DRAFT RISK ANALYSIS REPORT

APPLICATION A382

Food derived from insect-protected potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05

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

An application was received from Monsanto Australia Ltd on 30 April 1999 for the approval of food from genetically modified (GM) potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05. The potatoes have been genetically modified to provide protection against a range of insects, including the Colorado potato beetle (CPB). The potatoes are know commercially as New Leaf® potatoes. This report describes the scientific assessment of the application.

Issues addressed during assessment

i. Safety Evaluation

The New Leaf® potatoes have been evaluated according to ANZFA's safety assessment guidelines. This involves an extensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the new GM food. This approach can establish whether food produced from the New Leaf® potatoes is as safe and nutritious as food produced from non-GM varieties of potatoes.

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

Data on the potential toxicity and allergenicity of the proteins encoded by the transferred genes have been reviewed and indicates that the new proteins expressed in the New Leaf® potatoes are non- toxic and unlikely to be allergenic to humans.

Compositional analyses demonstrate no significant differences in key constituents between the New Leaf® potatoes and their conventional counterparts.

The impact on human health from the potential transfer of novel genetic material to cells in the human digestive tract has also been considered. The presence of novel genetic material, including two antibiotic resistance genes, in the New Leaf® Plus potatoes is not considered to pose any additional safety concerns.

In assessing all of the above data, ANZFA has concluded that the New Leaf® potatoes do not raise any safety concerns.

ii. Labelling

On the basis of the data considered in the safety evaluation, the New Leaf® potatoes were found to be substantially equivalent to non-GM potatoes therefore no mandatory labelling is required.

It should be noted that 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. This requirement will come into effect 12 months after the date of gazettal and may result in changes to the way in which GM foods, including those derived from New Leaf® potatoes, are labelled.

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 draft safety assessment report.

Conclusion

ANZFA considers that food from New Leaf® Plus potatoes is as safe for human consumption as food from other commercial potato varieties and is therefore proposing an amendment to the Australian *Food Standards Code* to give approval to such food. Based on the data submitted in the present application, food derived from the New Leaf® potatoes can be regarded as substantially equivalent to food derived from non-GM potatoes therefore no mandatory labelling is required, although as noted above this may change once the new labelling provisions of Standard A18 come into effect.

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

INVITATION FOR PUBLIC 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.* The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the *Food Standard Code,* 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 A382** at one of the following addresses:

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Submissions should be received by the Authority by **25 October 2000.**

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

INTRODUCTION

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

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

BACKGROUND TO THE APPLICATION

ANZFA received an application from Monsanto Australia Ltd on 30 April 1999 to amend the Australian *Food Standards Code* to include food produced from potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 in the Table to Clause 2 of Standard A18 – Food Produced using Gene Technology.

The five lines of Russet Burbank, Atlantic and Superior potatoes were genetically modified to be protected against a range of insects, including the Colorado potato beetle (CPB). These potatoes are known commercially as New Leaf® potatoes. The New Leaf® potatoes are protected against CPB through the transfer of the *cry3Aa* gene from the soil bacterium *Bacillus thuringiensis* subspecies *tenebrionis.*

New Leaf® potatoes are not grown in Australia or New Zealand and are currently not permitted to be imported into Australia or New Zealand as fresh produce. Rather, they currently enter into the market in imported processed food commodities such as processed potato crisps, pre-cooked French fries, potato flour and potato starch.

Direct benefits from the New Leaf® potatoes are likely to accrue mainly to the primary producer who will be able to substantially reduce costs for controlling CPB by reducing reliance on the use of agricultural chemicals. More general benefits, however, may also 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 Technological 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)

The safety assessment was performed according to the safety assessment guidelines prepared by ANZFA[1] and considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues.

Nature of the genetic modification

Three genes were transferred to the New Leaf® potatoes using *Agrobacterium tumefaciens*-mediated transformation – *cry3Aa*, *nptII,* and *aad*.

The *cry3Aa* gene is present in all five New Leaf® lines. Not all the New Leaf® lines, however, contain the *nptII* and *aad* genes. Line SPBT02-05 does not contain either the *nptII* or *aad* gene, and only line ATBT04-36 contains the *aad* gene.

The *cry3Aa* gene is one of several isolated from *B. thuringiensis*, which encode a group of toxins known as the *Bt* toxins. These toxins are selectively active against several groups of insects such as moths and butterflies, beetles, and flies and mosquitos. The *Bt* toxin produced by the *cry3Aa* gene is known as Cry3Aa and is selectively active against a narrow range of beetles, including CPB. When a susceptible beetle ingests Cry3Aa the toxin binds to the cells lining the insect gut causing their rupture and leading to gut paralysis. The insect stops feeding and eventually dies. Cry3Aa produces this toxic effect by binding to specific receptors in the gut of target insects. As there are no receptors for Cry3Aa on the surface of mammalian intestinal cells, humans are not susceptible to Cry3Aa.

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

The two other genes were used as markers to assist in the selection of transformed cells (i.e., cells to which the gene of interest has been transferred). The *nptII* gene encodes the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the antibiotics neomycin, kanamycin, and geneticin (G418). The *aad* gene encodes the enzyme streptomycin adenyltransferase and confers resistance to the antibiotics spectinomycin and streptomycin.

Where present, all three genes appear to be stably integrated and maintained in the potato plants over multiple generations.

General safety issues

The potato (*Solanum tuberosum* L.) is a major food crop throughout the world and has a long history of safe use as human food. The main food products to be derived from the New Leaf® Plus potatoes will be processed food commodities such as processed potato crisps, pre-cooked French fries, potato flour and potato starch.

Lines BT-06, ATBT04-06, ATBT04-31, and ATBT04-36 each express two novel proteins — Cry3Aa and NPT II — whereas line SPBT02-05 only expresses the Cry3Aa protein. The expression levels of both proteins are variable between lines but are consistently low. Expression levels range from between 0.00025 and 0.006% total tuber protein for Cry3Aa (0.05 – 1.29 μg/g fresh weight) and from <0.0002 to 0.02% total protein for NPTII (0.01 – 3.82 μg/g fresh weight). Line SPBT02-05 exhibited the highest Cry3Aa expression level and line ATB04-06 the highest NPTII expression level.

The impact on human health from the potential transfer of novel genetic material from New Leaf® potatoes to cells in the human digestive tract was evaluated. It was concluded that transfer was extremely unlikely to occur, and unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods. In the case of the two antibiotic resistance genes, it was concluded that even should transfer occur, the health impacts would be negligible because these antibiotic resistance genes are already commonly carried by bacteria found in the environment as well as inhabiting the human digestive tract.

Toxicological issues

The levels of naturally-occurring toxins in New Leaf® potatoes were assessed as well as the potential toxicity and allergenicity of the two novel proteins — Cry3Aa and NPTII.

The only naturally-occurring toxins in potatoes are the glycoalkaloids. For the majority of New Leaf® lines, the glycoalkaloid levels were either equivalent to or slightly lower than glycoalkaloid levels in the control. For one of the lines, BT-06, glycoalkaloid levels were slightly elevated compared to the control however the level reported was still at the lower end of the normal range reported for other commercial varieties of Russet Burbank potatoes. The slightly elevated glycoalkaloid level in line BT-06 does not raise any safety concerns.

Acute oral toxicity testing of Cry3Aa and NPTII proteins in mice demonstrated that both proteins are non-toxic to mammals, including humans. In addition, it is well known from the literature that Cry3Aa is toxic only to a narrow range of Coleopteran insects and is non-toxic to mammals, including humans. Dietary intake assessments also show that human exposure to both proteins, from the consumption of New Leaf® potatoes, will be low. On the basis of this evidence, it can be concluded that both proteins are non-toxic to humans.

Both proteins are also unlikely to be allergenic to humans because neither possess any of the physical characteristics common to allergens, both are rapidly degraded in the proteolytic and acid conditions of simulated gastric fluid suggesting neither would survive mammalian digestion, neither of the proteins have any significant similarity to known allergens, nor are they present in large amounts in potato tubers. Furthermore, humans have a prior history of exposure to these proteins with no recorded instances of allergenicity. On the basis of this information it was concluded that both proteins are highly unlikely to be allergenic.

Nutritional issues

Compositional analyses were done to establish the nutritional adequacy of the New Leaf® potatoes, and to compare them to non-modified control lines. Analyses were done of total solids, dextrose, sucrose, soluble protein, proximate (total protein, fat, crude fibre, ash, total carbohydrates and calories), and vitamin C content. On the basis of the data submitted with the present application, the New Leaf® potato lines can be regarded as compositionally equivalent in terms of these key constituents.

Two animal feeding studies with birds and rats were provided as additional supporting data for New Leaf® Russet Burbank line BT-06. The results of these studies confirm that food from this line is nutritionally adequate.

Conclusion

Based on the data submitted in the present application, New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 are equivalent to other commercially available potato cultivars in terms of their safety and nutritional adequacy.

2. Labelling of food derived from insect-protected potatoes

Clause 3 of Standard A18 prescribes mandatory labelling of a food produced using gene technology when it contains new or altered genetic material and where it is not substantially equivalent in any characteristic or property of the food. As the New Leaf® potatoes have been found, on the basis of data submitted with the present application, to be equivalent to other commercial varieties of potatoes there is no requirement for mandatory labelling under the current standard.

It should be noted that 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. This requirement will come into effect 12 months after the date of gazettal and may result in changes to the way in which GM foods, including those derived from New Leaf® potatoes, are labelled.

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) Use of Bt toxins

Mr A.Ward and the Health Department of Western Australia raised concerns about the effect of *Bt* toxin on humans. The New Zealand Ministry of Health stated that ANZFA's assessment report should explain the biochemistry of the *Bt* protein and why it is unlikely to cause any harmful effects when consumed by humans.

Response

The *Bt* toxins are a related group of proteins produced by different types of *B. thuringiensis* during sporulation. The *Bt* toxin being used in the New Leaf® potatoes is known as Cry3Aa. Cry3Aa has been shown to be selectively active against a narrow spectrum of beetles, such as CPB.

The mode of action of the *Bt* toxin is reviewed in the draft safety assessment report and its potential toxicity to mammals is also addressed (Attachment 2). It was concluded that Cry3Aa is non-toxic to mammals, including humans. This conclusion is based on a number of different pieces of evidence.

Firstly, direct experimental evidence on the absence of toxicity in mice was provided. Doses of up to 5000 mg protein/kg body weight were used. This dose has been calculated to be at least 1.2 million times higher than estimated dietary intakes.

Secondly, the mode of action of the *Bt* toxins has been thoroughly studied. When ingested by susceptible insect species, *Bt* toxins cause lysis of midgut epithelial cells in the insect gut, which leads to gut paralysis, cessation of feeding and the eventual death of the insect. The binding of the toxin to the insect gut is essential for the onset of toxicity. Binding is mediated through specialised receptors on the cell surface. If the receptors are not present on the cell surface, the toxin will not bind and will not be able to exert its toxic effect. This is why certain types of *Bt* toxins can be toxic to some insects and not others e.g., why Cry3Aa is toxic to some types of beetles, but not toxic to moths and butterflies. The *Bt* toxins also have not shown any ability to be able to bind to mammalian gut tissue. It can therefore be inferred from the results of these studies that the *Bt* toxins are highly unlikely to exert any toxic effects in mammals, including humans, because the cells lining the human gut lack the receptors necessary for the binding of the toxin.

Lastly, microbial preparations containing Cry3Aa have been in commercial use as an insecticide on crops such as eggplant, potatoes and tomatoes in the United States since 1989 with no reports of any adverse effects in humans.

(ii) Estimation of dietary intakes of novel proteins

The New Zealand Ministry of Health submitted that the insect and virus-protected potatoes are likely to be a highly consumed foodstuff and suggested that the dietary intakes of the *Bt* and viral protein present in the potatoes should be estimated.

Response

When food substances are known to be hazardous, an estimate is made of the dietary intake to determine the likely human exposure to the hazard. If exposure is likely to be low there may be less cause for concern than if exposure is likely to be high.

The *Bt* protein is not considered hazardous, that is, it is non-toxic to mammals, including humans. Because of the absence of any hazard, an estimate of the dietary intake of the *Bt* protein was not considered essential for the safety assessment. However, it is recognised that such information may be useful in providing reassurance to the community that exposure to a novel protein is low and/or that the novel protein is likely to be present in the diet at levels well below those found to be safe in animal toxicity studies.

Cry3Aa is expressed in the New Leaf® potato tubers levels ranging from 0.05 to 1.29 µg protein/g fresh weight. Therefore, if certain assumptions are made about market penetration of the New Leaf® potato products, it is possible to estimate the dietary intake of the *Bt* protein.

Australian and New Zealand consumption data is available for crisps, instant mashed potato and commercial potato fries but is not currently available for potato flour or potato starch. Excluding potato flour and starch, the average total consumption[2] of processed potato products per person is 19.4 g/day in Australia, and 21.5 g/day in New Zealand. If, however, the consumption figures are based only on those in the population who report consuming potatoes then the average total consumption is 118.2 g/day and the 95th percentile consumption is 300 g/day.

