05/02
10 October 2001

FINAL ASSESSMENT REPORT
(INQUIRY - SECTION 17)

APPLICATION A372

OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT CANOLA LINES TOPAS 19/2 AND T45 AND OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA LINES MS1, MS8, RF1, RF2 AND RF3.

TABLE OF CONTENTS

EXECUTIVE SUMMARY ... *3*
BACKGROUND ... 3
ISSUES ADDRESSED DURING ASSESSMENT ... 3
CONCLUSIONS ... 4
1. BACKGROUND TO THE APPLICATION ... *5*
2. PUBLIC CONSULTATION ... *5*
3. NOTIFICATION OF THE WORLD TRADE ORGANIZATION ... *6*
4. ISSUES ADDRESSED DURING THE ASSESSMENT ... *6*
4.1 SAFETY ASSESSMENT ... 6
4.2 LABELLING OF OIL DERIVED FROM LIBERTYLINK® AND INVIGOR® CANOLA ... 6
4.3 ISSUES ARISING FROM PUBLIC SUBMISSIONS ... 7
4.4 RISK MANAGEMENT ... 14
4.5 REGULATORY IMPACT ASSESSMENT ... 15
5. CONCLUSIONS ... *15*
ATTACHMENT 1: DRAFT VARIATION TO THE FOOD STANDARDS CODE ... *18*
ATTACHMENT 2: SAFETY ASSESSMENT REPORT ... *19*
ATTACHMENT 3: REGULATORY IMPACT ASSESSMENT ... *56*
ATTACHMENT 4: WORLD TRADE ORGANIZATION AGREEMENTS ... *58*
ATTACHMENT 5: SUMMARY OF PUBLIC SUBMISSIONS ... *61*
ATTACHMENT 6: GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS ... *77*
ATTACHMENT 7: STATEMENT OF REASONS ... *87*

EXECUTIVE SUMMARY

Background

An application was received from Aventis CropScience Pty Ltd on 12 March 1999 seeking approval for oil derived from the seeds of genetically modified (GM) canola plants. Two lines of open pollinated canola have been engineered for tolerance to the herbicide glufosinate ammonium. Five lines of canola have been engineered for use in hybrid seed production by the addition of genes controlling pollen production in addition to the gene conferring tolerance to glufosinate-ammonium. In North America, the canola is known commercially as Liberty Link® open pollinated and InVigor® hybrid canola. These genetically modified canola lines are currently not grown in either Australia or New Zealand but oil from these lines may be present in imported processed foods.

Issues addressed during assessment

(i) *Safety evaluation*

Oil derived from the seeds of glufosinate-ammonium tolerant and pollination controlled canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 has been evaluated according to the safety assessment guidelines prepared by ANZFA. The assessment considered the following issues: (1) the nature of the genetic modifications; (2) general safety issues such as novel protein expression and the potential for transfer of antibiotic resistance genes to microorganisms in the human digestive tract; (3) toxicological issues; and (4) nutritional issues.

On the basis of the available information, it is concluded that oil derived from these genetically modified canola lines is as safe and wholesome as oil from other commercial canola varieties. A detailed food safety report on these foods has been prepared.

(ii) *Labelling information for consumers*

Under the current Standard A18, which remains in effect until 7 December 2001, oil derived from glufosinate-ammonium and pollination controlled canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 does not require labelling as it is regarded as substantially equivalent to oil from non-genetically modified canola varieties.

When the amended Standard comes into effect on 7 December 2001, food products containing canola oil derived from any of these genetically modified lines will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

(iii) *Public consultation*

ANZFA undertook two rounds of public consultation in relation to this application and a total of 68 submissions were received overall – 45 submissions in the first round and 23 submissions in the second round. The majority of submissions were not supportive. Those opposing the application did so primarily on the basis that they perceive GM food to be unsafe or environmentally unsound. The food safety concerns raised in submissions have been addressed by the safety assessment report.

Conclusions

- There are no public health and safety concerns associated with the genes introduced into glufosinate-ammonium tolerant and pollination controlled canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3.

- Oil derived from genetically modified canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 is as safe and wholesome as that from other commercially available canola varieties.

