxicity of the CP4-EPSPS protein expressed in sugar beet GTSB77 has also been addressed by ANZFA in other safety assessments of foods assessed under Standard A18 (see A338 Roundup Ready soybeans, A355 Roundup Ready cotton, A362 Roundup Ready corn and A363 Roundup Ready canola). The safety of the GUS protein and Protein 34550 have not been addressed in other applications. Certain aspects of the toxicity data have been published in the scientific literature as cited in the text.

The *cp4-epsps*, *uidA* and *chimeric-gox* genes were cloned into *E. coli* in order to obtain sufficient amounts of each respective protein to assess its safety. CP4-EPSPS and GUS proteins derived from *E. coli* were shown to be equivalent for safety assessment purposes to the respective plant expressed proteins on the basis that:

- comparative Western blot analysis demonstrated similar immunoreactivity and equivalent molecular weights (the latter also shown by SDS-PAGE analysis);
- positive correlation occurred between quantity and immunological dose-response in ELISA assays;
- comparative functional enzyme assays demonstrated correlative activities (in the case of CP4-EPSP and GUS); and
- homology of the N-terminal sequence of amino acids through 15 positions; and
- there are no differences in glycosilation patterns.

As no Protein 34550 was identified in sugar beet GTSB77 tissue, no equivalence of the *E. coli* cloned Protein 34550 could be established. PCR analysis using primers specific to the *truncated-gox* gene did, however, show that the sizes of the genes in both sugar beet GTSB77 and *E. coli* are equivalent at approximately 1273 bp.

4.2.1 Potential human exposure

- **Protein levels in sugar products produced from sugar beet**

The high temperatures and precipitation methods used in the production of sugar from sugar beets are known to reduce protein levels significantly with the highest level of protein detected in refined sugar being 1.2 µg per g (Potter, *et al.,* 1990). Since CP4-EPSPS and GUS proteins are expressed at low levels in the sugar beet root (CP4-EPSPS ranging between 32-76 µg per g fresh weight and GUS ranging between 0.08-0.8 µg per g fresh weight), the level of these proteins in refined sugar is extremely low or absent (i.e. less than 2 ppb and 4 ppb respectively, as discussed in Section 3.4).

It is concluded that little if any CP4-EPSPS or GUS protein would be consumed as a consequence eating food containing sugar derived from sugar beet GTSB77.

- **History of human exposure to CP4-EPSPS**

The CP4-EPSPS protein has a specific catalytic function in the aromatic acid shikimate pathway of plants, bacteria and fungi. This pathway is not present in mammals. The CP4-

EPSPS protein shows high amino acid sequence homology to the other EPSPS enzymes found in common food crops (for example, soybean and tomato) that have a long history of safe human consumption, or that are present in fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*) or *Bacillus subtilis*. Thus, CP4-EPSPS is a member of a family of closely related proteins from plants and microbes that are commonly found in human foods.

- **History of human exposure to GUS**

The *uidA* gene was originally isolated from *E. coli* which is a commensal bacterium found in the gut microflora of many animals including humans (Jefferson *et al.,* 1986). The GUS protein is an acid hydrolase that catalyses the cleavage of certain β-glucuronides and has been used extensively as a visible marker in evaluating putative genetic transformation events (Jefferson *et al.*, 1987). Apart from its expression in animals, GUS activity has also been detected in a number of plant tissues including sugarbeet (Wozniak and Owens, 1994). The GUS protein is thus a common component of bacteria native to humans and animals regularly associated with a range of foods and feeds.

- **History of human exposure to the chimeric *gox* gene product; Protein 34550**

The native *gox* gene is derived from *Ochromobactrum anthropii* strain LBAA [formerly *Achromobacter sp*], a commonly found bacterium in soil and is likely to occur on food plants. Southern and PCR analysis identified that a truncated chimeric version of the *gox* gene was present in sugar beet GTSB77. When cloned into *E. coli* the truncated chimeric *gox* gene expressed a new protein which was labelled Protein 34550. Protein 34550 is composed of the 89 amino acids of the CTP1 genetic element, 299 amino acids of the N-terminus of the GOX protein and 43 amino acids encoded by sugar beet DNA. Protein 34550 has no GOX enzymatic activity. If Protein 34550 were expressed in sugar beet GTSB77 it would represent a novel protein not previously found in the human diet.

Western and ELISA analysis for the presence of Protein 34550 in sugar beet GTSB77 revealed that it is not expressed in either leaves, roots or in derivative food components (refined sugar, molasses and dietary fibre). While no evidence of the presence of Protein 34550 was found in sugar beet GTSB77, toxicity studies of the protein were still carried out.

4.2.2 Sequence similarity to known protein toxins

Patterns of amino acid sequence or shared regions between proteins may provide insight into the biological significance of a protein including its toxicity and allergenicity. When compared to the amino acid sequences of protein toxins in the PIR, EMBL, SwissProt and GenBank databases, the amino acid sequence of the CP4-EPSPS and GUS proteins, and Protein 34550, showed no significant similarities to any of the 1,935 protein toxins, or toxin-associated proteins, listed in these databases.

4.2.3 Digestive fate of novel proteins in simulated gastric and intestinal fluids

Most proteins are readily degraded upon exposure to gastric and intestinal fluids in the digestive tract with 50% of solid food emptying from the human stomach in 2 hr (Sleisenger and Fordtran, 1989). The rate of degradation of novel proteins in simulated gastric (SGF) and intestinal fluids (SIF) thus enables a prediction of their fate in digestion.