For calculation of the dietary intake of the novel proteins, the highest potato consumption figure (300 g/day) and the highest protein concentration (1.29 µg protein/g fresh weight) was used. This represents a 'worst-case' estimate.

To do the calculation, assumptions about the proportion of processed potato products derived from the New Leaf® potatoes must be made. In 1998, the New Leaf® potatoes comprised approximately 4% of the United States potato acreage (USEPA/USDA 1999), this has since declined to less than 1% as these varieties have been superseded. It is possible therefore to make two dietary intake estimates — one using a very worst case estimate where it is assumed that all potato products on the market are derived entirely from New Leaf® potatoes and the other, more realistic estimate, where it is assumed that 10% of potato products are derived from New Leaf® potatoes. The dietary intake estimates are provided in the table below:

[2] Calculated for all respondents

Market penetration	Estimated dietary intake	
	µg /day	µg/kg BW/day[1]
100 %	387	5.95
10 %	38.7	0.595

[1] assuming a body weight of 65 kg.

The very worst-case estimate is at least 0.9 million times less than the dose found to have no adverse effects in mice (5220 mg Cry3Aa/kg BW). Therefore, even if all processed potato products were to be derived from the New Leaf® potatoes, a very large margin of safety exists.

The potato consumption data and estimated dietary intakes of the novel proteins are included with the draft safety assessment report (Attachment 2).

4. Risk management

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

On the basis of the conclusions of the safety assessment report, together with a consideration of the public submissions, it is proposed that Table 1 to Clause 2 of Standard A18 be amended to include food from New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05. The proposed amendment is provided in Attachment 1.

In relation to labelling of the food, the safety assessment report found, based on the data submitted in the present application, that New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 are substantially equivalent to other commercially available potatoes in terms of their safety and nutritional adequacy. Therefore, under the current standard, no mandatory labelling is required.

In relation to the concerns raised in the public submissions with regard to gene technology and GM food, ANZFA has prepared a public discussion paper on the safety assessment process for GM food[3]. This is widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

REGULATORY IMPACT ASSESSMENT

The benefits and costs associated with the proposed amendment to Standard A18 have been analysed in a draft Regulatory Impact Assessment (Attachment 3). The benefits of the

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

proposed Standard A18 amendment to approve food from New Leaf® potatoes primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

CONCLUSIONS

It is concluded that:

- the introduced genes in New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 are not considered to produce any additional public health and safety risk;

- based on the data submitted in the present application, New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 are equivalent to other commercial varieties of potatoes in terms of their safety and nutritional adequacy;

- food derived from New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 does not require labelling under the current provisions of Standard A18 as it is substantially equivalent to food derived from non-GM potatoes. Recently agreed amendments to the labelling provision of Standard A18 may result in some New Leaf® potato food products being labelled in the future; and

- the benefits to government, consumers and industry associated with the proposed amendment outweigh the costs.

ATTACHMENTS

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

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A382 - FOOD DERIVED FROM INSECT-PROTECTED POTATO LINES BT-06, ATBT04-06, ATBT04-31, ATBT04-36, AND SPBT02-05

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

Food derived from insect-protected potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05

ATTACHMENT 2

DRAFT SAFETY ASSESSMENT REPORT

A382 – FOOD DERIVED FROM INSECT-PROTECTED POTATO LINES BT-06, ATBT04-06, ATBT04-31, ATBT04-36, AND SPBT02-05

SUMMARY AND CONCLUSIONS

Nature of the genetic modification

Fives lines of three different potato cultivars (Russet Burbank line BT-06, Atlantic lines ATBT04-06, ATBT04-31, and ATBT04-36, and Superior line SPBT02-05) were protected against the Colorado potato beetle through the *Agrobacterium tumefaciens* mediated transfer of the *cry3Aa* gene from the soil bacterium *Bacillus thuringiensis* subspecies *tenebrionis* (*B.t.t.*). The insect-protected potato lines are known as New Leaf® potatoes.

The *cry3Aa* gene is responsible for the production of the Cry3Aa protein which is toxic to a narrow range of beetles, including the Colorado potato beetle. When ingested by a susceptible beetle, Cry3Aa causes lysis of midgut epithelial cells in the insect gut, leading to gut paralysis, cessation of feeding and the eventual death of the insect. A number of microbial pesticide products based on Cry3Aa are commercially available in the United States, with some being in use since 1989.

Other genes transferred along with the *cry3Aa* gene to the New Leaf® potatoes were the *nptII* gene (in all but line SPBT02-05) and the *aad* gene (in line ATBT04-36 only). The *nptII* gene is a marker used for selection of transformed plant lines during the potato transformation procedure. It codes for the enzyme neomycin phosphotransferase II (NPT II) and confers resistance to the antibiotics neomycin, kanamycin, and geneticin (G418). The *aad* gene is a marker used to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It codes for the enzyme streptomycin adenyltransferase, which confers resistance to the antibiotics spectinomycin and streptomycin.

The *cry3Aa* gene in the New Leaf® potatoes appears to be stably integrated and all lines are phenotypically and genotypically stable over multiple generations.

General safety issues

The potato (*Solanum tuberosum* L.) is a major food crop throughout the world and has a long history of safe use as human food. The main food products to be derived from the New Leaf® potatoes will be processed food commodities such as processed potato crisps, pre-cooked French fries, potato flour and potato starch.

Lines BT-06, ATBT04-06, ATBT04-31, and ATBT04-36 each express two novel proteins — Cry3Aa and NPT II — whereas line SPBT02-05 only expresses the Cry3Aa protein. The expression levels of both proteins are variable between lines but are consistently low. Expression levels range from between 0.00025 and 0.006% total tuber protein for Cry3Aa (0.05 – 1.29 μg/g fresh weight) and from <0.0002 to 0.02% total protein for NPTII (0.01 – 3.82 μg/g fresh weight). Line SPBT02-05 exhibited the highest Cry3Aa expression level and line ATB04-06 the highest NPTII expression level.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells in the human digestive tract. Much of the concern in this regard is with antibiotic resistance genes. In the case of the New Leaf® potatoes, it was concluded that the *nptII* and *aad* genes would be extremely unlikely to transfer to bacteria in the human digestive tract because of the number

and complexity of the steps that would need to take place consecutively. More importantly, however, in the highly unlikely event that transfer did occur, the human health impacts would be negligible because both these resistance genes are already commonly found in the environment as well as inhabiting the human digestive tract. Transfer of other novel genetic material from the New Leaf® potatoes to human cells via the digestive tract was also considered to be equally unlikely. As the amount of novel genetic material in the New Leaf® potatoes is minute compared to the total amount of DNA present it is unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Toxicological issues

The levels of naturally-occurring toxins in New Leaf® potatoes were assessed as well as the potential toxicity and allergenicity of the two novel proteins — Cry3Aa and NPTII.

The only naturally-occurring toxins in potatoes are the glycoalkaloids. For the majority of New Leaf® lines, the glycoalkaloid levels were either equivalent to or slightly lower than the glycoalkaloid levels found in the control. For one of the lines, BT-06, glycoalkaloid levels were slightly elevated compared to the control however the level reported was still at the lower end of the normal range reported for commercial varieties of Russet Burbank potatoes. The slightly elevated glycoalkaloid level in line BT-06 does not raise any safety concern.

Acute oral toxicity testing in mice demonstrated that both Cry3Aa and NPTII have very low oral toxicity, with no adverse signs being seen in mice at doses up to 5220 mg/kg body weight. Human dietary exposure to both proteins is estimated to be well below this level. In terms of their allergenicity, while both proteins are within the size range of known allergens, neither possess any of the other physical characteristics which are common to allergens, neither have any significant similarity to known allergens, nor are they present at high levels in potato tubers and both proteins are readily degraded in conditions that simulate mammalian digestion. Furthermore, humans have a prior history of exposure to these proteins with no recorded instances of allergenicity. Therefore it was concluded that Cry3Aa and NPTII are unlikely to be allergenic to humans.

Nutritional issues

Compositional analyses were done to establish the nutritional adequacy of the New Leaf® potatoes, and to compare them to non-modified control lines. Analyses were done of total solids, dextrose, sucrose, soluble protein, proximate (total protein, fat, crude fibre, ash, total carbohydrates and calories), and vitamin C content. These analyses showed that, in terms of these key constituents, the New Leaf® potato lines are compositionally equivalent to other commercial potato cultivars.

Two animal feeding studies with birds and rats were provided as additional supporting data for New Leaf® Russet Burbank line BT-06. The results of these studies confirm that this line is nutritionally adequate.

Conclusion

Based on the data submitted in the present application, New Leaf® potato lines BT-06, ATBT04-06, ATBT04-31, ATBT04-36, and SPBT02-05 are equivalent to other commercially available potato cultivars in terms of their safety and nutritional adequacy.

1. BACKGROUND

Monsanto Australia Ltd have made an application to ANZFA to amend Standard A18 of the Australian *Food Standards Code* to include food derived from potatoes which have been genetically modified to be protected against the Colorado potato beetle (*Leptinotarsa decemlineata* Say.), one of the principle pests of potatoes in North America. The potatoes are known commercially as New Leaf® potatoes.

Protection against the Colorado potato beetle is achieved through expression in the plant of the insecticidal protein, Cry3Aa. Cry3Aa is produced naturally by the *tenebrionis* subspecies of the spore-forming soil bacterium *Bacillus thuringiensis* (*B.t.t.*). The majority of described *B. thuringiensis* strains produce insecticidal proteins active against lepidopteran insects (larvae of moths and butterflies) and a few are reported to have activity against dipteran insects (mosquitos and flies). The Cry3Aa protein, however, is toxic to a narrow spectrum of coleopteran insects (beetles) and shows no activity against other groups of insects such as the lepidopterans or dipterans (Herrnstadt *et al* 1986).

Two microbial pesticide products (M-One® and Foil®), which are based on *B.t.t,* are commercially available in the United States and have been in use since 1989. In addition, a bio-insecticide known commercially as MYX 1806 comprising Cry3Aa genetically engineered into the bacterium *Pseudomonas fluorescens,* which has been rendered non-viable, has been commercially available in the United States since 1991.

New Leaf® potatoes are not grown in Australia or New Zealand and are currently not permitted to be imported into Australia or New Zealand as fresh produce. Rather, they currently enter into the market in imported processed food commodities such as processed potato crisps, pre-cooked French fries, potato flour and potato starch.

2. DESCRIPTION OF THE MODIFICATION

2.1 Methods used in the genetic modification

A total of fourteen transformed potato lines were produced using *Agrobacterium*-mediated transformation of stem tissue. Plasmid PV-STBT02 was used to generate seven Russet Burbank and two Superior potato lines and plasmid PV-STB-04 was used to generate five Atlantic potato lines. The two plasmids differ only in the promoter region for one of the genes.

2.2 Function and regulation of the novel genes

The transformation of the potatoes with either plasmid PV-STBT02 or PV-STBT04 resulted in the transfer of two gene expression cassettes — *cry3Aa* and *nptII*.

The gene expression cassettes are described in Table 1 below.

Table 1: Description of the gene expression cassettes in PV-STBT02 and PV-STB-04

Cassette	Genetic element	Source	Function
cry3Aa	enhanced 35S promoter (PV-STBT02)	The cauliflower mosaic virus (CaMV) 35S promoter region (Odell *et al* 1985) with duplicated enhancer region (Kay *et al* 1987).	A promoter of high level constitutive gene expression in plant tissues.
	ArabSSU1A promoter (PV-STB04)	The Arabidopsis thaliana ribulose-1,5-bisphosphate carboxylase (Rubisco) small subunit *ats*1A promoter (Almeida *et al* 1989, Wong *et al* 1992).	Constitutive plant promoter.
	cry3Aa	Coding region of the *B.t.t.* Band 3 protein (Perlak *et al* 1993).	Confers protection against a narrow spectrum of Coleopterans, including Colorado potato beetle.
	E9 3' terminator	The 3' non-translated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) E9 gene (Coruzzi *et al* 1984).	Contains signals for termination of transcription and directs polyadenylation.
nptII	35S promoter	The 35S promoter region of CaMV (Gardner *et al* 1981, Sanders *et al* 1987).	A promoter of high level constitutive gene expression in plant tissues.
	nptII	The gene coding for neomycin phosphotransferase II from Tn5 in *Escherichia coli* (Beck *et al* 1982).	Confers resistance to the antibiotics kanamycin and neomycin. Used as a selectable marker for plant transformation (Horsch *et al* 1984, DeBlock *et al* 1984).
	NOS 3'	The 3' terminator region of the nopaline synthase gene from the Ti plasmid of *Agrobacterium tumefaciens* (Depicker *et al* 1982, Bevan *et al* 1983).	Contains signals for termination of transcription and directs polyadenylation.