- From 7 December 2001, food products containing oil from genetically modified (glufosinate-ammonium tolerant and pollination controlled) canola will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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

1. BACKGROUND TO THE APPLICATION

Canola (*Brassica napus*) has been genetically engineered for tolerance to the herbicide glufosinate-ammonium and for the production of higher yielding fertile hybrids. In this application, seven glufosinate-ammonium tolerant canola lines (Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3) have been generated. Two of these, Topas 19/2 and T45, are open pollinated canola lines known commercially as LibertyLink® canola. The Ms and Rf lines are pollination-controlled parental breeding lines for use in hybrid production and are known commercially as InVigor® hybrid canola.

All seven lines are tolerant to the broad-spectrum herbicide glufosinate-ammonium, the active constituent of the proprietary herbicides Basta, Finale, Buster, Harvest and Liberty. Tolerance to glufosinate-ammonium (also known as phosphinothricin) is conferred by the transfer of one of two bacterial genes – either *bar* or *pat*. These genes produce the enzyme phosphinothricin acetyl transferase (PAT), which breaks down phosphinothricin into an inactive form, allowing the modified plants to tolerate application of the herbicide. The *bar* gene is derived from the soil bacterium *Streptomyces hygroscopicus* and the *pat* gene is derived from a closely related species *Streptomyces viridochromogenes*.

In addition to *bar* or *pat*, the male sterile (Ms) lines contain the *barnase* gene derived from the bacterium *Bacillus amyloliquefaciens*. The presence of this gene in the plant results in abnormal development of the parts of the flower that produce pollen. The fertility restorer (Rf) lines express the *barstar* gene (derived from the same bacterial species), which counteracts the effects of the barnase. The presence of the barstar gene has no direct effect on the plants and is only evident when an Rf line is crossed with one of the Ms lines to produce hybrid plants in which both genes are expressed at the same stage in development. These plants exhibit greater vigour than either of the parental lines and are fully fertile yielding greater amounts of seed.

Canola seed, a genetic variation of rape-seed, was first developed in Canada through traditional plant breeding techniques that specifically aimed to maximise nutritional value. The seeds are crushed to obtain canola oil for human consumption, while the remainder is processed into canola meal, which is used as a high-protein livestock feed. Canola oil contains the lowest level of saturated fatty acids of any vegetable oil, and is used in table spreads and for cooking purposes. At present, the key markets for canola production are Canada and the US, however, because of export food markets, oil produced from genetically modified canola may enter the Australian and New Zealand market in imported processed food products.

The direct benefits of the new genetic modifications outlined in this application are likely to accrue mainly to the primary producer by way of increased choice of commercially available, higher yielding varieties of canola. More general benefits may also flow to the community as a result of reduced primary production costs.

2. PUBLIC CONSULTATION

ANZFA completed an Initial Assessment (formerly referred to as the Preliminary Assessment Report) upon receipt of the application and invited submissions from the public between 3 November 1999 and 12 January 2000. A total of 45 submissions were subsequently received and a summary of these is included in this report at Attachment 5.

ANZFA subsequently conducted an assessment of the application, including a safety evaluation of the food, taking into account the comments received. A Draft Assessment Report (formerly termed a Full Assessment Report) of this assessment was released for public comment on 7 March 2001 until 20 April 2001, resulting in a further 23 submissions being received.

This report, known as the Final Assessment Report, finalises the assessment by ANZFA and again takes into account comments received from the public. ANZFA's recommendation will then be transmitted to the Ministerial Council for consideration. Attachment 5 contains a summary of all submissions received.

3. NOTIFICATION OF THE WORLD TRADE ORGANIZATION

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

As there is significant international interest in the safety of GM foods, the proposed amendments are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters and were therefore notified to the WTO.

4. ISSUES ADDRESSED DURING THE ASSESSMENT

4.1 Safety assessment

Edible oil from the glufosinate-ammonium tolerant and pollination-controlled canola lines have been evaluated according to the safety assessment guidelines prepared by ANZFA[1]. The assessment 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. On the basis of the available information, ANZFA concluded that oil from the seven lines of herbicide-tolerant and pollination-controlled canola is as safe and wholesome as oil from other commercial varieties of canola. The full safety assessment report can be found at Attachment 2 to this document.