The degradation of C4 EPSPS, GUS and Protein 34550 were followed, either through Western blot or enzyme activity assays, in both SGF and SIF at 37°C. CP4-EPSPS protein was shown to have a half-life of 15sec in SGF and 10min in SIF. No detectable GUS protein was present after 15sec exposure to SGF and over 50% had been degraded in SIF after 60 to 120min. Over 90% of GUS activity had dissipated after 4h in SIF. Protein 34550 was degraded within 15 seconds exposure to SGF and within 60 seconds exposure to SIF, moreover no intermediate stable protein fragments larger that 2kD were generated by digestion of Protein 34550 in either SGF or SIF.

On the basis of these data all three novel proteins were demonstrated to be readily digestible.

4.2.4 Acute oral toxicity of novel proteins mice

To directly assess the potential toxicity of the CP4-EPSPS, GUS and 34550 proteins acute gavage tests were undertaken in mice using purified forms of each protein. The dosage of administration, treatment-related findings, and the safety factors relative to the highest potential human consumption are shown in Table 5. Note, the safety factors were calculated on the basis of the level which humans would be exposed if each protein were expressed in soybean, corn, tomato and potato assuming no loss of protein due to processing.

Table 5. Acute gavage studies of CP4-EPSPS, GUS and Protein 34550 toxicity in mice and exposure safety factor.

Protein	Max. dosage (mg/kg body wt)	Significant treatment-related effects[4]	Human exposure safety factor[5]
CP4-EPSPS[1]	572	None	1,300
GUS[2]	100	None	>1,000
34550[3]	20	None	10^{10}- 10^{11}

[1] Administered as a single dose by gavage to groups of 10 mice per sex at dosages of 49, 154 and 572 mg/kg (Harrison *et al.,* 1996).
[2] Administered as a single dose by gavage to groups of 10 mice per sex at dosages of 1, 10 and 100 mg/kg.
[3] Administered as a single dose by gavage to groups of 10 mice per sex at dosages of 0.2, 2 and 20 mg/kg.
[4] In-life observations included body weights, food consumption and signs of toxicity. Post-mortem observations included internal and external examinations, kidney weights and kidney histopathology. No statistical differences were observed outside those expected by chance alone at $p \leq 0.05$.
[5] Based on the potential exposure to humans if the protein were expressed in soybean, corn, tomato and potato assuming no loss due to processing.

Despite the level of all three proteins being well in excess of the likely human dietary exposure factor, no mortality or morbidity 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 with any protein were observed.

4.3 Levels of naturally occurring allergenic proteins

Allergic reactions to foods arise from an immune reaction to a particular protein that 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.

Very rare instances in the literature describe allergic response to beet sugar taken orally and to beet sugar solutions administered by injection (Randolf and Rollins, 1950; Richter *et al.*, 1976). One report (Richter *et al.*, 1976) pointed to certain polysaccharide components as the potential allergen. However, the identity of the immunogenic substances in sugar beet sugar has not been positively established. The instances of sugar beet sugar induced allergic responses are very rare and as refined sugar is generally recognised as safe, further investigation of putative naturally occurring allergens is not considered necessary.

4.4 Potential allergenicity of novel proteins

Although there are no predictive assays available to assess the allergic potential of proteins, a number of characteristics are common among many of the allergens that have been characterised. Known allergens tend to be:

- glycosylated proteins with a molecular weight of 10–70 kD,
- heat stable,
- resistant to proteolytic digestion and the acidic conditions of the stomach, and
- expressed as major proteins in commonly consumed foods.

The CP4-EPSPS, GUS and 34550 proteins were evaluated for potential allergenicity against well-accepted criteria for allergens (Taylor, 1992a; Taylor *et al.*, 1987; 1992b). These were whether:

- the source organism(s) has a history of allergenicity;
- the proteins are stable to digestion;
- the proteins are stable to food processing;
- the proteins are similar in amino acid sequence to known protein allergens; and
- the proteins are a principal component of the food.

4.4.1 Allergenicity of source organisms

The CP4-EPSPS protein was obtained from the naturally occurring soil-borne plant-symbiotic bacterium *Agrobacterium sp.* strain CP4. The *gox* coding region was obtained from the common soil bacterium *Ochrobactrum anthropi.* This coding region is responsible for the production of protein 34550. The *uidA* gene was obtained from *E. coli,* a bacteria prevalent in the gastrointestinal tract of animals including humans. None of these source organisms are known to be allergenic to humans.

4.4.2 Stability of protein to digestion

As reported above in Section 4.2.3, the CP4-EPSPS, GUS and 34550 proteins are degraded rapidly after exposure to simulated gastric fluid. Such rapid digestion would severely limit the amount of protein absorbed via the intestine and thus restrict any potential immunological response.

4.4.3 Stability of the protein to food processing

The levels of CP4-EPSPS and GUS proteins in sugar beet GTSB77 are extremely low in the root (0.005% and 0.00006% respectively) and Protein 34550 was undetectable at a limit of

detection of 100ppb. None of these proteins were detectable in molasses and refined sugar components derived from sugar beet GTSB77 at limits of detection ranging from 2 – 100ppb. CP4-EPSPS and GUS were detected at levels of 50ppm and 1ppm respectively in sugar beet pulp. Beet pulp is used to a limited degree in the manufacture of refined soluble food fibre that could potentially be used as an additive at less than 1% in some specific foods (breakfast cereals etc.). While no direct analysis of these proteins was undertaken in soluble fibre derivatives, it is expected that they would be at negligible concentrations due to the processing steps involved.