The *cry3Aa* gene

The *cry3Aa* gene was isolated from the DNA of *B.t.t* strain BI 256-82 (Krieg *et al* 1983). A full length clone and complete nucleotide sequence of the *cry3Aa* gene has been published (McPherson *et al* 1988, Perlak *et al* 1993). The gene is one of several that have been isolated from *B. thuringiensis* and which encode a group of toxins known as the δ-endotoxins or the crystal proteins. These toxins are selectively active against several Orders of insects such as the Lepidoptera, Coleoptera, and Diptera. The crystal proteins are produced by the bacterium during sporulation. The protein product of the *cry3Aa* gene, Cry3Aa, is selectively active against a narrow spectrum of Coleoptera (MacIntosh *et al* 1990). When ingested by susceptible insect species, the crystal proteins cause lysis of midgut epithelial cells in the insect gut, which leads to gut paralysis, cessation of feeding and the eventual death of the insect (Höfte and Whiteley 1989). Cytolytic effects on the midgut cells are mediated by binding of the activated toxin to specialised receptors on the cell surface. This binding of the

toxin to specialised receptors has been shown to be essential for the onset of toxicity (Wolfersberger 1990, Ferré *et al* 1991). Following binding of activated toxin to the receptors, a rapid change in permeability of midgut cells is observed where there is an influx of ions and water in the cell, resulting in its eventual lysis (Knowles and Ellar 1987).

The *nptII* gene

The *nptII* gene is widely used as a selectable marker in the transformation of plants (Kärenlampi 1996). The gene functions as a dominant selectable marker in the initial, laboratory stages of plant cell selection following transformation (Horsch *et al* 1984, DeBlock *et al* 1984). It codes for the enzyme neomycin phosphotransferase II (NPTII) and confers resistance to the aminoglycoside antibiotics, neomycin, kanamycin, and geneticin (G418). The *nptII* gene is transferred along with the *cry3Aa* and *PLRVrep* genes, enabling those plant cells successfully transformed with the *cry3Aa* and *PLRVrep* genes to grow in the presence of kanamycin. Those cells that lack the *nptII* gene, and hence the *cry3Aa* and *PLRVrep* genes, will not grow and divide in the presence of kanamycin.

Other genetic elements

The plasmid vectors, PV-STBT02 and PV-STBT04, are double border binary plant transformation vectors, which differ only in the non-translated promoter region of the *cry3Aa* gene. Both plasmid vectors contain well characterised DNA segments required for selection and replication of the plasmids in bacteria as well as the right and left borders delineating the region of DNA (T-DNA) which is transferred into the plant genomic DNA. This is the region into which the gene of interest, and the plant cell selectable marker, is inserted. DNA residing outside the T-DNA region does not normally get transferred into plant genomic DNA (Zambryski 1992). The genetic elements are described in Table 2 below.

Table 2: Description of other genetic elements contained within PV-STBT02 and PV-STBT04

Genetic element	Source	Function
aad (resides outside the T-DNA)	Gene coding for streptomycin adenyltransferase from transposon Tn7 in *Escherichia coli* (Fling *et al* 1985).	Confers resistance to the antibiotics spectinomycin and streptomycin.
LB	A 0.45 kb fragment of the octopine Ti plasmid pTi5955, which contains the 24 bp T-DNA left border (LB) region (Barker *et al* 1983).	Terminates the transfer of the T-DNA from *A. tumefaciens* to the plant genome.
oriV (resides outside the T-DNA region)	A 1.3 kb origin of replication region derived from the broad-host range RK2 plasmid of *Agrobacterium* (Stalker *et al* 1981).	Allows plasmids to replicate in *A. tumefaciens*.
***ori-322/rop* region** (resides outside the T-DNA region)	A 1.8 kb segment of the plasmid pBR322 which contains the origin of replication region and the *bom* site for the conjugational transfer.	Allows for maintenance of plasmids in *E. coli* and their conjugal transfer into *A. tumefaciens* cells (Bolivar *et al* 1977, Sutcliffe 1978).
RB	A 0.36 kb fragment from the pTiT37 plasmid containing the 24 bp nopaline-type T-DNA right border (RB) region. (Depicker *et al* 1982).	The RB region is used to initiate T-DNA transfer from *A. tumefaciens* to the plant genome.

The *aad* gene is derived from the bacterial transposon Tn7 (Fling *et al* 1985) and is used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It encodes the enzyme streptomycin adenyltransferase, which confers resistance to the antibiotics spectinomycin and streptomycin. Only those bacterial cells that have been transformed with the plasmid containing the *aad* gene, and hence the gene of interest (in this case the *cry3Aa* gene) will grow. The *aad* gene is under the control of a bacterial promoter and would therefore not be expressed in transformed plant cells.

The host for all DNA cloning and vector construction was *E. coli* strain MM-294, a derivative of the common laboratory *E. coli* K-12 strain.

2.3 Characterisation of the genes in the plant

Studies submitted by Monsanto:

Keck, P.J. (1993). Molecular characterisation of CPB resistant Russet Burbank potatoes. Monsanto Study No. MSL-12784

The technique of Southern blotting was used to characterise the genes that had been transferred to the potato plants. Southern blotting is a sensitive technique that enables the detection of specific sequences among DNA fragments separated using gel electrophoresis (Southern 1975). The size and overall pattern of hybridising bands can be used to characterise the nature of the T-DNA insertion into the genome (e.g. how many sites in the genome the T-DNA has inserted into, whether the inserted T-DNA copies are intact).

Southern blotting was done on genomic DNA isolated from the fourteen lines of transformed potatoes in order to characterise the inserted T-DNA in terms of the number of integration sites in the genome and the number of T-DNA copies inserted at a particular site.

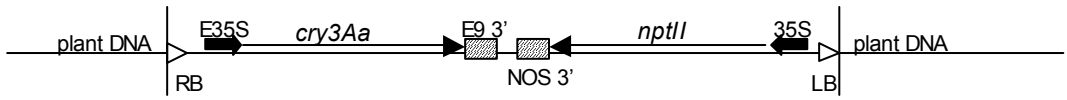
Five of the fourteen lines of transformed potato were selected for commercialisation on the basis that they contained single copies of the inserted T-DNA at a single site within the potato genome. These lines are listed in Table 3 below.

Table 3: Commercialised New Leaf® potato lines

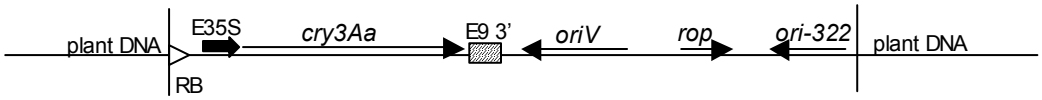
Name	Plasmid vector	Cultivar
BT-06	PV-STBT02	Russet Burbank
ATBT04-06	PV-STBT04	Atlantic
ATBT04-31	PV-STBT04	Atlantic
ATBT04-36	PV-STBT04	Atlantic
SPBT02-05	PV-STBT02	Superior

Each of these lines were further analysed to determine the gene organisation as well as confirm the absence of DNA from outside the T-DNA borders. A diagram of the gene organisation in the insertion site for each line is presented below.

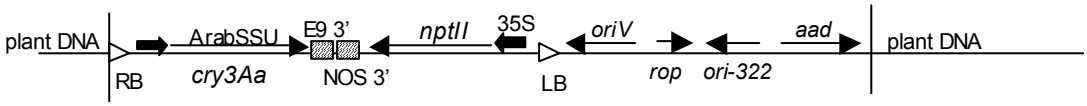
BT-06, ATBT04-06 and ATBT04-31



SPBT02-05



ATBT04-36



Although DNA residing outside the T-DNA generally does not get transferred into the plant genome, occasionally, insertion of DNA beyond the classically defined T-DNA region is known to occur (Zambryski 1992). The *aad* gene, the *oriV* and *ori322* regions are all located outside the T-DNA borders therefore they would not be expected to be transferred in the transformation. Southern blotting of the transformed potato lines indicates, however, that some or all of these three elements have been transferred into the genome of lines SPBT02-05 (*oriV* and *ori322*) and ATBT04-36 (*oriV*, *ori322* and *aad*).

Conclusion

The genetic elements that have been transferred to each of the New Leaf® lines are summarised in Table 4 below.

Table 4: Genetic elements transferred to the New Leaf® lines

	Transferred genetic elements				
Line No.	***cry3Aa***	***nptII***	***oriV***	***ori-322***	***aad***
BT-06	√	√			
SPBT02-05	√		√	√	
ATBT04-06	√	√			
ATBT04-31	√	√			
ATBT04-36	√	√	√	√	√

2.4 Stability of the genetic changes

The New Leaf® potato lines were evaluated over several generations of vegetative propagation used to generate potato seed to determine if they expressed consistent levels of the Cry3Aa protein in leaf tissue. Expression of the Cry3Aa protein was found to be highly stable across multiple generations in all the lines tested. The results of the expression studies are summarised in Table 5 below.

Table 5: Cry3Aa expression in New Leaf® potatoes over multiple generations

	Mean protein expression levels (µg/g tissue fresh weight)		
Potato line	**Generation 1**	**Generation 2**	**Generation 3**
ATBT04-36	12.8	13.3	14.6
ATBT04-06	52.2	42.7	ND
ATBT04-31	ND	7.1	8.3
SPBT02-05	ND	8.3	7.9
BT-06	5.7	6.2	7.5

New Leaf® Russet Burbank line BT-06 exhibited insect-resistance under field conditions when it was first planted in 1991. The applicant reports that this trait has been stably maintained through subsequent generations of plant propagation and breeding under different environmental conditions in potatoes grown in subsequent field trials and on a commercial scale beginning in 1996 in the United States and Canada. The Superior variety of New Leaf® potato (SPBT02-05) and the Atlantic varieties (ATBT04-36, ATBT04-06, ATBT0-31) were first planted in 1992 and the applicant reports that the trait in these lines has also been consistently expressed since that time.

Conclusion

The *cry3Aa* gene in the New Leaf® potatoes is stably integrated and all lines appear to be phenotypically and genetically stable over multiple generations.

3. GENERAL SAFETY ISSUES

The New Leaf® potatoes have been assessed according to ANZFA's safety assessment guidelines relating to Group D foods, i.e., plants or animals that contain new or altered genetic material (ANZFA 1999).

3.1 History of use

The potato (*Solanum tuberosum* L.) is a major food crop throughout the world (Simmonds 1976). It was introduced into Europe from South America in the 16th century and is cultivated for the production of underground tubers.

Potatoes are generally consumed either cooked (as a fresh vegetable) or processed into crisps, potato flour or potato starch. They are rarely consumed raw because of the indigestibility of ungelatinised potato starch and the presence of protease inhibitors (Burton 1989).

3.2 Nature of novel protein

Cry3Aa

Cry3Aa is a protein of 644 amino acids (molecular weight 73 kDa), which is produced by *B. thuringiensis* during sporulation. The protein is encoded by the *cry3Aa* gene, which is isolated from *B. thuringiensis* subsp. *tenebrionis* (*B.t.t*) strain BI 256-82. In addition to the full length Cry3Aa protein, *B.t.t* also produces a smaller form of the protein known as *B.t.t* band 3 (McPherson *et al* 1988). *B.t.t* band 3 has a molecular weight of 68 kDa (597 amino acids) and results from an internal translation initiation event within the same gene starting at an internal methionine codon at amino acid position 48. This protein has been shown to

possess the same insecticidal activity and selectivity to Colorado potato beetle larvae as the full-length Cry3Aa protein.

The gene encoding *B.t.t* band 3 protein was engineered for plant expression by being completely re-synthesised to substitute the existing bacteria-preferred codons with plant-preferred codons (Perlak *et al* 1993). The genetic code is degenerate, meaning that a given amino acid may be specified by more than one codon. For example, four different codons can be used to specify the amino acid alanine. It has been found that plants often prefer different codons to bacteria to specify the same amino acid, and this can affect the expression levels of bacterial genes when they are transferred to plant cells. It has been shown that the plant expression of bacterial genes can be improved if the bacteria-preferred codons are substituted with plant-preferred codons (Perlak *et al* 1990). The re-synthesis of the gene encoding the band 3 protein, to substitute plant-preferred codons for bacteria-preferred codons, changed 399 out of 1791 nucleotides without altering the amino acid sequence. The re-synthesised *cry3Aa* gene therefore expresses a protein that is identical to that produced by *B. thuringiensis* subsp. *tenebrionis*.

Neomycin phosphotransferase II

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

3.2 Expression of novel protein in the plant

***In planta* expression of Cry3Aa**

Studies submitted by Monsanto:

Bartnicki, D.E. *et al* (1993). Characterisation of the major tryptic fragment from Colorado potato beetle active protein from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*). Monsanto Study No. 92-01-37-15.

Bartnicki, D.E. *et al* (1993). Equivalence of microbially-produced and plant-produced *B.t.t.* protein also called Colorado potato beetle active protein form *Bacillus thuringiensis* subsp. *tenebrionis*. Monsanto Study No. 92-01-37-07.

Rogan, G.J. and Lavrik, P.B. (1994). Compositional comparison of Colorado potato beetle (CPB) active *Bacillus thuringiensis* subsp. tenebrionis (*B.t.t.*) proteins produced in CPB resistant potato plants and commercial microbial products. Monsanto Study No. 92-01-37-17.