4.2 Labelling of oil derived from LibertyLink® and InVigor® canola

On 28 July 2000 the Australia New Zealand Food Standards Council agreed to a revised standard which requires labelling of food where novel DNA and/or protein is present in the final food and also where the food has altered characteristics. The revised standard (A18 in Volume 1 of the *Food Standards Code*, 1.5.2 in Volume 2 of the *Food Standards Code*) was gazetted on 7 December 2000 and will come into effect 12 months from the date of gazettal.

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

Until the new labelling requirements take effect in December 2001, the provisions in the original Standard A18 apply. Under these provisions, oil derived from the seven genetically modified canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 does not require labelling as it is regarded as substantially equivalent to oil derived from non-genetically modified canola varieties.

4.3 Issues arising from public submissions

General issues

Many of the submissions received in both the first and second rounds of public comment raised matters 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 more general issues raised can be found in Attachment 6.

However, in light of the rapid developments in this field, some general issues raised in the second round of public consultation have been addressed again taking into account more recent outcomes of intensive deliberations on gene technology issues, such as the publishing of the report of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on "New Biotechnology Food and Crops: Science, Safety and Society", and the deliberations of various Codex Alimentarius and OECD taskforces and FAO/WHO Expert Consultations.

1. *ANZFA's processes*

Several criticisms of ANZFA's general processes for the risk assessment of GM foods were raised by submitters including: the Public Health Association of Australia (PHAA), the GeneEthics Network, the National Council of Women of Australia (NCWA), Consumer's Institute of New Zealand, GE Free New Zealand, Paul Elwell-Sutton, Sandra Jacobs, Brian Lister and Lorraine Leader, Claire Bleakely, Julian Yates, Oraina Jones, Leila Huebner and Dr Kate Clinch-Jones.

<u>Response</u>

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

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

2. Substantial equivalence

Several submitters (PHAA, GeneEthics, Dr Kate Clinch-Jones, Consumer's Institute of New Zealand) raised concerns with the use of the concept of substantial equivalence

Response

On the issues of the appropriate use of the concept of substantial equivalence, ANZFA reiterates that it uses this tool as a starting point in the safety assessment process for GM foods as supported by international bodies such as Codex Alimentarius, OECD, FAO/WHO, other regulators such as the UK, the EU, Japan, Canada and the recent report of the Canadian Royal Society.

3. *Antibiotic resistance marker genes*

Several submitters (PHAA, GeneEthics Network, Dr Kate Clinch-Jones) raised some concerns about the use of antibiotic resistance marker genes (ARMGs) in the development of GM foods. In particular, the PHAA submission asserts that ANZFA is "remarkably out-of-step with scientific opinion…" and quotes the JETACAR Report as evidence of this.

Response

The JETACAR Report states (page 117 referring to a specific gene called *nptII*) that the use of antibiotic resistance genes in GM foods is unlikely to contribute in any significant way to the spread of antibiotic resistance in humans. The issue of the use of antibiotic resistance marker genes in GM foods was discussed at the recent Ministerial Council meeting held in Adelaide in late July 200. At that meeting, Professor John Turnidge, former Chair of JETACAR and now Chair of the NHMRC Expert Advisory Group on Antibiotic Resistance (EAGAR) appeared at the Council meeting to present his expert advice on the safety of the use of ARMGs in GM foods in support of ANZFA's views on this issue.

4. *Source of data*

Some submitters (PHAA, GeneEthics) raised concerns over the independence of the source of the data submitted to ANZFA.

Response

It is a requirement of the ANZFA assessment process that raw data from experiments supporting the safety of a GM food are submitted to ANZFA for assessment. These data are assessed in detail by ANZFA scientists and then the assessment report undergoes a robust process of internal review by ANZFA's own scientific experts and external review by ANZFA's expert panel and senior health officials from State and Territory and New Zealand Health Departments. The quality and sources of the data supplied to ANZFA in support of applications for approval of GM foods was the subject of particularly intense scrutiny during ANZFA's evidence at the New Zealand Royal Commission on Genetic Modification. ANZFA submitted a full data package (15 volumes of raw data on Roundup Ready Soybeans) to the Commission for inspection.