4.4.4 Sequence similarity to known protein allergens

Amino acid sequence similarity with known allergens is a useful indicator of the allergenic potential of novel proteins. The amino acid sequence of the CP4-EPSPS, GUS and 34550 proteins were compared to the amino acid sequences of 219 known allergens present in public domain databases (eg GenBank, EMBL, Swissprot, PIR). Sequence similarity was defined as a sequence identity of greater than seven contiguous amino acids. No biologically significant similarity was found between any of the novel proteins with any of allergens listed in these databases.

4.4.5 Level of novel protein in the final food

Allergenic proteins in known allergenic foods (such as milk, soybeans and peanuts) exist as major proteins (Metcalfe *et al.*, 1996). There is very limited potential for the novel proteins from sugar beet GTSB77 to act as allergens in final food products as:

- none of the novel proteins could be detected in either sugar or molasses derived from sugar beet GTSB77, and
- dietary fibre refined from pulp derived from sugar beet GTSB77 would contain extremely low levels of CP4-EPSPS and GUS as these are expressed in the crude pulp at very low levels (50ppm and 1ppm respectively). Protein 34550 was not detected in pulp samples.

4.5 Conclusions regarding toxicological issues

There is no evidence to indicate that there is any potential for the CP4-EPSPS, GUS or Protein 34550 to be either toxic or allergenic to humans. Proteins from the EPSPS or GUS family of proteins are naturally present in our food supply or expressed in human intestinal microflora. Although the truncated GOX protein (Protein 34550) is not present in foods normally, it does not possess any of the characteristics common to many allergens or toxins or any significant sequence similarity to any known allergens or toxins. Furthermore:

- none of the proteins are detectable in the principal food components sugar and molasses, and
- Protein 34550 was absent and CP4-EPS PS and GUS are detectable at very low levels in sugar beet pulp (50ppm and 1ppm respectively) - dietary fibre derived from pulp is highly refined and used at very low levels in food products (<1%v/v)
- none of the three proteins were detected in refined sugar or molasses derived from sugar beet GTSB77,
- all three proteins are rapidly digested in conditions that mimic human digestion,

- none of the proteins had toxic effects on mice given acute doses of the equivalent bacterially produced proteins,

From these data it can be concluded that the food products derived from sugar beet GTSB77 should pose no greater risk as a source of toxins or allergens than food products derived from conventional sugar beets.

5. NUTRITIONAL ISSUES

Monsanto have submitted the following reports:

Andersen, A., Dideriksen, T.B., Knudsen, D. and E. Smed. 1996. Compositional analysis of beet with Roundup Ready gene from 1995 field trials. Danisco Technical Report RR SB 01. Holeby, Denmark.

Andersen, A., Dideriksen, T.B., Knudsen, D. and E. Smed. 1997. Compositional analysis of beet with Roundup Ready gene from 1996 field trials. Danisco Technical Report RR SB 02. Holeby, Denmark.

Mueth, M. 1996. Compositional analysis of Roundup Ready™ sugar beet (line #77) from 1996 field trials. Monsanto Company/CEREGEN;Environmental Sciences. Study No. 96-01-49-01. Monsanto Company, St. Louis ,USA, 63198.

Nickson, T.E. and M.T. Gies. 1996. Analysis of roots, leaves and tops from glyphosate-tolerant sugar beet from the 1995 field trial. Monsanto Technical Report MSL-14561. Monsanto Company, St. Louis, USA, 63198.

Taylor, M.L., Mueth, M.G. and T.E. Nickson. 1997. Analytical and compositional analyses of Roundup Ready™ sugar beet (line #77) from 1996 US field trials. Monsanto Technical Report MSL-15048. Monsanto Company, St. Louis ,USA, 63198.

5.1 Compositional analysis

In order to determine the equivalence of sugar beet GTSB77 to conventional sugar beet, a broad range of compositional analyses were undertaken on samples of GTSB77 root and top tissue obtained from five trials in the USA in 1996 and 20 trials across Europe in 1995 (6 trials), 1996 (6 trials) and 1997 (8 trials). As root tissue is the only component of sugar beet used in food production, only compositional and quality data for this tissue is presented. Roots were processed into brei – shredded roots used in the first step of sugar processing.

The analyses included proximate values for:

- crude ash;
- crude fibre;
- crude protein;
- carbohydrate;
- dry matter
- crude fats were also determined in tops.

Additional quality components were measured included:

- invert sugar (glucose + fructose) content;
- sodium;
- amino nitrogen.
- polarisation (% sucrose);
- potassium;

Data on saponins, the principal toxicant in sugar beet root, were considered previously under Section 4 - Toxicological Issues.

The effect of applications of glyphosate on the level of these components was also assessed in all trials. Glyphosate was applied at the suggested agronomic concentration of 0.75 lb (active equivalents) per acre.

Analyses in the USA were conducted by Monsanto Co. in St. Louis, MO and at the Research Centre of American Crystal Sugar Co. in Moorehead, MN. Samples in the European trials were analysed by DANISCO Seeds in Holeby, Denmark and the DANISCO Sugar Development Centre in Nakskov, Denmark.

5.1.1 Proximate and quality component analysis

Mean values and ranges of proximate constituents and quality components for root/brei tissue from all field trials, both untreated and treated with glyphosate, are summarised in Table 6.

Table 6: Mean values and ranges for the proximate and quality component analyses of sugar beet GTSB77 roots/brei from various field trials*.