An initial study was done, using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting to analyse the physical properties of potato tuber-expressed Cry3Aa using immunoaffinity chromatography purified Cry3Aa from New Leaf® Russet Burbank tubers. When the tuber-purified Cry3Aa was run on a polyacrylamide gel it was possible, using Coomassie blue stain, to visualise three polypeptides. The major protein band had an apparent molecular weight of 68 kDa and a minor band just below it had an apparent molecular weight of 63 kDa. The third band represented about 20-30% of the tuber-purified Cry3Aa protein and had an apparent molecular weight of 55 kDa.

Western blotting was used to further characterise the major polypeptides. Western blotting is an extremely specific immunological technique that allows for comparisons of apparent molecular weights of proteins possessing immunological cross-reactivity. Western blotting, using a rabbit polyclonal antibody against Cry3Aa isolated from *B.t.t*, demonstrated similar apparent molecular weights and staining for the 68 kDa protein, and similar staining intensity of the 55 kDa fragment. The presence of the 55 kDa band did not appear to be an artefact of the purification process as the 55 kDa band has previously been reported following digestion of the 68 kDa Cry3Aa protein by incubation in insect gut fluid or trypsin, and shown to retain full bioactivity against target insects (Carroll and Ellar 1989).

In further experiments, Western blotting was used to examine extracts of tuber and leaf tissues from Russet Burbank line BT-06 and compare them to extracts of commercial *B.t.t* preparations (M-One® and Foil®). Two major protein bands were detected in the extract from Russet Burbank line BT-06. The more abundant of the two proteins co-migrated with a 68 kDa size marker and the second band, which was about half as abundant, co-migrated with the 55 kDa size marker. Identical bands to those observed in the potato extract were also seen in the extract prepared from the M-One® microbial preparation except in this preparation the 55 kDa species was the more abundant. The extract from the other microbial preparation (Foil®) only contained the 68 kDa band.

These data confirm that the 68 kDa protein product of the *cry3Aa* gene transferred into potato plants undergoes processing or degradation in the plant cell into a smaller protein of 55 kDa. The two protein products seen in the plant cell appear identical to that observed in commercial *B.t.t* preparations.

Protein expression levels

Studies submitted by Monsanto:

Rogan, G.J. *et al* (1993) Determination of the expression levels of *B.t.t* and NPTII proteins in potato tissues derived from field grown plants. Monsanto Study No. 92-01-37-02.

Duff, D.A. (1993). Development and validation of an enzyme-linked immunosorbent assay (ELISA) for detection and quantitation of *Bacillus thuringiensis* subsp. *tenebrionis* Colorado potato beetle active protein in genetically modified potato plants. Monsanto Study No. 92-01-37-02, Report No. 12735.

Anderson, J.S and Rogan, G.J. (1993). Development and validation of an enzyme-linked immunosorbent assay (ELISA) for detection and quantitation of neomycin phosphotransferase II in Colorado potato beetle resistant potatoes. Monsanto Study No. 92-01-37-02, Report No. 12330.

Lavrik, P.B. and Grace, A.M. (1995). Expression levels of *B.t.t.* and NPTII proteins in Colorado potato beetle resistant potato tissues derived from Superior potato plants grown under field conditions. MSL No. 14415.

Lavrik, P.B. and Grace, A.M. (1996). Expression levels of *B.t.t.* and NPTII proteins in Colorado potato beetle resistant potato tissues derived from Atlantic potato plants grown under field conditions. MSL No. 14659.

The levels of Cry3Aa and NPTII proteins expressed in the leaf and tuber tissue of all five New Leaf® lines were determined by enzyme-linked immunosorbent assay (ELISA). ELISA is a technique that uses highly specific antibodies to identify proteins. The assay system is capable of quantifying proteins in crude tissue extracts. Novel protein expression in individual New Leaf® potato lines was determined using tissues isolated from a number of different field trials conducted in different years.

Tissues from Russet Burbank line BT-06 were collected from plants grown in field trials during the summer of 1992 at seven locations. A randomised non-replicated arrangement of treatments was used at five of the field locations, and a six replicate randomised complete block design was used at the remaining two field locations. The sampling regimen was designed to obtain tissue samples of the same relative physiological stage from all of the field locations. One whole plant was taken per plot at replicated sites and three plants per plot were taken at non-replicated sites. Protein expression data was determined for leaves, whole plant and tubers and is summarised in Table 6a below.

Table 6a: Protein expression data for Cry3Aa and NPTII in New Leaf® Russet Burbank line BT-06

Sample	Mean expression (ug/g tissue fresh weight)	Range	% total protein[1]
Cry3Aa:			
Whole plant (early season)	3.33	2.025-6.258	
Whole plant (late season)	4.297	0.526-5.644	
First leaf sample	16.364	12.15-20.823	0.076-0.13%
Second leaf sample	11.617	7.868-15.635	0.049-0.098%
Third leaf sample	11.094	5.395-13.111	0.034-0.082%
Tubers	0.664	0.404-0.955	0.002-0.005%
NPTII:			
Whole plant (late season)	0.446	0.038-0.862	
First leaf	2.063	1.344-2.431	0.008-0.015%
Tubers	0.353	0.173-0.599	0.0009-0.003%

[1] using total protein levels of 1.6 and 2.0% for leaf and tuber fresh weight, respectively

These data show that Cry3Aa and NPTII are expressed at higher concentrations in leaf tissue compared to tubers — a maximum of 0.13% total protein in leaf for Cry3Aa compared to a maximum of 0.005% total protein in tubers, and a maximum of 0.015% in leaf for NPTII compared to 0.003% in tubers.

Tissues from Superior line SPBT02-05 and one control Superior plant line were collected from plants grown in two field locations in the United States during 1994. A four replicate randomised complete block design was used at both locations. Leaf samples were collected at approximately 6 weeks post planting from one of the field locations and tuber samples were collected at harvest from the other site from three of the four replicated plots. Protein expression data for Cry3Aa and NPTII is summarised in Table 6b below.

Table 6b: Cry3Aa and NPTII expression[1] in New Leaf® Superior and control lines

Tissue	Cry3Aa expression			NPTII expression		
	Mean	Range	% total protein[2]	Mean	Range	% total protein
SPBT02-05[3]:						
Leaf	11.542	8.90-15.49	0.055-0.097	0.003	0.00-0.01	< 0.0001
tuber	1.146	1.00-1.29	0.005-0.006	0.016	0.01-0.03	<0.0002
Superior control:						
Leaf	0.038	0.00-0.01		0.004	0.00-0.01	
tuber	0.042	0.03-0.05		0.034	0.03-0.04	

[1] values are ug/g tissue fresh weight
[2] using total protein levels of 1.6 and 2.0% for leaf and tuber fresh weight, respectively
[3] line SPBT02-05 does not contain a copy of the *nptII* gene

These data confirm, as expected, that NPTII expression in line SPBT02-05 is equivalent to the background levels found in the control line. Cry3Aa expression is highest in the leaves, at a maximum representing nearly 0.1% of total leaf protein. In tubers, Cry3Aa expression is considerably lower, representing at a maximum only 0.006% of total tuber protein.

Tissues from Atlantic lines ATBT04-36, ATBT04-06, and ATBT04-31 and a control Atlantic line were collected from plants grown at six field locations in the United States and Canada in 1993 through to 1995. The field trials were arranged either in a four, six, twelve or fifteen replicate randomised complete block design. Leaf samples were collected at approximately six, ten and fourteen weeks post planting from a single location in the 1993 field trials with each line replicated six times and at approximately six weeks post planting from a single location in the 1994/1995 field trials with each line replicated four times. Tuber samples from each line were collected at harvest from four different locations in the 1995 field trials. Four replicated plots for each line were harvested from each site. Protein expression data for Cry3Aa and NPTII is summarised in Table 6c below.

Table 6c: Cry3Aa and NPTII expression[1] in New Leaf® Atlantic and control lines

	Cry3Aa expression			NPTII expression		
Tissue	**Mean**	**Range**	**% total protein[2]**	**Mean**	**Range**	**% total protein**
ATBT04-06:						
Leaf	59.336	29.48-88.67	0.18-0.55	36.564	21.54-47.63	0.13-0.3
Tuber	0.528	0.26-0.71	0.001-0.004	2.864	2.06-3.82	0.01-0.02
ATBT04-31						
Leaf	15.694	8.23-19.81	0.05-0.12	4.994	3.23-5.59	0.02-0.035
Tuber	0.140	0.07-0.26	0.0003-0.001	0.726	0.27-1.75	0.0013-0.009
ATBT04-36						
Leaf	20.278	8.90-32.22	0.06-0.2	12.156	5.46-23.02	0.034-0.14
Tuber	0.126	0.05-0.27	0.00025-0.001	0.583	0.29-0.80	0.0015-0.004
Atlantic control:						
Leaf	0.050	0.01-0.13		0.093	0.01-0.41	
Tuber	0.029	0.01-0.07		0.035	0.00-0.18	

[1] values are ug/g tissue fresh weight
[2] using total protein levels of 1.6 and 2.0% for leaf and tuber fresh weight, respectively

Expression of Cry3Aa and NPTII is quite variable both within each line and between lines but in all cases is highest in the non-edible portion of the plant – the leaf tissue. Cry3Aa expression in the tuber ranges from 0.00025 to 0.004% total protein and NPTII tuber expression ranges from 0.0013 to 0.02% total protein. Line ATBT04-06 exhibited the highest tuber expression levels for both proteins.

Conclusion

The expression levels of Cry3Aa and NPTII in potato tubers from the New Leaf® lines are quite variable but consistently low. Expression levels range from between 0.00025 and 0.006% total tuber protein for Cry3Aa and from <0.0002 to 0.02% total protein for NPTII. Line SPBT02-05 exhibits the highest Cry3Aa expression level and line ATB04-06 the highest NPTII expression level.

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

The human health considerations in this regard depend on the nature of the novel genes and must be assessed on a case-by case basis.

In 1991, the World Health Organization (WHO) issued a report of a Joint FAO[4]/WHO Expert Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). It was concluded by that consultation that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

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

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

Two antibiotic resistant genes have been transferred to the New Leaf® potato lines — the *nptII* gene and the *aad* gene. The *nptII* gene confers resistance to the aminoglycoside antibiotics, neomycin, kanamycin, and geneticin (G418) and the *aad* gene confers resistance to the antibiotics spectinomycin and streptomycin. These antibiotics only have very limited clinical use. Neomycin is not used orally because of its toxicity but is still used topically in certain circumstances (Davis *et al* 1980). Streptomycin has mostly been replaced by newer aminoglycosides, although it is still used for special indications, such as in the treatment of tuberculosis and brucellosis (Kärenlampi 1996) and spectinomycin is rarely used clinically.

Line SPBT02-05 was shown, by Southern blotting, not to contain either of the antibiotic resistance genes (see Section 2.3). Lines BT-06, ATBT04-06, ATBT04-31 and ATBT04-36, however, all contain the *nptII* gene, under the control of the 35S promoter, meaning it will be expressed in plant cells, and line ATBT04-36 also contains a copy of the *aad* gene, under the control of a bacterial promoter, meaning it will not be expressed in plant cells.

The first issue that must be considered in relation to the presence of the *nptII* and *aad* genes in the New Leaf® potatoes is the probability that these gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract. The following steps are necessary for this to occur:

- excision of DNA fragments containing the antibiotic resistance gene;
- survival of DNA fragments containing the antibiotic resistance gene in the digestive tract;
- natural transformation of bacteria inhabiting the digestive tract;
- survival of the bacterial restriction system by the DNA fragment containing the antibiotic resistance gene;

[4] Food and Agriculture Organization.

- stable integration of the DNA fragment containing the antibiotic resistance gene into the bacterial chromosome or plasmid;

- maintenance and expression of antibiotic resistance gene by the bacteria. In the case of the *nptII* gene this would have to involve the acquisition of a bacterial promoter.

In the case of line ATBT04-36, there may be a slightly higher probability of horizontal gene transfer of the *aad* and *nptII* genes because of the transfer to the plant genome of the linked *Escherichia coli* origin of replication (*ori322*) and the origin of replication for the broad host-range *Agrobacterium* plasmid (*oriV*). Depending on the integrity of these components, the presence of these elements on the same DNA fragment could lead to the reconstitution of almost the entire PV-STBTO4 plasmid. A plasmid is more likely to be successfully taken up than an isolated fragment of DNA. This however, would still be an extremely unlikely event.

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

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

In the case of transfer of the *nptII* gene and the *aad* gene, the human health impacts are considered to be negligible. In the case of *nptII*, this gene occurs naturally in bacteria inhabiting the human digestive tract therefore the additive effect of an *nptII* gene entering the human gastrointestinal flora from a genetically modified plant would be insignificant compared to the population of kanamycin resistant microorganisms naturally present. In the case of the *aad* gene, this gene is common and can be found at high frequencies in natural populations of bacteria as well as clinical isolates (Shaw *et al* 1993). Natural populations of streptomycin resistant bacteria are far more likely to be sources of transferred antibiotic resistance than ingested plant material.