The Commission states that it looked closely at the quality of this data and came to the view that ANZFA did receive and assess raw data and that its processes were not wanting in this regard.

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

5. *Imported GM foods versus GM crops*

Some submitters (GeneEthics Network, National Council of Women of Australia) have argued that approvals for GM foods for import, is a tacit approval for the GM crop to be grown in Australia.

Response

The regulatory framework for approval by ANZFA of safety of GM foods (imported foods and derived from GM crops grown in Australia) is separate from that of the Office of the Gene Technology Regulator (OGTR), which has responsibility for approving the environmental release of GM crops. ANZFA's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard A18 of the Food Standards Code (Standard 1.5.2 of the joint Australia New Zealand Food Standards Code) is not, and would never be, a tacit approval for the environmental release of the crop in Australia since the environmental issues are completely separate and entirely different to food safety issues.

6. *Compositional studies*

The PHAA commented that some of the components of the genetically modified plant line under assessment were statistically different to the control line and that therefore the GM line is not comparable to the control line.

Response

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

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

Issues raised in first round of public comment, which were previously discussed in the Draft Assessment Report (see Attachment 5 for summary)

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

(i) Use of the herbicide glufosinate-ammonium

Several submitters including the Consumers' Association of South Australia Inc. and the National Council of Women of Australia raise the issue of herbicide toxicity and contend that the use of glufosinate-ammonium tolerant canola may lead to increased use of the herbicide on the crop, which in turn may necessitate an increase in the Maximum Residue Limit (MRL) for glufosinate-ammonium.

Response

The Australian Food Standards Code (Standard A14 – Maximum Residue Limits) lists the maximum allowable limits for agricultural and veterinary chemical residues present in food. The herbicide glufosinate-ammonium is permitted at particular levels in a range of foods, which are listed in Schedule 1, however there is no listing for vegetable oils *per se* or canola oil in particular. Accordingly, currently there must be no detectable residue of glufosinate-ammonium in the oil from any canola crop, whether the crop is genetically modified or conventionally grown.

The process by which an MRL is set for a herbicide, such as glufosinate-ammonium, is discussed more fully in Attachment 6 – General issues raised in public comments, at discussion point 14. From this information, it can be seen that the permitted level of residue is therefore dependent on the type of food under assessment and is based on a raft of scientific information including traditional toxicological studies, agricultural use patterns and food consumption patterns.

In relation to the herbicide glufosinate-ammonium, extensive toxicological analysis in laboratory animals (rats, mice and dogs) has been assessed, including studies on the plant metabolite N-acetyl-glufosinate. The mechanism of action of glufosinate-ammonium is to inhibit the enzyme glutamine synthetase, a key enzyme involved in the metabolism of nitrogen in plants.

The result of the inhibition is an over-accumulation of ammonia in the plant leading to cell death. In contrast, animals are not dependent on glutamine synthetase activity to achieve homeostatic control of ammonia but possess alternative metabolic pathways. This biochemical difference between plants and animals reduces the degree of toxicity of glufosinate-ammonium in animals. In addition, N-acetyl-glufosinate demonstrates low toxicity after repeated oral administration to mice, rats or dogs and is not carcinogenic at the highest doses tested (equal to 1200 mg/kg bw per day in mice and 1000 mg/kg bw per day in rats), nor is it genotoxic or teratogenic.

The available evidence indicates that exposure to glufosinate-ammonium under normal conditions of use does not present a significant health risk to humans. Toxicity assessments on glufosinate-ammonium, N-acetyl-glufosinate and another plant metabolite[2] indicate that the toxicity of the metabolites was comparable to or less than that of the parent compound, and that all were considered of low acute toxicity. In monitoring the effects of human exposure, there were no adverse findings reported in workers in glufosinate-ammonium production plants.

Issues raised in second round of public comment (see Attachment 5 for summary)

(i) Properties of the barnase gene

The Public Health Association of Australia as well as Sandra Jacobs (NZ) have submitted comments in relation to the safety assessment of this application that specifically relate to the toxicity of the herbicide, to the nature of the ribonuclease expressed in the parental Ms lines, and to the relevance of the animal feeding studies. For clarification of the first issue, ANZFA's discussion on the assessment of toxicity of the herbicide is reproduced above. Discussion of the issue relating to animal feeding studies is included in Attachment 6. Information on the ribonucleases is presented below.