	Control		GTSB77 – untreated		GTSB77 – treated[2]		Literature Range[3]
ROOTS/BREI	Mean	Range	Mean	Range	Mean	Range	
Crude Ash							
1995 Europe	3.4	2.7-4.9	3.4	2.7-5.1	3.0	2.3-4.0	1.1-17.7
1996 Europe	2.5	2.0-3.2	2.5	2.1-3.4	2.7	2.3-3.2	
1996 USA	5.5	4.6-6.3	6.6	4.8-9.0	8.8	4.9-15.6	
1997 Europe	2.7	2.0-3.8	2.7	2.0-4.0	2.8	2.0-4.4	
Crude Fibre							
1995 Europe	4.1	3.5-5.2	4.0	3.1-5.3	3.6	3.0-4.8	2.9-7.4
1996 Europe	4.2	3.9-4.6	4.2	3.9-4.6	4.2	3.6-4.8	
1996 USA	4.1	2.8-5.0	4.0	3.3-4.7	4.1	3.3-4.8	
1997 Europe	4.2	3.7-4.7	4.2	3.5-5.1	4.1	3.7-4.9	

Table 6: continued

Invert Sugar							
1995 Europe	1.7	0.3-3.7	1.8	0.4-4.24	1.0	0.3-1.9	0.3-2.7
1996 Europe	0.4	0.3-0.5	0.4	0.3-0.5	0.4	0.3-0.6	
1996 USA	n/d	n/d	n/d	n/d	n/d	n/d	
1997 Europe	0.6	0.3-1.7	0.7	0.3-2.6	0.5	0.3-1.0	
Amino Nitrogen							
1995 Europe	2.8	2.0-4.0	2.9	2.0-3.9	2.5	0.6-4.2	0.9-5.1
1996 Europe	1.6	0.7-2.8	1.6	0.8-2.5	2.0	0.7-2.8	
1996 USA	5.6	2.7-7.6	5.7	3.4-7.2	5.9	4.3-7.7	
1997 Europe	2.6	1.0-4.3	2.5	0.8-3.8	2.5	0.9-4.0	
Crude Protein							
1995 Europe	6.2	4.8-8.2	6.3	4.9-7.9	5.3	3.4-7.0	1.2-12.4
1996 Europe	4.3	3.0-5.4	4.3	3.0-5.2	4.8	3.1-5.9	
1996 USA	6.3	3.4-9.5	5.6	2.4-8.0	5.8	3.9-8.0	
1997 Europe	5.0	3.1-6.9	4.9	3.0-6.6	4.8	3.2-6.6	
Dry Matter							
1995 Europe	20.5	14.1-23.5	20.5	13.6-23.1	21.3	14.5-23.8	19.8-23.0
1996 Europe	23.9	19.2-26.4	23.9	19.5-26.2	23.5	18.9-26.0	
1996 USA	19.4	17.8-22.6	21.1	19.4-22.6	21.0	19.1-22.8	
1997 Europe	22.7	20.9-24.9	22.4	20.2-24.4	22.5	21.3-24.6	
Carbohydrate							
1995 Europe	86.3	81.7-88.9	86.3	81.7-88.7	88.2	86.6-90.0	67.3-91.0
1996 Europe	89.0	87.1-91.1	89.0	87.6-90.9	88.3	86.5-91.1	
1996 USA	84.1	80.3-87.2	84.1	79.0-88.1	82.0	74.0-86.0	
1997 Europe	88.1	84.9-91.0	88.2	85.1-91.1	88.3	85.2-91.1	
Polarisation							
1995 Europe	14.4	8.4-17.4	14.5	7.9-17.2	15.6	9.9-18.2	10.8-20.7
1996 Europe	17.3	13.8-19.4	17.3	14.1-19.4	16.8	13.1-18.9	
1996 USA	14.8	12.9-17.1	14.6	12.7-16.2	14.7	13.4-15.9	
1997 Europe	16.6	14.7-18.9	16.2	14.3-18.5	16.4	14.7-18.7	
Sodium							
1995 Europe	1.7	0.5-3.1	1.8	0.4-3.5	1.1	0.4-2.2	0.4-5.5
1996 Europe	0.5	0.3-0.8	0.5	0.2-0.8	0.5	0.3-1.2	
1996 USA	1.5	1.0-2.3	1.5	1.3-1.9	1.6	0.8-2.2	
1997 Europe	0.7	0.3-1.6	0.9	0.4-2.2	0.8	0.3-0.6	
Potassium							
1995 Europe	5.3	4.6-5.9	5.3	4.2-6.0	5.2	3.4-6.6	4.2-10.2
1996 Europe	4.9	4.1-6.0	5.0	4.0-6.4	5.2	3.8-5.9	
1996 USA	8.2	6.8-11.7	8.0	6.7-11.5	8.4	6.2-12.5	
1997 Europe	4.6	3.8-6.2	4.7	3.9-6.3	4.7	3.3-6.3	

*All units in g/100g dry weight except dry matter and polarisation (g/100g fresh weight). Sodium, Potassium, invert sugar (glucose+fructose) and Amino Nitrogen expressed as mmol/100g fresh weight. Analyses performed according to published methods.
[1] Samples taken from single plots at six (Europe '95&'96), five (USA '96) or eight (Europe '97) geographically different field trials. Non-expressing isogenic lines grown adjacent to trial plots acted as controls. Analyses undertaken according to standard published methods.
[2] Treated with three applications of 0.75lb active equivalents per acre glyphosate as recommended for agronomic purposes.
[3] See references Marlander *et al.,* 1996; Smed *et al.,* 1996; Augustinussen and Smed, 1979, and DLG, 1991.