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

Conclusion

It is extremely unlikely that the *nptII* or *aad* genes would transfer from the New Leaf® potatoes to bacteria in the human digestive tract because of the number and complexity of steps that would need to take place consecutively. In the highly unlikely event that the genes were transferred the human health impacts would be negligible because both antibiotic resistance genes are already commonly found in bacteria in the environment as well as

inhabiting the human digestive tract and both antibiotics have very little, if any, clinical use in Australia and New Zealand.

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

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally-occurring toxins

Wild tuberous *Solanum* species contain high concentrations of the toxic glycoalkaloids, which are very bitter in taste. The presence of glycoalkaloids in *Solanum* species is generally believed to be a natural plant defense mechanism against pests and diseases (Conner 1995). Modern potato cultivars accumulate high glycoalkaloid concentrations in green shoot tissue and in tubers upon exposure to light. In some cultivars, significant concentrations of glycoalkaloids can also accumulate in tubers not exposed to light. The variation in glycoalkaloid content of tubers can be attributed to both genetic effects and the environmental conditions under which the plants are grown and stored following harvest (van Gelder 1990). The concentration of glycoalkaloids in potato tubers in advanced lines of modern breeding programs is usually routinely monitored (Morris and Lee 1984).

Studies submitted by Monsanto:

Dodson, H.C. *et al* (1998). Composition analysis of potato tubers from Colorado potato beetle resistant Atlantic potato plants grown under field conditions. Monsanto Study No. 98-01-37-01.

Dodson, H.C. *et al* (1998). Composition analysis of potato tubers from Colorado potato beetle resistant Superior potato plants grown under field conditions. Monsanto Study No. 98-01-37-12.

Lavrik, P.B. and Love, S.L. (1994). Composition and quality analysis of potato tubers derived from field-grown Colorado potato beetle resistant potato plants. Monsanto Study No. 92-01-37-19.

Atlantic potato lines ATBT04-06, ATBT04-31, and ATBT04-36 were grown in field trials during the 1997 season at three locations in the United States. Superior potato line SPBT02-05 was grown in field trials during the summer of 1997 at eight locations in the United States and Canada. Russet Burbank potato line BT-06 was grown during the 1992 season at six locations in the United States. In all cases, tubers were collected from four areas of each plot at each site and analysed for glycoalkaloid content. The controls were tubers isolated from the non-transformed potato line grown at the same field location. A summary of the results is present in Table 7 below.

Table 7: Glycoalkaloid content[1] in New Leaf® potato lines.

Line	Total glycoalkaloids	Literature range
Atlantic:		4.2-10.5
ATBT04-06 (n=16)	8.7 (5.58-13.09)	
ATBT04-31 (n=16)	8.1 (3.82-15.53)	
ATBT04-36 (n=16)	7.6[#] (5.00-12.52)	
Control (n=16)	9.4 (5.27-16.78)	
Superior:		Not available
STBT02-05 (n=9)	5.2 (2.5-10.4)	
Control (n=6)	4.8 (1.9-7.3)	
Russet Burbank:		3.1-16.1
BT-06 (n=6)	6.6[#] (3.4-14.3)	
Control (n=6)	4.5 (2.7-6.7)	

[1] the values provided are mg/100g fresh weight and are the mean values with the range in parentheses
[#] significantly different compared to the control

Lines ATB04-06, ATBT04-31, and STBT02-05 have glycoalkaloid levels that are equivalent to the non-transformed controls and line ATBT04-36 has glycoalkaloid levels that are decreased compared to the control. Slightly decreased glycoalkaloid levels do not raise any safety concerns and all the values reported are within the literature reported range for glycoalkaloids. Line BT-06 has glycoalkaloid levels that are slightly elevated compared to the control. The increase however is minor and the value reported is at the lower end of the normal range reported in the literature for glycoalkaloids, therefore the slight increase does not raise any safety concerns.

4.2 Potential toxicity of novel protein

The New Leaf® potato lines all express the Cry3Aa protein, and all lines, with the exception of SPBT02-05, also express the NPTII protein (see Section 3.3). Therefore this section will address the potential toxicity of these two proteins.

Studies submitted by Monsanto:

Berberich, S.A. *et al* (1993). Preparation and verification of dose for a mouse acute oral toxicity study with neomycin phosphotransferase II protein (NPTII). Monsanto Study No. ML-91-409.

Naylor, M.W. (1992). Acute oral toxicity study of neomycin phosphotransferase (NPT II) in albino mice. Monsanto Study No. ML-91-409.

Naylor, M.W. (1993). Acute oral toxicity study of *B.t.t*. protein in albino mice. Study No. ML-92-407.

Lavrik, P.B. *et al* (1993). Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein dose formulation, dose confirmation, and dose characterisation for albino mice acute toxicity study. Monsanto Study No. ML-92-407.

Lavrik, P.B. (1993). Characterisation of Colorado potato beetle active *Bacillus thuringiensis* subsp. *tenebrionis* protein produced in *Escherichia coli*. Monsanto Study No. 92-01-37-10.

Bartnicki, D.E. *et al* (1993). Equivalence of microbially-produced and plant-produced *B.t.t.* protein also called Colorado potato beetle active protein form *Bacillus thuringiensis* subsp. *tenebrionis*. Monsanto Study No. 92-01-37-07.

Bartnicki, D.E. *et al* (1993). Equivalence of microbially-produced (*Escherichia coli*) and plant-produced (Colorado potato beetle resistant potato) neomycin phosphotransferase II (NPTII). Monsanto Study No. 92-01-37-08.

Cry3Aa

Cyr3Aa is insecticidal only to Coleopteran insects (MacIntosh *et al* 1990) and its specificity of action is directly attributable to the presence of specific receptors in the target insects (Wolfersberger 1990, Ferré *et al* 1991). There are no receptors for the δ-endotoxins of *B. thuringiensis*, including Cry3Aa, on the surface of mammalian intestinal cells (Hubert *et al* 1995), therefore, humans, as well as other mammals, are not susceptible to this protein.

An acute oral toxicity study was done to confirm the absence of mammalian toxicity of Cry3Aa. Acute tests are used because it is known that protein toxins generally act via acute mechanisms (Jones and Maryanski 1991). The test protein was produced in a single fermentation batch of *E. coli* (Batch No. 5192101) and purified to greater than 95% protein. The test protein was produced in *E. coli* because the plant lines did not express enough protein for purification of large quantities for toxicity testing. Data was presented to indicate that the bacterially produced Cry3Aa is equivalent to the plant produced Cry3Aa in terms of its molecular mass, N-terminal amino acid sequence, lack of glycosylation, and biological activity. Therefore the *E. coli* produced Cry3Aa is a suitable substitute for plant produced Cry3Aa in toxicity testing.

The Cry3Aa protein (Batch No. 5192101) was administered by gavage to groups of 10 CD-1 mice (10/sex/dose) at levels of 0, 500, 1000 and 5220 mg/kg body weight. Another group of mice were dosed with 5000 mg/kg bovine serum albumin and controls were administered the same volume of dosing vehicle given to the high dose mice. Mice were observed twice daily for signs of toxicity and food consumption was recorded daily. Body weights were recorded pre-test and on day 7 after dosing. All animals were necropsied 7 days after dosing.

Results

Two animals died during the test — a vehicle control female on day 1 and a low dose male on day 3. The death of the control female was attributed to accidental gavaging of the vehicle into the lung. No cause of death could be determined for the low dose male. As there were no deaths in other treated mice, or at higher exposure levels, the death is not considered to be treatment related.

No abnormal clinical signs were observed in any of the other mice during the study that could be attributed to the treatment. No significant differences were observed in body weight, cumulative body weight or food consumption. Several minor pathologic changes were observed at necropsy. The minor pathologic changes were randomly distributed among all groups and the applicant reports that these are commonly seen for the strain of mice used by the testing laboratory.

Conclusion

No adverse effects were observed when Cry3Aa was administered by gavage to mice at doses up to 5220 mg/kg. Cry3Aa is therefore considered to have low oral toxicity in mice.

Neomycin phosphotransferase II

An acute oral toxicity study was done to assess the potential mammalian toxicity of neomycin phosphotransferase II. The test protein was produced in a single fermentation batch of *E.*

coli (Batch No. NBP 4821020) and purified to greater than 95% protein. The test protein was produced in *E. coli* because the plant lines did not express enough protein for purification of large quantities for toxicity testing. Data was presented to indicate that the *E. coli* produced NPTII is equivalent to the plant produced NPTII in terms of its molecular mass, N-terminal amino acid sequence, lack of glycosylation, and biological activity. This study has also been published (Fuchs *et al* 1993). The *E. coli* produced NPTII is therefore a suitable substitute for plant produced NPTII in toxicity testing.

The NPTII protein was administered by gavage to groups of CD-1 mice (10/sex/dose) at doses of 0, 100, 1000, and 5000 mg/kg body weight. The test material was dissolved in a 0.1M carbonate buffer vehicle and given as a solution. Control mice were administered vehicle only. Clinical observations, body weights and food consumption measurements were performed. All animals were necropsied 8-9 days after dosing.

Results

When compared to controls, there were no statistically significant differences between group mean body weights or cumulative body weight gain in any of the treated groups. Among males, there were no statistically significant differences in food consumption but among females food consumption was significantly lower compared to controls. However, food consumption by the female control mice was nearly twice that of any male group. The applicant speculates that the high apparent food consumption by the control females reflects spillage due to digging behaviour. This being the case, the observed differences in food consumption among females could not be attributed to any toxicity of the test material. No abnormal clinical signs were noted and there were no unscheduled deaths. There were no differences between group mean terminal body weights and no gross lesions were observed at necropsy.

Conclusion

No adverse effects were observed when NPTII was administered by gavage to mice at doses up to 5000 mg/kg. NPTII is considered to have low oral toxicity in mice.

4.3 Levels of naturally-occuring allergenic proteins

Potatoes are not generally regarded as major sources of food allergy, although patatin, the main storage protein of potatoes, has recently been reported to induce an allergic reaction in some individuals (Seppälä *et al*., 1999). The clinical importance of patatin as a food allergen has yet to be confirmed.

As potatoes are not classified as major sources of food allergy, and there have yet to be any confirmed potato allergens described, an assessment of the naturally-occurring allergenic proteins of New Leaf® potatoes is unnecessary.

4.4 Potential allergenicity of novel proteins

The concerns regarding potential allergenicity of novel proteins are two fold. Firstly, there are concerns that the ability to express new or different proteins in food will result in the transfer of allergens from one food to another, thereby causing some individuals to develop allergic reactions to food they have not previously been allergic to. Secondly, there are

concerns that the transfer of novel proteins to food will lead to the development of new allergies in certain individuals. The former is more easily addressed than the latter because if an allergen is already known it is possible, using human sera or human skin tests, to test if it has been transferred. There are no reliable tests or animal models, however, which enable the prediction of the allergenic potential of novel proteins. Instead, potential allergenicity can only be indicated by examination of a number of characteristics of the novel protein, such as whether it is derived from a known allergenic source, its physical/chemical characteristics (most allergens have molecular weights between 10 and 70 kDa, are glycosylated, and are resistant to acid and protease degradation), whether it has any sequence similarity to any known allergens, and whether it is likely to be present in large amounts in the food as consumed and therefore have potential for allergic sensitisation.

There are two new proteins expressed in the New Leaf® potatoes – Cry3Aa and NPTII. The potential allergenicity of these two proteins will therefore be considered.

Studies submitted by Monsanto:

Keck, P.J. and Sims, S.R. (1993). Assessment of the metabolic degradation of the Colorado potato beetle (CPB) active proteins in simulated mammalian digestive models. Monsanto Study No. 389270.

Ream, J. (1993) Assessment of degradation of neomycin phosphotransferase II in *in-vitro* mammalian digestion models. Monsanto Study No. IRC-91-ANA-06.

Cry3Aa

Source of the protein

Cry3Aa is encoded by the *cry3Aa* gene, which is derived from the soil bacterium *B. thuringiensis* subsp. *tenebrionis* (*B.t.t.*) and is expressed in the bacterium during sporulation. Microbial formulations of *B.t.t.* have been in use since 1989 where they are used on a wide variety of crops such as eggplant, potato and tomato. These formulations have been shown to contain the Cry3Aa protein (see section 3.3.2) and to date there have been no reports of any allergic reactions.

Physical and chemical characteristics

The Cry3Aa protein has a molecular weight of 68 kDa, which is in the size range of known allergens. Cry3Aa is not glycosylated, however, as it is expressed in the plant cell cytoplasm. For glycosylation of Cry3Aa to occur, the protein would need to be transported through the endoplasmic reticulum and Golgi bodies (Taiz and Zeiger 1991). This requires the presence of specific targeting sequences on the protein and none of these were included in the *cry3Aa* gene construct. The absence of glycosylation of Cry3Aa has also been determined experimentally (see section 4.2).