Response

Ribonucleases are enzyme proteins that chemically digest ribonucleic acid (RNA) and are ubiquitous in nature, including in animal and plant tissues eaten as foods. The precise mode of action of any particular ribonuclease within this class of proteins varies somewhat according to its biological setting, but many individual ribonuclease enzymes from vertebrate, bacterial and fungal sources have been very well characterised at the molecular and biochemical level. Although within the enzyme class, ribonucleases may be either exonucleases or endonucleases depending on their specific activity, all hydrolyse RNA molecules usually *in vitro* as well as *in vivo* and therefore, when present in a cell, may disrupt biochemical processes leading to the death of the cell (Concise Encyclopaedia Biochemistry and Molecular Biology, 3rd Edition, Walter de Gruyter & Co. 1997). Particular ribonucleases (for example from chicken liver, bovine pancreas, *Physarum polycephalum* or *Aspergillus oryzae*) are used widely in the laboratory as tools for manipulation of nucleic acids (DNA and RNA).

[2] 3-[hydroxy(methyl)phosphinoyl]propionic acid

The genes encoding ribonucleases are also widely distributed in nature and are therefore commonly digested in foods from a variety of sources. In this case, the source of the bacterial ribonuclease gene, *barnase*, is the organism *Bacillus amyloliquefaciens* which is a dominant source of industrial α-amylase production and accounts, together with *B. licheniformes*, for much of the total world output of industrial enzymes. *B. amyloliquefaciens* has no known pathogenicity and can be used in the brewing, bread-making and food industry as a whole.

The specificity of the expression of the *barnase* gene in the transformed canola, outlined in the Draft Risk Analysis Report, is convincingly described in the published scientific literature (Roth, 1962; Smeaton *et al*., 1967; Mauguen *et al*., 1982; Hartley *et al*., 1973; Hill *et al*., 1983; Hartley 1989; Mariani *et al*., 1990; Yen *et al*., 1991; Mariani *et al*., 1992; Guillet *et al*., 1993). From its obvious phenotypic effects, the expression of the *barnase* gene in the plant can be precisely monitored. In the transformed canola plants, linked to the anther specific promoter, *barnase* disrupts pollen production by destroying a specific layer of cells in the developing flower. This is completely reversible by crossing a plant expressing barnase (Ms) with a plant expressing the barstar gene (Rf), encoding a protein that inactivates it. There is no toxicity associated with the presence of the gene in the canola plants and the protein encoded by the gene only affects those cells in the flower of the plant where it is present.

In the event that the barnase protein was ingested, the digestibility studies clearly demonstrate that it is inactivated by normal digestive proteases in the human gastrointestinal tract. This information, coupled with the extremely low likelihood of human exposure to canola seed proteins, provides compelling evidence that there are no health and safety concerns associated with the use of the *barnase* gene in canola plants used for the production of food for humans or feed for animals.

(ii) Compositional studies on canola oil

The Food Branch of the South Australian Department of Human Services has noted the range of parameters tested as part of the compositional studies on the GM canola oil. This submission claims that canola oil is an important dietary source of tocopherols, and that the data relating to these substituents should have been presented.

Response

The key nutritional considerations with respect to canola oil relate to the fatty acid composition of the oil. This is reflected in the Codex Standard for Named Vegetable Oils (Codex-Stan 210, Codex Alimentarius Commission, 1999) in which standards for fatty acid ranges for low erucic acid rapeseed oil are presented. Tocopherols and sterols are not considered to be major constituents of canola oil by the OECD Task Force for the Safety of Novel Foods and Feeds (OECD, 1999), nor is canola regarded as a major source of these compounds in the diet.

Tocopherols (the alpha isomer is also known as Vitamin E) are natural antioxidants and their level in plants is governed by the level of unsaturated fatty acids. The distribution of natural tocopherols varies with the different vegetable oils both quantitatively and in the amount of different isomers, numbering at least four.