Two-sided pooled-variance *t*-tests (and in some cases parametric and non-parametric statistical analyses) of proximate and quality values were undertaken for the European trials comparing values of non-transgenic control sugar beet (which was isogenic to the GTSB77 background) to sugar beet GTSB77 both untreated or treated with glyphosate at recommended agronomic levels (three applications of 0.75lb active equivalents per acre glyphosate). No statistical analysis was performed on the US trial.

In the European trials no statistically significant differences were found for any value at the 5% level of significance between untreated GTSB77 and its non-transgenic control within any trial year. Only two significant differences were found in glyphosate-treated GTSB77 compared to non-transgenic control plants; crude fibre in 1995 trials (-13.2%), and protein in 1996 trials (+12.0%). As no broadly consistent differences occur in compositional or quality parameters for glyphosate-treated GTSB77 compared to the non-transgenic control these values are most probably outliers. Given that these data show no consistent effect, and that the component types affected are not significant to food products derived from sugar beet, it is concluded that applications of glyphosate to sugar beet GTSB77 pose no human health and safety issue with respect to proximate or quality components.

None of the mean values for either non-transgenic control sugar beet or GTSB77 either untreated or treated with glyphosate falls outside the literature range except for higher amino nitrogen levels in the 1996 US trial. These outliers occurred for both the non-transgenic control and sugar beet GTSB77 (both untreated and treated with glyphosate). As both the isogenic control line and the transgenic line were affected, this anomaly may reflect a differential agronomic practice being applied in the US trial. Given that this outlier occurs for only one characteristics (amino nitrogen) which is not significant to the food products derived from sugar beet, this finding is not considered significant to the human health and safety of sugar or ancillary food products derived from sugar beet GTSB77 under different conditions.

On the basis of the data provided it is concluded that sugar beet GTSB77, both untreated or treated with glyphosate at recommended agronomic application rates, is equivalent to conventional sugar beet with respect to composition and quality values relevant to the human health and safety of final food derivatives.

5.2 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 glyphosate-tolerant sugar beet GTSB77, no significant differences regarding nutritional and toxicological parameters were evident and no feeding studies were thus undertaken.

Another important factor in the assessment of sugar beet GTSB77 to support growth and well-being is that the principal human food derivative is highly refined sugar composed of 96-99% sucrose and 0.6-1.2% other sugars such as glucose and fructose. Refined sugar from any source has a history of safe use and is generally recognised as safe for human consumption.

5.3 Conclusions of Nutritional Analysis

On the basis of the compositional data submitted in the present application sugar beet GTSB77 is equivalent to other commercially available sugar beet in terms of its composition and nutritional adequacy.

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ATTACHMENT 3

DRAFT REGULATORY IMPACT ASSESSMENT

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

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

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

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • no benefits were identified.	**Costs** • the governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • a prohibition on food produced using gene technology in Australia and New Zealand could result in retaliatory trade measures from other countries. • there may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	**Costs** • food manufacturers and producers will be unable to use the processed food fractions from foods produced using gene technology thus requiring the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be $A 207m in Australia and $NZ 37m in New Zealand[4]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

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

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

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

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

Conclusion of the regulatory impact assessment

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

ATTACHMENT 4

WORLD TRADE ORGANISATION AGREEMENTS

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

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

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

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

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

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

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

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

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

SPS Notifications

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

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;

- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

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

- to prevent or limit other damage from the entry, establishment or spread of pests.

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

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

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

TBT Notifications

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

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

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

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

ATTACHMENT 5

SUMMARY OF PUBLIC COMMENTS

National Genetic Awareness Alliance (Aus)

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

Pola Lekstan and Anna Clements (Aus)

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

Arnold Ward (Aus)

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

Australian GeneEthics Network

- Believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
  - Direct health effects of pesticide residues
  - Possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria

  - The possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
  - Insertion of viral DNA could create new and virulent viruses
  - The possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
  - The threat to GE-free status export markets
- Believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

Public and Environmental Health Service (Aus)

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

David Grundy (Aus)

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

Leesa Daniels (Aus) Member of the Genetic Engineering Action Group

- Believes that:
  - Scientific research although limited, has brought concerns to light
  - Substantial equivalence is a subjective principal

- Comprehensive and mandatory labelling must be urgently implemented
- The Cauliflower Mosaic Virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
- Antibiotic marker genes could lead to increase in antibiotic resistance
- Several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

Australian Food and Grocery Council

- Fully endorses the policy of minimum affective regulation, supports these applications, and considers that food manufacturers should make their own choice with regard to use of GM crops or products derived from them
- Believes that since the growth of GM crops has been approved overseas, they would support their growth in Australia if approved through the GTAC/GMAC/OGTR process
- Considers it unfortunate that ANZFA has not negotiated "equivalence" agreements for products already approved overseas to enable approval without having to carry out its own safety assessment. In the absence of such an agreement it supports the ANZFA safety assessment process.
- Believes that an appropriate information and labelling scheme would enable consumers to make an informed choice.

New Zealand Ministry of Health

- Referred preliminary report to New Zealand Health Research Council, who stated concern that all safety aspects should be carefully considered in the ANZFA process.

Nestle Australia Ltd.

- Supports the continued approval of glufosinate ammonium-tolerant canola, and believes that manufacturers would be disadvantaged were approval not to be granted.