Digestibility

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

The digestibility of Cry3Aa was determined experimentally using *in vitro* mammalian digestion models. *In vitro* studies with simulated digestion solutions have been used as

models for animal digestion for a number of years and have had wide application. For example, they have been used to investigate the digestibility of plant proteins (Nielsen 1988, Marquez and Lajolo 1981), milk proteins (Zikakis *et al* 1977), and flavouring substances (Tilch and Elias, 1984); to assess protein quality (Akeson and Stahmann 1964); and to study digestion in pigs and poultry (Fuller 1991).

Purified Cry3Aa protein (68 kDa - Batch No. 5192101) was added to simulated gastric and intestinal fluids and incubated at 37ºC. The 55 kDa protein of Cry3Aa was prepared by trypsinization of the purified 68 kDa protein. The protein used was from the same batch that had been produced in *E.coli* for acute toxicity testing in mice. This protein has been shown experimentally to be equivalent to plant expressed Cry3Aa protein (see Section 4.2). The degradation of the protein in the digestion fluid was assessed over time by Western blot analysis. An insect bioassay was used as an additional means of monitoring Cry3Aa degradation in the digestion fluids. The simulated digestion fluids were prepared according to procedures outlined in the United States Pharmacopeia (1990). The simulated gastric fluid contained 3.2g pepsin/L and 2.0g NaCl/L at pH 1.2 and the simulated intestinal fluid contained 10g pancreatin/L in phosphate buffer at pH 7.5.

The 68 kDa and 55 kDa species of Cry3Aa were shown to be readily degraded in simulated gastric fluid, with neither protein species able to be detected by Western blot after thirty seconds of incubation. Samples were removed from the incubation at 0 and 10 minutes for bioassay with Colorado potato beetle. Intermediate time points were not taken. The results of the bioassay correlate with those of the Western blot where the level of bioactivity of the Cry3Aa proteins was reduced from between 60-100% mortality of Colorado potato beetle at 0 minutes to background levels (2-4% mortality) after 10 minutes of incubation.

Western blot analysis of samples incubated with simulated intestinal fluid show that about 10-20% of the 68 kDa species of Cry3Aa is converted to two smaller proteins of about 63 and 55 kDa within 30 seconds. The smaller proteins are consistent with the protein bands seen on Western blots of tuber extracts from line BT-06 (Section 3.3). After two hours of incubation, most of the 68 kDa protein had degraded to the 55 kDa protein product which persisted for up to 14 hours. The experiment was repeated with the isolated 55 kDa protein species which was also shown to persist for up to 14 hours, although a small amount of degradation could be observed. No decrease in bioactivity of the Cry3Aa protein was observed over the 14 hours of incubation, as would be expected from the Western blot data. The predominant proteolytic component of simulated intestinal fluid is trypsin. It has been previously reported that a 55 kDa protein product is generated from the 68 kDa protein upon incubation with trypsin or insect gut fluids (Carroll and Ellar 1989). The 55 kDa protein product therefore appears to be trypsin resistant and so would be expected to persist in the simulated intestinal fluid. Other Cry proteins are also known to have trypsin-resistant cores (Hofte and Whitely 1989).

Similarity to known allergens

The amino acid sequence of the Cry3Aa protein was compared to the 121 amino acid sequences that have been reported for allergens in the three current protein databases – Genpept, Pir protein and Swissprot. No significant similarity to any of the 121 amino acid sequences was found. There was also no greater similarity of the Cry3Aa protein to any of the 121 amino acid sequences for the allergenic proteins than for a scrambled sequence of the same amino acids that comprise the Cry3Aa protein.

Presence of the protein in food as consumed

One of the factors contributing to the allergenicity of certain proteins is their high concentration in foods that elicit an allergenic response (Taylor *et al* 1987, Taylor 1992, Taylor *et al* 1992). This is true for milk (Baldo, 1984, Taylor *et al* 1987), soybean (Shibasaki *et al* 1980, Burks *et al* 1988, Pendersen and Djurtoft 1989) and peanuts (Barnett *et al* 1983, Sachs *et al* 1981, Barnett and Howden 1986, Kemp *et al* 1985).

The Cry3Aa protein, in contrast, is expressed at very low levels in the tubers of New Leaf® potatoes. For example, in Russet Burbank line BT-06 the levels of Cry3Aa in the tuber range between 0.4 and 0.95 μg/g tissue fresh weight (equivalent to 0.002 - 0.005% of total protein).

Neomycin phosphotransferase II

The allergenicity of NPTII has previously been considered by the WHO (1993) and by the Nordic Council of Ministers (Kärenlampi 1996).

In relation to the potential allergenicity of proteins derived from marker genes, the conclusion of the WHO Workshop was that *unless the marker gene is derived from a source known to cause food allergy, there is no reason to believe that marker gene proteins per se would cause allergenic reactions*. The Nordic Council of Ministers concluded that NPTII is not allergenic to humans because it has no significant similarity to known allergens, it is not glycosylated, it is rapidly degraded in the gastrointestinal tract thereby minimising its potential for absorption by the gut, and importantly, it is a protein which is not novel to humans as NPTII producing kanamycin resistant bacteria are present in normal gut flora.

The applicant also provided additional studies confirming that the NPTII protein is not glycosylated *in vivo* in New Leaf® potato plants (see section 4.2) and that it is rapidly degraded in simulated gastric and intestinal digestion fluids.

Purified NPTII (Batch No. NBP4821020) was added to simulated gastric and intestinal fluids and incubated at 37ºC. The protein used was from the same batch that had been produced in *E.coli* for acute toxicity testing in mice. The *E.coli* produced NPTII protein has been shown experimentally to be equivalent to the plant expressed protein (see Section 4.2). The degradation of the NPTII protein in the digestion fluid was assessed over time by Western blot analysis. Measurements of NPTII enzymatic activity were used as an additional means to monitor the degradation of NPTII. The simulated digestion fluids were prepared according to procedures outlined in the United States Pharmacopoeia (1990). The simulated gastric fluid contained 3.2g pepsin/L and 2.0g NaCl/L at pH 1.2 and the simulated intestinal fluid contained 10g pancreatin/L in phosphate buffer at pH 7.5.

NPTII was readily digested in simulated gastric fluid with no protein able to be detected by Western blotting after only ten seconds of incubation. In simulated intestinal fluid, NPTII was shown to have a half-life of between 2 and 5 minutes. The enzymatic activity of NPTII had been completely destroyed by two minutes in the simulated gastric fluid and by 15 minutes in the simulated intestinal fluid. These data correlate with the Western blot observations.

Conclusion

Both the Cry3Aa and the NPTII proteins are within the size range of known allergens, however, neither are glycosylated and both are rapidly degraded in the proteolytic and acid conditions of simulated gastric fluid suggesting neither would survive mammalian digestion. Neither of the proteins has any significant similarity to known allergens, nor are they present in large amounts in potato tubers. On the basis of this data and on the basis that humans have a prior history of exposure to these proteins with no recorded instances of allergenicity, it can be concluded that Cry3Aa and NPTII are unlikely to be allergenic to humans.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

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

In undertaking a compositional analysis of potatoes there are a number of key defining nutrients and constituents that should be measured as part of that analysis. They are: total tuber solids (measured as tuber dry matter); sugars; protein; and vitamin C. Tuber solids are an important quality factor for processing and are also the single most important determinant of culinary appeal (Murphy *et al* 1967). Approximately 75% of the dry matter content of potatoes consists of starch. The remainder is composed of sugars, protein, and assorted cell and cell wall components (Storey and Davies, 1992). The major sugars in potatoes are sucrose as well as the reducing sugars fructose and glucose. They are present in small quantities and are inconsequential as a source of energy. However, like total solids, they are a very important factor in processed food quality. Potatoes also contain measurable amounts of proteins, fats, carbohydrates, and numerous vitamins and minerals. However, they are only a significant dietary source for two of these constituents – protein and vitamin C (Storey and Davies 1992, Pennington and Wilkening 1997). Potato proteins are highly digestible, have a fairly good balance of amino acids and are especially high in the essential amino acid lysine. Measurement of total protein is considered more informative than measurement of individual amino acids as nearly all of the proteins in potato tubers (albumin, globulin, glutelin, and prolamin) have a similar amino acid composition, therefore, changes in their respective ratios will have little impact on the amino acid profile (Storey and Davies, 1992).

The applicant undertook compositional analyses of tubers from New Leaf® potato lines grown in a series of separate field trials across the United States.

Studies submitted by Monsanto:

Lavrik, P.B and Love, S.L. (1994). Composition and quality analysis of potato tubers derived from field-grown Colorado potato beetle resistant potato plants. Monsanto Study No. 92-01-37-19.

Dodson, H.C. *et al* (1998). Composition analysis of potato tubers from Colorado potato beetle resistant Atlantic potato plants grown under field conditions. Monsanto Study No. 98-01-37-01.

Dodson, H.C. *et al* (1998). Composition analysis of potato tubers from Colorado potato beetle resistant Superior potato plants grown under field conditions. Monsanto Study No. 98-01-37-12.

Lavrik, P.B. (1996). Proximate analysis of potato tubers from Colorado potato beetle resistant Russet Burbank potatoes grown under field conditions. Monsanto Study No. 96-02-37-23.

Dodson, H.C. (1998). Proximate analysis of potato tubers from Colorado potato beetle resistant Superior line SPBT02-05 potato plants grown under field conditions. Monsanto Study No. 98-01-37-13.

Dodson, H.C. (1998). Proximate analysis of potato tubers from Colorado potato beetle resistant Atlantic potato plants (lines ATBT04-06, ATBT04-31 and ATBT04-36) grown under field conditions. Monsanto Study No. 98-01-37-15.

Russet Burbank line BT-06

New Leaf® Russet Burbank line BT-06 and Russet Burbank non-transformed control plants were grown during the 1992 field season. The study was done it two parts. In Part I, tubers were grown in six locations using six replicated plots per line for two of the locations, and a single plot per line at the remaining four locations. Tubers harvested from these sites were analysed for total solids, sugars (dextrose and sucrose), vitamin C, and total protein. In part II, tubers were grown in two locations using six replicated plots per line at each site. Composite tuber samples from each of the two sites were analysed for proximate composition (total protein, fat, carbohydrate, total dietary fibre, calories and ash) and for minor constituents — thiamine (vitamin B_1), pyridoxine (vitamin B_6), folic acid, niacin (vitamin B_3), riboflavin (vitamin B_2), and minerals (calcium, copper, iodine, iron, magnesium, phosphorous, sodium, zinc and potassium).

The compositional data from all locations was combined. A summary of the data appears in Table 8 below.

Table 8: Compositional data[1] for Russet Burbank potato line BT-06

Constituent	Line BT-06	Control line	Normal range
values reported below are means, with ranges in brackets (n=6)			
Total solids (%)	20.5 (16.0-23.9)	19.9 (16.2-22.3)	16.8-24.5
Sugars:			
Dextrose (%FW)	0.18[#] (0.11-0.26)	0.15 (0.05-0.21)	0.04-0.52
Sucrose (%FW)	0.13 (0.03-0.20)	0.12 (0.06-0.18)	0.1-0.88
Vitamin C (mg/100g FW)	8.8 (6.2-11.7)	9.0 (5.9-12.3)	10.3-22.0
Protein (%DW)	4.9 (4.1-6.0)	5.2 (3.7-7.5)	3.4-7.3
values reported below are means of two analyses from two composite samples			
Proximate data:			
Protein (g/100g)	10.45, 10.8	10.10, 10.7	7.1-14.6
Moisture (g/100g)	4.55, 4.6	3.3, 5.2	Not available
Fat (g/100g)	0.3, 0.3	0.3, 0.25	0.2-0.8
Ash (g/100g)	4.35, 5.2	4.35, 5.25	2.2-9.5
Total dietary fibre (g/100g)	6.65, 7.5	6.25, 7.1	5-13
Carbohydrates (g/100g)	80.35, 79.1	81.95, 78.6	84.5 (avg)
Calories (kcal/100g)	366, 362.5	371, 359.5	350 (avg)
Minor constituents:			
Thiamine-HCl (mg/100g)	0.38, 0.42	0.35, 0.41	0.35-0.70
Pyridoxine-HCl (mg/100g)	0.42, 0.55	0.45, 0.53	0.7-1.4
Folic acid (mg/100g)	0.031, 0.023	0.044, 0.026	0.02-0.1
Niacin (mg/100g)	6.72, 8.95	6.58, 8.31	4-8
Riboflavin (mg/100g)	0.085, 0.135	0.080, 0.125	0.05-0.45

Calcium (mg/100g)	68.7, 58.9	64.85, 59.4	30-90
Copper (mg/100g)	0.38, 0.3	0.38, 0.67	0.4-1.0
Iron (mg/100g)	5.19, 517	5.24, 4.96	2.5-10
Iodine (µg/100g)	<10.0, <10.0	<10.0, 13.65	2-60
Magnesium (mg/100g)	87.2, 68.1	96.5, 67.1	60-140
Phosphorous (mg/100g)	91.6, 139.5	118, 149.5	150-300
Sodium (mg/100g)	18.4, 17.6	20.0, 15.8	4-26
Potassium (mg/100g)	1960, 2175	1965, 2190	1700-3000
Zinc (mg/100g)	0.98, 1.15	0.99, 1.20	0.6-2.4

[1] composition based on dry matter, except for moisture content
[#] statistically significantly different compared to the control

The level of dextrose in line BT-06 was slightly elevated compared to the control line, however, the difference is minor and the level reported is within the normal range for Russet Burbank cultivars. There were no significant differences between line BT-06 and the control line for any of the other analysed constituents and for the majority of constituents the levels reported were comparable to the normal ranges for Russet Burbank cultivars. The exceptions to this were for vitamin C, pyridoxine-HCl and phosphorous content, which were all low, compared to the literature reported values, for both the transformed and control lines.