Amongst the vegetable oils, safflower, sunflower and cottonseed oils are more significant sources of alpha-tocopherol than canola (The Lipid Handbook, Second Edition, Chapman and Hall, 1994). Processing of canola oil, in particular the high temperatures used during the deodorisation step, generally reduces the level of tocopherol present in the oil. The available data on tocopherols from control and transformed lines of canola show that the levels of this group of compounds are virtually the same for all lines tested.

(iii) Lines recommended for approval

The Food Branch of the South Australian Department of Human Services noted that the canola oil for human consumption is produced from the hybrid canola seed and not directly from either of the parental Ms or Rf lines and stated that the legal drafting of proposed changes to the Standard should reflect the source of the food.

Response

The safety assessment process undertaken by ANZFA for GM foods requires that compositional data relate directly to the plant lines that have been genetically modified. Once the assessment had been completed and food products derived from the assessed line have been approved, the particular transformed line is regarded as any other commercial crop line and may be used by plant breeders in conventional breeding programs to generate a variety of hybrid lines. As the hybrids can be generated from a large number of different combinations of crosses, the most comprehensive means of assessment of the genetic modification applies to the original transformed parental lines, which are also the lines for which the molecular characterization data are also readily available.

(iv) Terminator technology

Submissions from the National Council of Women of Australia, the Nelson GE Awareness Group (NZ), Kate Clinch Jones (Aust) and Leila Huebner (NZ) contend that this application features the use of the so-called terminator technology, which results in farmers' dependence on the purchase of new seed at the end of each growing season.

Response

This application encompasses seven lines of canola although only the Ms and Rf lines feature genetic changes, which can exert an influence on the fertility of the canola plants. The two open pollinated lines, Topas 19/2 and T45, do not contain either of the two genes used to control pollen production in the Ms and Rf lines.

It is important to note that this application **does not** equate with the terminator technology, which has not been introduced into any commercially produced crops. Rather, the *barnase*/*barstar* gene system has been used in canola plants to take greater advantage of the natural genetic phenomenon widely known as hybrid vigour. The genetic superiority of hybrid seed in many crop plants of commercial importance is well demonstrated and plant breeding practices reflect widespread acceptance, knowledge and manipulation of the genetic determinants that lead to hybrid vigour in later generations.

In this application, the system involves male sterile (female parent) and fully fertile (male parent) elite breeding lines as the parents of the first generation hybrids (F1). The seeds from this cross are commercially required by farmers. The hybrid seeds are fully fertile and the plants that grow from these seeds, due to their specific parentage, feature the desired agronomic characteristics of both parents, and in turn produce large yields of oil-bearing seeds in the next generation. In contrast, the aims of the terminator technology are to *prevent* seed germination by expression of novel genes (possibly *barnase*, depending on the specific patent) in the seeds of the plant. This key difference has been scientifically misinterpreted in articles (for example, by Ho, Cummins and Bartlett, 2001) that have been widely disseminated in the public domain.

Hybrid canola seed is a constant requirement to maintain levels of primary production for farmers. This goal of plant breeding, to combine higher yielding varieties with other desirable plant traits (such as drought tolerance, herbicide resistance, etc) has always existed and does not exclusively apply to recently engineered GM varieties. Before the advent of the *barnase*/*barstar* system, the parental lines used to generate non-genetically modified hybrid seed were developed by other methods that were not as accurate or specific.

It is important to note also that planting hybrid seeds for many generations results in the loss of hybrid vigour as a result of natural genetic processes. The generation of new hybrid seeds with increased vigour is therefore a necessity for primary producers and must continually occur whether the parental lines are genetically modified or conventionally modified.

(v) Consumption of canola seeds

The Nelson GE Awareness Group in New Zealand raised the issue of the consumption of whole canola seeds in certain bakery products and therefore claims that the novel protein(s) will be consumed by humans and other animals.

Response

The safety aspects of the novel proteins present at very low levels in canola seeds from the lines under assessment have been investigated, and the conclusions presented in the Safety Assessment (Attachment 2 to this report). The safety of the proteins from a human food perspective has been established from consideration of the biochemical data submitted and other relevant information including an evaluation of potential toxicity and allergenicity.