Consumers' Association of South Australia Inc. & National Council of Women of Australia (CASA supports submission of NCWA)

- Believe that current testing procedure is inadequate and that human trials are the only adequate method, as with testing of new drugs. Also that physiological and neurological effects as well as the toxicological and allergenic effects should be looked at, and that an independent body should be responsible for testing.
- Do not support the use of antibiotic markers, since they believe they may pose a threat to efficacy of antibiotics in humans.
- State that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans confirming the inadequacy of the term 'substantial equivalence'.
- Raise the point that although these crops have been approved elsewhere, this situation may change with consumer pressure.

- Do not accept that it is impossible to source food to ascertain whether or not it contains GM ingredients. Believe that if McCain and Sanitarium can do it, then others should also be able to.
- State general concern about the risk that MRLs will be raised as a result of herbicide-tolerant crops being developed, and feel that the calculations used are flawed and are not based on safety criteria.
- Believe that the use of GM crops in animal feed should also be regulated. A378
- State concern over possible increase in glyphosate use (it is apparently confirmed in one reference that herbicide use increases with herbicide resistant crops), referring to studies that link the chemical to Hodgkin's lymphoma, and the possibility that Europe may ban it due to adverse effects on beneficial insects**.** They are particularly concerned that glyphosate is not looked at by the same regulatory body as that looking at GM foods.

A379, A38

- State concern over the persistence and toxicity of bromoxynil, and consider that these have not been adequately assessed by the US FDA. They understand that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself, and believe that a safety assessment needs to be done on this too. This is apparently the main residue, and they believe that this may appear in cotton oil and linters.

A372, A375, A380, A381, A386

- With respect to glufosinate ammonium, state concern about toxicity, neurotoxicity, teratogenicity and residues in food, soil and water. They believe that Monsanto is likely to apply for an increase in the MRL, and that such increases are likely to constitute a health hazard.

A380, A382, A383, A384, A385, A386

- Raise issues of adverse effects of Bt toxins on non-target insects and think that it needs more study.

A387

- Believe that raising the amount of a nutrient in a food may have unknown drawbacks e.g. affecting the efficacy of other nutrients.

Health Department of Western Australia

- Highlights various health and environmental concerns:
  - the use of antibiotic resistance genes as markers may transfer resistance to animals via gut bacteria
  - the possibility that microbial gene sequences may contain fragments of other virulent genes, and also that ingesting Bt toxins may be harmful to humans
  - the possibility that insects may be more prone to developing resistance to Bt, since Bt toxins have been found to be released into the soil
- Believes that both safety data and gene sequences should be available for public scrutiny

Meat New Zealand

A379

- Concerned at how labelling regulations will apply to sausage casings that may contain cotton linters even if they are not to be eaten, i.e. are effectively a processing aid. Think that labelling should only be used to advise the sausage manufacturer not consumers.

BRI Australia

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety.

Food Technology Association of Victoria Inc.

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety.

Diane Davie (Aus)

- Believes all 13 applications should be rejected, since they have not undergone human safety testing here or overseas, and have not been assessed on their ethical merits
- Believes that risks include:
  - Bacterial and viral vectors which could affect human physiology
  - Herbicide and insect-resistance genes, which could increase allergies and antibiotic resistance
  - Environmental risks
- Also believes that ANZFA must heed the concerns of consumers opposed to GM foods.

Martin Hurley, David Hook, Ian Smillie, Margaret Dawson, Tee Rodgers-Hayden, David Lovell-Smith (Natural Law Party), Barbara Brown, Ngaire Mason, Robert Anderson (member, Physicians and Scientists for Responsible Genetics), Louise Carroll, Gilbert Urquart, Caroline Allinson-Dunn, Megan Lewis, Peter Barnes, James Harlow, Gabrielle Dewan, Scott Young, Virginia Murray, Stephanie Chambers, Kay Dyson, Peter Fenwick, Joanne Xerri, Paul True, Josh Gill, James & Peysha Charlwood, Mitta Hirsch, Alan Florence, Nicole Paul, Lawrence Clarke, David Snowman, Reg Paling, Mark and Johanna Blows, David and Bev Semour, Richard and Sharon Moreham (see also below), Stuart Drury and Helen Murphy (All Aus), Brennan Henderson (NZ) – Generic e-mail objection.

- Believe that most Australians and New Zealanders do not want GM foods, there are no benefits, and deferral would not be disadvantageous. Approval should be delayed until they are proven safe.

- Feel that there is insufficient time to assess these applications thoroughly, and there are so many products under development that there is a high overall risk of a major disaster
- Believe that GM foods encourage pesticide use, and applications have made for commercial purposes only, and also that here could be commercial benefit to Australia and New Zealand in remaining GM-free.

Richard and Sharon Moreham (see also above)

- In addition to the points above, also think that it is unfortunate that the NZ government agreed to joint approval of food, as the Australian public are less educated about the issues surrounding GM foods
- Think that approval would only prove that ANZFA serves the interests of large multinational companies rather than those of the public.

Vicky Solah (Aus)

- Is for GM foods if the safety evaluation is carry out using approved, validated methods by an independent body, if the results are made available to consumers, and if all GM food is labelled
- Is concerned that transformation may lead to disruption of another gene, and that more research is needed before it is clear whether the process is safe
- With regard to herbicide tolerant crops, is concerned that consumers may not be aware of the need to wash products that have been sprayed, and that this therefore impacts on food safety. Also concerned about environmental impact of these chemicals, and of the possibility of resistance necessitating higher pesticide use in the future.

Dr Rosemary Keighley (Aus)

- Will not purchase foods unless they are certified GM-free. Believes that Australian producers who do not actually use GM products, but who fail to label them as such, will suffer.