Atlantic potato lines ATBT04-06, ATBT04-31, and ATBT04-36

The New Leaf® Atlantic potatoes and non-transformed Atlantic potatoes were grown during the summer of 1997 at three locations in the United States and Canada. Four replicate plots per line were grown at two of the sites, and eight replicated plots per line were grown at the remaining site. Tubers were collected at harvest and analysed for total solids, dextrose, sucrose, vitamin C and proximate composition (total protein, fat, crude fibre, ash, total carbohydrate and calories). A summary of the results of the compositional analyses is presented in Table 9 below.

Table 9: Compositional data[1] from Atlantic potato lines ATBT04-06, ATBT04-31, and ATBT04-36

Constituent	ATBT04-06	ATBT04-31	ATBT04-36	Control	Literature Range
Total solids (%FW)	24.6 (23.1-26.5)	24.6 (23.1-25.9)	23.3 (21.9-25.0)	24.0 (22.5-26.9)	22.0-26.8
Sugars:					
Dextrose (%FW)	0.31 (0.12-0.51)	0.35 (0.10-0.60)	0.37 (0.15-0.65)	0.35 (0.09-0.58)	0.03-0.14
Sucrose (%FW)	0.66 (0.34-1.02)	0.69 (0.35-1.14)	0.66 (0.38-1.01)	0.57 (0.39-0.70)	0.11-0.62
Vitamin C (mg/100 g FW)	8.3 (7.7-9.3)	9.4 (8.4-11.7)	8.9 (7.8-10.2)	8.3 (7.8-8.9)	15.4-19.4
Proximate data[2]:					
Protein	9.7 (8.2-10.7)	10.0 (9.5-10.9)	9.9 (8.6-10.4)	9.3 (8.6-10.2)	7.1-14.6
Fat	0.32 (0.15-0.40)	0.33 (0.27-0.44)	0.37 (0.29-0.46)	0.37 (0.26-0.45)	0.2-0.8
Ash	4.9 (4.1-5.6)	4.6 (3.7-5.4)	4.84 (4.1-5.5)	4.7 (3.9-5.4)	2.2-9.5
Crude Fibre	1.4 (1.3-1.6)	1.5 (1.3-1.7)	1.4 (1.3-1.5)	1.5 (1.1-1.7)	Not available
Carbohydrates	85.1 (84.0-86.1)	85.1 (84.1-86.3)	84.9 (84.2-85.6)	85.6 (84.5-86.4)	84.5 (avg)
Calories/100g	382 (379-386)	383 (380-387)	383 (379-385)	383 (379-386)	350 (avg)

[1] values reported as means, range in brackets (n=16)
[2] Except for calories, reported values are in g/100 g dry weight.

No significant differences in the key constituents were found between the Atlantic potato lines and the non-transformed control. With the exception of vitamin C, the values reported were comparable to the normal ranges for Atlantic cultivars. The levels of vitamin C were reduced compared to literature reported values in both the New Leaf® lines and the control lines.

Superior potato line SPBT02-05

New Leaf® Superior potato line SPBT02-05 and a control (non-transformed) Superior potato line were grown at eight locations in the United States and Canada during the summer of 1997. Tubers were grown in non-replicated plots at each of the eight sights. Tubers were collected at harvest from four areas of each plot at each site and analysed for total solids, sugars, vitamin C and proximate (total protein, fat, ash, crude fibre, total carbohydrates and calories). A summary of the results of the compositional analyses is presented in Table 10 below.

Table 10: Compositional data[1] from Superior potato line SPBT02-05

Constituent	SPBT02-05	Control line	Literature range
Total solids (%FW)	19.5 (16.6-23.8)	19.7 (18.0-21.7)	Not available
Sugars:			
Dextrose (%FW)	0.62 (0.30-0.98)	0.60 (0.51-0.72)	"
Sucrose (%FW)	0.23 (0.08-0.34)	0.20 (0.10-0.32)	
Vitamin C (mg/100 g FW)	8.42# (7.28-9.44)	9.33 (8.49-10.55)	"
Proximate data[2]:			
Total protein	10.9# (7.1-13.2)	12.0 (9.2-14.0)	7.1-14.6
Fat	0.55 (0.38-0.76)	0.51 (0.33-0.70)	0.2-0.8
Ash	4.98# (4.1-5.4)	4.70 (3.7-5.1)	2.2-9.5
Crude fibre	2.31 (1.67-2.92)	2.10 (1.77-2.43)	Not available
Total carbohydrate	83.6 (80.3-87.5)	82.8 (80.6-85.5)	84.5 (avg)
Calories	383 (381-387)	384 (382-388)	350 (avg)

[1] values reported as means, range in brackets (n=8)
[2] Except for calories, reported values are in g/100 g dry weight. Calories are reported in calories/100 g.
statistically significantly different compared to the control

Literature ranges for total solids, sugars and vitamin C are not available for the Superior cultivar of potato. The literature ranges for these constituents determined for other potato cultivars is presented in Table11 below.

Table 11: Literature ranges for major constituents of potato cultivars

Constituent	Atlantic	Gemchip	Norchip	Russet Burbank
Total solids	22.0-26.8	19.3-25.4	17.3-22.7	16.8-24.5
Sugars:				
Dextrose	0.03-0.14	0.08-0.28	0.03-0.39	0.04-0.52
Sucrose	0.11-0.62	0.05-0.44	0.09-0.64	0.10-0.88
Vitamin C	15.4-19.4	15.9-18.0	15.9-20.3	10.3-22.0

Significant differences between the New Leaf® Superior line and the non-transformed control line were noted for vitamin C, total protein and ash. The level of total protein and vitamin C in SPBT02-05 was slightly decreased compared to the control and the level of ash was slightly elevated. The slight differences in total protein and ash content in line SPBT02-05 compared to the control are not biologically significant and the values reported are within the literature reported ranges.

In relation to the differences in vitamin C content, this vitamin is reported to decrease during storage (Burton 1987), therefore, the low levels in both the transformed and control Superior lines may be explained if the tubers used for the analyses had been stored for prolonged periods. The New Leaf® and control Atlantic lines also exhibited decreased vitamin C levels compared to literature reported ranges (see above). An alternative explanation for the lower vitamin C content could be that the Superior cultivar naturally has lower levels of vitamin C compared to other potato cultivars. The applicant reports (data not provided) that in

reviewing its previous studies using the Superior cultivar, both the transformed and non-transformed controls typically produced low levels of vitamin C compared to other varieties. Therefore, it appears that lower vitamin C content could be a varietal characteristic of the Superior cultivar. The small but significant difference in vitamin C content between the New Leaf® Superior line and the Superior control does not pose a safety concern and, although potatoes are a significant source of vitamin C, the levels in line SPBT02-05 are still considered to be nutritionally adequate.

Conclusion

On the basis of the data provided, New Leaf® lines BT-06, ATBT04-06, ATBT04-31, and ATBT04-36 are compositionally equivalent to other commercial lines of potatoes. The New Leaf® Superior line had a slightly lower vitamin C content compared to its non-transformed control however the control line also had a vitamin C content that was significantly lower than the normal range seen in commercial potato varieties. Lower vitamin C content may be a characteristic of the Superior cultivar. The slightly reduced level of vitamin C observed in line SPBT02-05 is not a cause for concern and the levels of vitamin C in this line are considered to be nutritionally adequate.

5.2 Levels of anti-nutrients

The only known anti-nutrient present in potato is trypsin inhibitor. Trypsin inhibitors are classed as anti-nutrients because they interfere with the digestion of proteins leading to decreased animal growth. Trypsin inhibitors are heat labile and are destroyed during the cooking process or during processing when heat treatment is applied.

As trypsin inhibitor is inactivated by heating, its presence is only an issue when raw potatoes are consumed. Humans rarely consume raw potatoes due to the indigestibility of the ungelatinised starch.

5.3 Ability to support typical growth and well-being

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

In the case of the New Leaf® potatoes, the extent of the compositional and other data provided in the application is considered adequate to establish the safety of the food. Nonetheless, the applicant also provided two animal feeding studies to compare the wholesomeness of New Leaf® Russet Burbank line BT-06 with control lines. Although not considered essential for establishing safety in this instance, these animal feeding studies have been reviewed as additional supporting data.

Studies submitted by Monsanto:

Cambell, S.M. *et al* (1993). A dietary toxicity study with Russet Burbank potatoes in the Northern Bobwhite. Wildlife International Ltd Study No. 139-356.

Cambell, S.M. *et al* (1993). A dietary toxicity study with Russet Burbank potatoes in the Northern Bobwhite. Wildlife International Ltd Study No. 139-357.

Naylor, B.S. (1983). One month feeding study with CPB (Colorado potato beetle) control potatoes in Sprague-Dawley rats. Monsanto Study No. 92209.

The applicant also submitted four other feeding studies using insect-protected Shepody potatoes. These additional studies were not considered in this assessment because the genetically modified Shepody potato lines are not the subject of this application therefore this data is not relevant to confirming the safety of the New Leaf® potatoes.

Bird feeding study

Potato tubers were obtained from 1992 field trials, conducted with Russet Burbank New Leaf® line BT-06 and a non-transformed Russet Burbank line, in the United States.

Ten-day-old northern bobwhite quail chicks (30/group of mixed sex) were randomly assigned to either the New Leaf® line or the parental control line. The chicks were fed dietary concentrations of 50,000 ppm (5% w/w) lyophilised potato powder mixed into a basal ration. The applicant has calculated that this is equivalent to approximately 90 g potatoes/kg body weight. In addition to the parental control line, a group of 30 chicks were also fed the basal ration without any potato supplementation. Each group was fed the diet for 5 days and then switched to basal diets for the last 3 days of the study. Food consumption was recorded for each pen, with food and water provided *ad libitum*. Individual body weights were recorded at the commencement of the study, on study day 5 and at study termination. Birds were observed twice daily for mortality or signs of toxicity.

Results

No mortality and no differences in food consumption or body weight gains between the treated and control groups were observed. One bird in the negative control group (basal diet only) was observed as nostril picked on day 6 and one bird in the Russet Burbank (control) group (fed parental line of potatoes) was noted as toe picked on day 8. Two birds fed New Leaf® line BT-06 were observed to be toe picked on days 4 and 5. All other birds were reported as appearing normal.

Conclusion

No treatment related mortality or differences in food consumption, body weight gain or behaviour occurred between birds fed 50 000ppm lyophilised New Leaf® Russet Burbank potato powder in the diet and birds fed the same level of Russet Burbank control potatoes or birds fed basal diets. New Leaf® Russet Burbank line BT-06 is equivalent to Russet Burbank control potatoes in its ability to support the typical growth and well-being of bobwhite quail chicks over the time period measured.

Rat feeding study

The origin of the potato tubers used in this study is not clearly specified in the report provided but appear to originate from the 1992 field trial of the New Leaf® Russet Burbank potatoes (line BT-06).

Six-week-old Sprague-Dawley rats (10/sex) were each given one fresh raw potato approximately every 2-3 days of the study for one month. It is not possible to calculate the dose/kg body weight from the information provided. The control was potatoes from the non-transformed Russet Burbank line. The form in which potato was fed to the rats is not apparent from the data provided in the study. Rodent Chow was continuously available to the test and control groups. Clinical observations and determinations of body weights and food consumption were done. All animals were necropsied at the termination of the study (days 29-31). Liver, kidneys and testes were weighed and tissues retained.

Results

Consumption of potatoes (both control and test group) was slightly reduced for the first 3 days of the study, however, both group's potato consumption increased rapidly and remained high for the rest of the study. Consumption of Rodent Chow was similar for both the test and control group. Cumulative weight gains were normal and equivalent to the control group. No adverse clinical signs were observed. Gross pathology revealed a number of abnormal findings, such as enlarged lymph nodes, hydronephrosis and enlarged adrenals, however, these findings were observed in both the test and control groups and could not be related to the test substance. There were no significant differences in absolute or relative organ weights of the kidney, liver or testes for the test group when compared to the control.

Conclusion

There are no significant differences in the measured parameters between control animals fed the parent line of Russet Burbank potatoes and those fed Russet Burbank line BT-06. Therefore, Russet Burbank line BT-06 is equivalent to control Russet Burbank potato lines in its ability to support typical growth and well-being.

6. OTHER ISSUES

6.1 Estimation of dietary intake of novel proteins

If the concentration of a substance in a food is known and data is available on the human consumption of that food then it is possible to estimate the dietary intake of that substance for the population. In safety assessments, dietary intakes are usually only estimated in circumstances where a substance is considered to be hazardous. In this way it is possible to determine the likely human exposure to the hazard and thus ascertain whether there is cause for concern.