Whole rapeseeds are currently not considered to be used widely in human food because of the presence of naturally occurring toxins in the seed meal. The presence of the toxins in the seed meal however, does not represent a feeding hazard for non-human animals, on the basis that ‘double-nought’ varieties of rapeseed, meeting canola quality specifications, are used for animal feed.

4.4 Risk management

Under Standard A18 (Standard 1.5.2), a GM food must undergo a safety assessment in accordance with ANZFA’s safety assessment guidelines.

On the basis of the conclusions of the safety assessment, together with a consideration of the public submissions, it is recommended that Table 1 to clause 2 of Standard A18/Standard 1.5.2 be amended to include oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45, and oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines Ms1, Ms8, Rf1, Rf2 and Rf3. The recommended variation to the Standard is provided in Attachment 1.

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.

4.5 Regulatory impact assessment

The benefits and costs associated with the proposed amendment to the *Food Standards Code* have been analysed in a Regulatory Impact Assessment (see **Attachment 3**). The benefits of the proposed amendment to approve canola oil from the genetically modified canola lines known commercially as Liberty Link® and InVigor® primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

5. CONCLUSIONS

- There are no public health and safety concerns associated with the genetic modifications used to produce canola lines Topas 19/2, T45, Ms1, Ms 8, Rf1, Rf2 and Rf3.

- Canola oil derived from glufosinate-ammonium tolerant and pollination controlled transformation events Topas 19/2, T45, Ms1, Ms 8, Rf1, Rf2 and Rf3 is equivalent to oil from other commercially available canola lines in terms of its safety for human consumption and its nutritional adequacy.

- On 7 December 2001, food products containing oil derived from Liberty Link® or InVigor® canola will require labelling if it can be shown that novel DNA and/or protein is present in the final food.

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

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

FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

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

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

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

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

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

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

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

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

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

FURTHER INFORMATION

Submissions: No submissions on this matter are sought as the Authority has completed its assessment and the matter is now with the Australia New Zealand Food Standards Council for consideration.

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

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

PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 4739942
email: anzfa.nz@anzfa.gov.au

Copies of assessment reports or other information papers are available on the website at www.anzfa.gov.au then <Food Standards> then < Recent Standards Development>. Further information should be addressed to the Authority's Information Officer at the above address, or e-mail info@anzfa.gov.au.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety assessment report
3. Regulatory impact assessment
4. World Trade Organization agreements
5. Summary of first and second round public submissions
6. General issues raised in public submission
7. Statement of Reasons

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A372 - OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA LINES TOPAS 19/2, T45, MS1, MS8, RF1, RF2 AND RF3.

To commence: on gazettal

[1] *Standard A18 of Volume 1 and Standard 1.5.2 of Volume 2 are varied by inserting in Column 1 of the Table to clause 2 -*

Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and glufosinate-ammonium tolerant and pollination controlled canola lines Ms1, Ms8, Rf1, Rf2 and Rf3.

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

APPLICATION A372

OIL FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA

SUMMARY AND CONCLUSIONS

This application is for the approval of seven lines of canola (Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3) that have been genetically modified to provide growers with a range of production and breeding lines that are tolerant to the herbicide glufosinate-ammonium. Both Topas 19/2 and T45 are open pollinated canola lines while the remaining modified lines (Ms1, Ms8, Rf1, Rf2 and Rf3) have been specifically developed for use in a plant breeding system for the purpose of generating hybrids with increased vigour.

1. Nature of the genetic modifications

The herbicide tolerance trait has been introduced into all seven genetically modified canola lines by the addition of one of two bacterial genes, *bar* or *pat*, to enable the plants to produce an enzyme, phosphinothricin acetyl transferase (PAT), which chemically inactivates the herbicide, phosphinothricin (also known as glufosinate-ammonium), in the plant. Therefore, plants expressing the PAT protein are able to function normally in the presence of the herbicide.

In conjunction with the herbicide tolerance trait, five of the genetically modified lines contain one or both of the bacterial genes, *barnase* and *barstar*. Expression of *barnase* in specific parts of the flower at a particular developmental stage gives rise to plants that are male sterile (Ms). Conversely, expression of *barstar* does not produce any change in phenotype in the plant unless it is expressed at the same time and place as *barnase*. When this occurs, the *barstar* expression product counteracts the effects of the *barnase* gene, and restores male fertility. Plant lines expressing *barstar* are thus referred to as fertility restorer (Rf) lines.