Nicola Roil (Aus)

- Believes that GM foods pose health threats and may contaminate non-modified crops.

Ian and Fran Fergusson (Aus) – also in generic email above

- Believe there has been inadequate testing, and are concerned about possible side-effects.

Lyndal Vincent (Aus)

- Urges delay of approval until proven safe by extensive testing. Considers that genetic material is being released without knowing what the effects are, and cannot be recalled.
- Believes that there is no benefit to the consumer, and that national economic interests are best served by maintaining a GM-free market.

Fay Andary (Aus)

- Does not want any of the 13 products covered by the applications to be approved for inclusion in the food supply.

John and Francesca Irving (Aus)

- Thinks that no GE foods should be approved for inclusion in the food chain.

Diana Killen (Aus)

- Believes that there is no proven benefit to consumers and in many instances nutritional value is actually lower in GM crops, and it is therefore irresponsible to push through approval without thorough assessment of their long-term safety for public health.
- Suggests that research has highlighted adverse allergic reactions and a lowered immune response in some individuals, and that there are health implications with crops designed to be grown with greater concentrations of pesticides.
- Thinks that labelling is essential for consumers to discriminate in purchasing, and that Australia has a unique opportunity in supply of organic and GM-free food.

Sheila Annesley (Aus)

- Does not want any of the 13 foods included in the food supply.

David and Edwina Ross (Aus)

- State concern for the future food supplies and well-being of their grandchildren.

Beth Schurr (Aus)

- Wishes to protest against the threat of GM foods, the possible future detrimental effects and the further endangering of the planet.

Beth Eager (Aus)

- As a parent is concerned that neither the long-term effects on health nor the environment are being considered.

Bruce Pont and Ljiljiana Kuzic-Pont (Aus)

- Believe that safety has not been, and cannot be satisfactorily determined, and that any party associated with GM foods could be legally liable should adverse health effects be seen. Thalidomide, smoking, 'Agent Orange' and asbestos all show that such things can affect subsequent generations
- Believe that an increase in use of pesticides will result from pesticide-tolerant crops, and that the emphasis should be on organic and/or safe agriculture
- Believe that GM-food is a retrograde step, contrary to nature and has the potential to destroy the human race.

Chitta Mylvaganum (Aus)

- Wishes to know what tests were done to assess negative effects on human and environmental health, how thorough they were, what the outcomes were, are the results publicly available, and what further avenues of inquiry are open to the public
- Requests the prevention of the import or release of any products until tests are carried out by unbiased scientists in order to prove the lack of health or environmental effects.

John Stevens (Aus)

- Would be concerned if approval were granted before sufficient research had been completed on potential impacts on human health and gene pools of nearby crops. Once grown, spread via pollen would be impossible to stop, and labelling would not prevent exposure by this route.
- Considers that utmost caution should be exercised and import approval denied indefinitely.

Tim Carr (Convenor of the Emergency Committee against GE Foods)

- Believes that GM-foods are produced using a radical and unpredictable new technology so should be subject to more rigorous testing.
- States that it is unknown how the introduced gene will interact with and influence genetic expression in the host genome, and could change the chemical nature of the food.
- Considers that health risks could result from the increased use of pesticides, and also that ANZFA should consider wider environmental, ethical and socio-economic issues.

Jan Kingsbury (Aus)

- Believes that GM-foods could result in loss of economic advantage for Australia and New Zealand since they are known internationally for pure and safe products.
- Believes that foods are being complicated and pushed by big internationals, and organic farmers are being contaminated by cross-pollination.

Teresa Sackett (Aus)

- Believes that:
  - The KPMG report on labelling was prepared in a ridiculously short time and provided limited analysis
  - The proposal of 'no label' for foods which 'may contain' or in which there is 'no evidence' of GM material is inadequate
  - Inadequate testing procedures should not be used to declare a product is GM-free just because material can't be detected. In fact testing methods have been developed that can be used to work out the GM content
  - Government and industry seem to be favouring the introduction of GM foods. This will result in:
  - Increased use of chemicals
  - Destruction of soil life
  - Organic farming pay high costs for producing healthy plants, while conventional farmers have little restriction on pollution of air, soil and water. Salinity problems, the death of the Great Barrier Reef, rivers and streams has resulted from ignorance in farming and broader community. Such problems will increase with GM foods.
  - The implication that the public will not understand the issues is wrong. Everyone needs to be fully informed.
- Asks the question of whether workers in the food industry are to be better informed, and also why no 'verification documents' are to be required by retailers? Believes that certification schemes should be on a par with those for Kosher foods and organics.

John and Sandy Price (Aus)

- Approval of GM foods and seeds should not be allowed, as it is an affront to the sovereignty of Australia and the dignity of the Australian people. The results of the experiment cannot be reversed.

John Scott (NZ)

- Encloses article from The Irish Times, which describes the restrictions that have been placed by the US EPA on the cultivation of GM corn. These appear to have resulted from fears that Bt crops may be harmful to Monarch butterflies and that resistance may develop to Bt.

R A Randell (NZ)

- Believes that all GM products should be placed under a moratorium until the Royal Commission of Inquiry has considered the issue, and until all scientific, philosophical, ethical and moral issues have been looked at.

National Council of Women of New Zealand

- Believes that:
  - approval of all 13 applications should be rejected, and that none should be approved for planting.
  - Independently-funded body should be responsible for safety assessments
  - If it is possible to segregate high-oleic soybeans, then RoundUp Ready soybeans should be segregated too
  - Consumers should be made aware of the extent of GM ingredients in their food
  - GM foods, additives or processing aids already on the market must be labelled comprehensively and without extra cost to the consumer – suggest 'GM unknown' rather than 'may contain'
- Appreciates that rejection may contravene the WHO agreement, but consider that the primary role of ANZFA is the assurance of health and safety.