The two novel proteins expressed in the New Leaf® potatoes are not considered to be hazardous therefore a dietary exposure assessment is unnecessary for determining if there is cause for concern. However, such information can provide additional assurance that exposure to the novel protein is low and/or that the novel protein is likely to be present in the diet at levels well below those which have been found to be safe in animal toxicity studies.

The concentration of Cry3Aa and NPTII in the New Leaf® Plus potatoes is known, therefore it is possible to estimate their dietary intake.

Cry3Aa is expressed in New Leaf® potato tubers at levels ranging from 0.05 to 1.29 µg protein/g fresh weight and NPTII is expressed at levels ranging from 0.01 to 3.82 µg protein/g fresh weight (see Table 6a-c, Section 3.3).

Australian and New Zealand consumption data is available for potato crisps, instant mashed potato, and potato fries, although no data is currently available for potato flour and potato starch. The consumption data is presented in Table 13 below.

Table 13: Estimated consumption of processed potato products in Australia and New Zealand.

		All respondents (g/day)	Consumers only (g/day)		
Food	**Country**	**mean**	**mean**	**median**	**95th percentile**
Potato crisps	Aus	2.8	38.8	25	100
	NZ	2.9	48.4	40	150
Instant mashed	Aus	-	-	-	-
potato	NZ	0.007	34.6	34.6	34.6
Potato fries,	Aus	16.6	132.5	113	264
commercial	NZ	18.6	141.2	142	300
Total potato	Aus	19.4	-	-	-
products	NZ	21.5	118	112.2	300

For calculation of the dietary intake of the novel proteins, the highest potato consumption figure (300 g/day) and the highest protein concentration was used. This represents a 'worst case' estimate and also makes allowances for the lack of consumption data for potato flour and potato starch.

To do the calculation, assumptions about the proportion of processed potato products derived from the New Leaf® potatoes must be made. In 1998, the New Leaf® potatoes comprised approximately 4% of the United States potato acreage (USEPA/USDA 1999) although this has since declined to less than 1% as this variety has been superseded by other New Leaf® varieties. Therefore it is possible to make two dietary intake estimates — one using a very worst case estimate where it is assumed that all potato products on the market are derived entirely from New Leaf® potatoes and the other, more realistic estimate, where it is assumed

that 10% of potato products are derived from New Leaf® potatoes. The two estimates of dietary intake for Cry3Aa and NPTII are presented in Table 14 below.

Table 14: Estimate of dietary intake of Cry3Aa and NPTII

Novel protein	Estimated dietary intake			
	100 % market penetration		10 % market penetration	
	µg /day	µg/kg BW/day[1]	µg /day	µg/kg BW/day[1]
Cry3Aa (0.05-1.29µg/g FW)	15-387	0.23-5.95	1.5-38.7	0.02-0.60
NPTII (0.01-3.82 µg/g FW)	3-1146	0.046-17.6	0.3-114.6	0.005-1.76

[1] assuming a body weight of 65 kg.

For Cry3Aa, the very worst case estimated intake is at least 0.9 million times less than the dose found to have no adverse effects in mice (5220 mg Cry3Aa/kg BW). For NPTII, the estimated dietary intake is at least 0.3 million times less than the dose found to have no adverse effects in mice (5000 mg NPTII/kg BW). Therefore, even if all processed potato products were to be derived from the New Leaf® potatoes, an incredibly large margin of safety exists for both proteins.

ACKNOWLEDGEMENTS

ANZFA gratefully acknowledges the expert comments on the safety assessment of food derived from insect and PLRV-protected potato lines RBMT21-129, RBMT21-350 and RBMT22-82 provided by Associate Professor Richard T. Roush, Centre for Weed Management Systems, Waite Institute, University of Adelaide, Glen Osmond 5064, and Professor Tony Connor, New Zealand Institute for Crop and Food Research Ltd and Soil, Plant and Ecological Sciences Division, Lincoln University, New Zealand.

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ATTACHMENT 3

DRAFT REGULATORY IMPACT ASSESSMENT

Regulatory Impact Assessment

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

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

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

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • no benefits were identified.	**Costs** • the governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • a prohibition on food produced using gene technology in Australia and New Zealand could result in retaliatory trade measures from other countries. • there may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	**Costs** • food manufacturers and producers will be unable to use the processed food fractions from foods produced using gene technology thus requiring the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be $A 207m in Australia and $NZ 37m in New Zealand[5]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

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

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

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

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

Conclusion of the regulatory impact assessment

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

ATTACHMENT 4

WORLD TRADE ORGANIZATION AGREEMENTS

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

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

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

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

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

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

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

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

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

SPS Notifications

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

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

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

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

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

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

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

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

TBT Notifications

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

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

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

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

ATTACHMENT 5

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS FOR APPLICATIONS A372, A375, A378, A379, A380, A381, A382, A383, A384, A385, A386, A387 & A388

1. **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.
2. **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.
3. **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.
4. **Australian GeneEthics Network**
   - believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
     - direct health effects of pesticide residues
     - possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
     - the possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
     - insertion of viral DNA could create new and virulent viruses
     - the possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
     - the threat to GE-free status export markets

- believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

5. **Public and Environmental Health Service (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, anti-nutritional factors, natural toxicants, and define when a change would be considered 'significant'
   - potential effect of introduced proteins on metabolic pathways should be addressed e.g. over-expression or inhibition of enzymes
   - data should include details of whether introduced proteins are detectable in whole commodities, processed products and highly processed derivatives
   - data should include details of toxicity and allergenicity tests to prove that food is safe, as well as address issues of specificity and potency of proteins. It should also address the ability to support typical growth and well-being
   - data for herbicide-tolerant plants should be derived from studies performed on plants treated with herbicide. They should address the human toxicity of the herbicide and whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.
6. **David Grundy (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
7. **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.
8. **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

9. New Zealand Ministry of Health

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

10. Nestle Australia Ltd.

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

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

- believe that current testing procedure is inadequate and that human trials are the only adequate method, as with testing of new drugs. Also that physiological and neurological effects as well as the toxicological and allergenic effects should be looked at, and that an independent body should be responsible for testing
- do not support the use of antibiotic markers, since they believe they may pose a threat to efficacy of antibiotics in humans
- state that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans confirming the inadequacy of the term 'substantial equivalence'
- raise the point that although these crops have been approved elsewhere, this situation may change with consumer pressure
- do not accept that it is impossible to source food to ascertain whether or not it contains GM ingredients. Believe that if McCain and Sanitarium can do it, then others should also be able to
- state general concern about the risk that MRLs will be raised as a result of herbicide-tolerant crops being developed, and feel that the calculations used are flawed and are not based on safety criteria
- believe that the use of GM crops in animal feed should also be regulated. A378
- state concern over possible increase in glyphosate use (it is apparently confirmed in one reference that herbicide use increases with herbicide resistant crops), referring to studies that link the chemical to Hodgkin's lymphoma, and the possibility that Europe may ban it due to adverse effects on beneficial insects**.** They are particularly concerned that glyphosate is not looked at by the same regulatory body as that looking at GM foods

A379, A388

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

A372, A375, A380, A381, A386

- with respect to glufosinate ammonium, state concern about toxicity, neurotoxicity, teratogenicity and residues in food, soil and water. They believe that Monsanto is

likely to apply for an increase in the MRL, and that such increases are likely to constitute a health hazard

A380, A382, A383, A384, A385, A386

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

A387

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

12. Health Department of Western Australia

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

13. Meat New Zealand

A379

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

14. BRI Australia

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

15. Food Technology Association of Victoria Inc.

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

16. Diane Davie (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

17. Martin Hurley, David Hook, Ian Smillie, Margaret Dawson, Tee Rodgers-Hayden, David Lovell-Smith (Natural Law Party), Barbara Brown, Ngaire Mason, Robert Anderson (member, Physicians and Scientists for Responsible Genetics), Louise Carroll, Gilbert Urquart, Caroline Allinson-Dunn, Megan Lewis, Peter Barnes, James Harlow, Gabrielle Dewan, Scott Young, Virginia Murray, Stephanie Chambers, Kay Dyson, Peter Fenwick, Joanne Xerri, Paul True, Josh Gill, James & Peysha Charlwood, Mitta Hirsch, Alan Florence, Nicole Paul, Lawrence Clarke, David Snowman, Reg Paling, Mark and Johanna Blows, David and Bev 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.

18. Richard and Sharon Moreham (see also above)

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

19. Vicky Solah (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.

20. 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.

21. Nicola Roil (Aus)

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

22. Ian and Fran Fergusson (Aus) – also wrote in the big lot above

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

23. 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.

24. Fay Andary (Aus)

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

25. John and Francesca Irving (Aus)

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

26. 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.

27. Sheila Annesley (Aus)

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

28. David and Edwina Ross (Aus)

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

29. Beth Schurr (Aus)

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

30. Beth Eager (Aus)

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

31. Bruce Pont and Ljiljiana Kuzic-Pont (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.

32. 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.

33. 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

34. 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.

35. 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

36. 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:
    (i) increased use of chemicals
    (ii) destruction of soil life
  - organic farming pay high costs for producing healthy plants, while conventional farmers have little restriction on pollution of air, soil and water. Salinity problems, the death of the Great Barrier Reef, rivers and streams has resulted from ignorance in farming and broader community. Such problems will increase with GM foods.
  - the implication that the public will not understand the issues is wrong. Everyone needs to be fully informed.
- asks the question of whether workers in the food industry are to be better informed, and also why no 'verification documents' are to be required by retailers? Believes that certification schemes should be on a par with those for Kosher foods and organics

37. John and Sandy Price (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.

38. 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

39. 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.

40. National Council of Women of New Zealand

- believes that:
  - approval of all 13 applications should be rejected, and that none should be approved for planting.
  - independently-funded body should be responsible for safety assessments

- if it is possible to segregate high-oleic soybeans, then RoundUp Ready soybeans should be segregated too
- consumers should be made aware of the extent of GM ingredients in their food
- GM foods, additives or processing aids already on the market must be labelled comprehensively and without extra cost to the consumer – suggest 'GM unknown' rather than 'may contain'

▪ appreciates that rejection may contravene the WHO agreement, but consider that the primary role of ANZFA is the assurance of health and safety

41. 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.

42. 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)

43. 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

44. 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.

45. 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 and asserted that food produced using this technology is unsafe for human. A number of general issues were raised in these submissions and are addressed below.

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

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

- *Evaluation*

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

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

The safety assessment focuses on the new gene product(s), including intentional and unintentional effects of the genetic modification, its properties including potential allergenicity, toxicity, compositional differences in the food and 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. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

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

If there is a need to examine the safety of a newly-expressed protein in a genetically-modified food, it is more appropriate to examine the safety of this protein alone in an animal study rather than when it is part of a whole food. For newly-expressed proteins in genetically-modified foods, the acute toxicity is normally examined in experimental animals. In some 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 when consumed as part of a food. Such experiments can provide more meaningful information than experiments on the whole food. Additional re-assurance regarding the safety of newly-expressed protein can be obtained by examining the digestibility of the new protein in *in-vitro* assays using conditions which simulate the human gastric system.

3. *Substantial equivalence*

A number of submitters expressed concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some rejected the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

- *Evaluation*

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

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

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

The concept of substantial equivalence was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*.'

The concept has been internationally recognised and embraced as a valuable tool in the safety assessment of foods produced using gene technology. The OECD also advocates an approach to safety assessment based on substantial equivalence as being '*the most practical to address the safety of foods and food components derived through modern biotechnology*.'

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

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

- *Evaluation*

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

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

5. *Potential toxins and allergens*

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

- *Evaluation*

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

6. *Antibiotic resistance*

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

- *Evaluation*

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

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

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

environment for positive selection. The majority of these genes provide for resistance to antibiotics whose use is confined to the laboratory and are not considered to be of major therapeutic use in humans.

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

7. Transfer of novel genes

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

- *Evaluation*

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

8. *Viral recombination*

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

- *Evaluation*

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

will be addressed by the Genetic Manipulation Advisory Committee (GMAC) on a case–by–case basis when it assesses such plants.

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

9. *Labelling of foods produced using gene technology*

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

- *Evaluation*

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

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

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

- *Evaluation*

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

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

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

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

11. *Public consultation and information about gene technology*

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

- *Evaluation*

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

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

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

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

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

12. *Maori beliefs and values*

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

- *Evaluation*

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

13. *Environmental concerns and the broader regulatory framework*

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

- *Evaluation*

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

derived did not have the appropriate clearance for general release from either GMAC (or its successor) or ERMA, as appropriate.

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

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

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

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

In Australia a new Office of the Gene Technology Regulator (OGTR) will complement the existing arrangements. OGTR will supersede the existing arrangements under the Genetic Manipulation Advisory Committee (GMAC), which advises on research and environmental release of GMOs. OGTR will regulate all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

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

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

14. Maximum residue levels of agriculture/veterinary chemicals

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

- *Response*

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

products have to meet the MRL, whether or not they are derived from genetically modified organisms. The MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

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