Safe Food Campaign (NZ)

- Believes that approval should be rejected, and a moratorium be put in place until after the Royal Commission of Inquiry, for various reasons:
  - Possible effects on non-target insects
  - Spread of GM pollen may cause contamination of non-GM (especially organic) crops, and may result in the spread of herbicide-tolerance genes and an increase in resistance development. Cross-pollination is considered a particular risk for canola (A372 & A388). Bt resistance development is noted as being a particular risk for A382, A383 & A384
  - Lack of long-term testing means health risks are not known
  - Use of broad-spectrum pesticides affects wild flowers and non-target insects.

Jocelyn Logan, Caroline Phillips (NZ)

- Oppose all 13 applications for the following reasons:
  - Testing has not been long-term or independent, precautionary principle should apply. Approval can happen later if GM is proven safe.
  - No clear public benefit, and lack of opportunity for informed choice (immoral and undemocratic). Labelling regulations also unsatisfactory in this respect.
  - Environmental concerns (increase in pesticides, threat to organic farming, Bt resistance)

Robert Anderson (member of Physicians and Scientists for Responsible Genetics - NZ)

- Considers that the GM issue should be reconsidered in the light of the release of internal FDA documents made available for a recent lawsuit aimed at amending their policy. Attached document (presentation given by Steven Druker, Alliance for Bio-integrity) suggests that:
  - Scientist's warnings have been ignored

- FDA policy may be illegal, violating the Food, Drugs and Cosmetic Act – Mr Druker believes that the term generally-regarded-as-safe (GRAS) cannot apply to foreign DNA

Stephen Blackheath (NZ)

- Argues that ANZFA's approach to safety assessments is scientifically unsound:
  - Antibiotic resistance marker genes have been cited as being potentially dangerous by groups other than ANZFA e.g. the Royal Society
  - Unanticipated toxins and allergens are a concern, and it is suggested that the ANZFA process does not adequately consider these possibilities
  - Doesn't address the question of whether risks exist that are unique to the GM process
  - It relies on data from the manufacturers themselves, with little sway given to evidence from public submissions. Companies have vested interests the results and cannot be trusted (also gives evidence of Monsanto's past dishonesty)
- Believes that ANZFA is subject to undue influence through the directors, and is biased towards being pro-GM
- Suggests that RoundUp Ready soybeans are not substantially equivalent as the stems have been found to be more brittle than traditional lines, and may be lower in phytoestrogen content
- Also cites the lawsuit being brought by the Alliance for Bio-integrity, and the internal FDA documents that suggest concern from FDA scientists, as evidence of the FDA ignoring important evidence.

Claire Bleakley (NZ)

- Believes that approval should be rejected for various reasons:
  - They may be against Maori views
  - Further long-term trials are needed and should be carried out by ANZFA themselves - certain trials have apparently shown effects on immune system, allergies and rare syndromes
  - Health concerns of pesticide overuse
  - The possibility of horizontal gene transfer with respect to antibiotic resistance transfer
  - Lack of labelling and the use of the unsatisfactory 'substantial equivalence' concept, which makes hazard difficult to assess
  - There is no substantial gain to consumers.

ATTACHMENT 6

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, expressed general views against the use of gene technology, asserted that food produced using this technology is unsafe for human consumption and expressed opposition to the sale of the food, irrespective of the type of food concerned or the particular genetic modification. An evaluation of these general issues raised by the submissions appears below.

1. The safety of genetically modified foods for human consumption

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

Evaluation

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

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

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

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

2. The need for long-term feeding studies

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

Evaluation

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects of importance to humans. Establishing a dose-response relationship is a pivotal step in toxicological testing. In this way it is possible, in most cases, to determine the levels of exposure at which adverse effects are not present and so establish safe upper limits through applying 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 some reason to question 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 case, 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. Such experiments can provide more meaningful information than similar experiments on the whole food. Additional re-assurance regarding the safety of newly-expressed protein can be obtained by considering 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. 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 of the new and traditionally-produced food. This can include phenotypic[2] characteristics and compositional factors, as well as 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 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 a 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 technical 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, it is proposed that genetic modification be used intentionally to improve the nutritional value of food. In this regard, GM foods may be able to assist in addressing the

[2] characteristics that are visible

general nutritional issues of the community and also specific dietary problems 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 has prepared a public discussion paper on the safety assessment process for GM foods called "GM Foods and the Consumer". This is available off the ANZFA website (www.anzfa.gov.au) or from the ANZFA Information Officer 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

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.

Evaluation

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

MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

It is important to note that MRLs are not direct public health and safety limits but rather, are primarily indicators of appropriate chemical usage. MRLs are always set at levels lower than, and normally very much lower than, the health and safety limits. The MRL is determined following a comprehensive evaluation of scientific studies on chemistry, metabolism, analytical methods and residue levels. In Australia, the National Registration Authority (NRA) applies to ANZFA to amend the MRLs in the Food Standards Code and the application is considered by ANZFA through its legislated decision making processes. In New Zealand MRLs are set by the Ministry of Health， generally following a request from, and in collaboration with, the Ministry of Agriculture and Forestry. Only following demonstration that the use of agricultural and veterinary chemicals will not result in unsafe residues will the MRL enter into food law through its inclusion in either the Food Standards Code in Australia, or the New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.