The *barnase* gene, expressed in the Ms lines, prevents pollen formation by producing a non-specific ribonuclease that destroys the cells in which it is expressed. This ribonuclease activity is specifically inactivated by the presence of the barstar protein expressed in the Rf lines. The hybrid system consists of crossing a Ms line (female parent) with a specific Rf line, giving rise to progeny that are fully fertile. The primary objective of these modifications is the production of a range of parental lines with superior agronomic performance that are to be used in a breeding system for producing hybrids yielding significantly more seed.

Four of the genetically modified canola lines also contain a bacterial antibiotic resistance marker gene, *nptII*, under the control of a plant promoter. The *nptII* gene is used for the selection of transformed plants in the laboratory as well as for identification purposes in the field. Apart from its use as a marker in the field, the gene serves no agronomic purpose in the crop.

General safety issues

A comprehensive set of analytical data has been evaluated for the safety assessment of food derived from the genetically modified canola. The seeds are used to produce two major products, canola oil and meal, but only the oil is used as a human food. Presently canola meal is used only as a protein supplement for animal feed.

The gene modifications in this application encompass four novel proteins: PAT, NPTII, barnase and barstar, however not all of these are present in any one transformed line. Only some crosses of the male and female lines (Ms x Rf) may express all four proteins. The enzyme responsible for herbicide tolerance, PAT, is expressed in all tissues of the plant including the seeds, but at such low levels that the specific enzyme activity was not detectable. The NPTII marker protein is expressed only in the lines Topas 19/2, Ms1, Rf1 and Rf2 and was detected at very low levels in the leaves, but not in the seeds. The two novel proteins, barnase and barstar, present in either the Ms or Rf lines used for hybrid production are restricted entirely to the developing anthers and are not expressed elsewhere in the plant, including the seeds. The patterns and levels of gene expression conformed to those predicted and intended by the modification process.

2. Toxicological issues

Traditional rapeseed is unsuitable as a source of food for either humans or animals due to the presence of two naturally occurring toxicants, erucic acid and glucosinolates. The name *canola* therefore is now confined to those cultivars that yield oil low in erucic acid and meal low in glucosinolates, so called “double low” varieties. In addition, as a quality control measure, no protein is allowed to be present in canola oil, which is the only product suitable for human consumption.

Compositional analyses showed that the levels of erucic acid in the oil (and glucosinolates in the meal) conformed to the compliance requirements for certification as canola. The modified lines were tested in a range of environmental situations and following treatment with commercial levels of glufosinate-ammonium, and demonstrated that the introduced genetic changes have not produced changes in the levels of natural toxicants in the food fractions of the seed.

Data were presented to demonstrate that the processing involved in the production of canola oil effectively removes all traces of protein. Consequently, consumers will not be exposed to plant proteins, including the novel proteins, through consumption of canola oil. Notwithstanding the absence of protein in the oil, there is no evidence to indicate that either PAT or NPTII, which are both expressed in the seed, are likely to be allergenic or toxic to humans. Neither of these proteins shows any chemical similarity with known allergens or protein toxins using data obtained from public genetic and protein databases. Further toxicological assessment determined that the PAT protein is present in the seed at low levels, and when used (in four lines only) the NPTII protein is below the limit of detection. In addition, both proteins were readily degraded in simulated digestive systems indicating that neither is likely to be allergenic.

Expression of the barnase and barstar proteins is tightly controlled in the plant and both of these proteins may only be found in the non-edible parts of the plant. For this reason, these

proteins are not considered to be of major significance with respect to allergenicity, nutritional properties or overall food safety.

The risk of horizontal DNA transfer is considered to be zero on the basis of evidence provided by the applicant, which demonstrates conclusively that there is no novel DNA present in canola oil.

3. Nutritional issues

The results of extensive compositional analyses of the oil obtained from the genetically modified canola seeds from both herbicide-treated and untreated plants demonstrate that the fatty acid profile, particularly the levels of erucic acid, show no differences when compared to the control cultivar and to an extensive range of published literature data for commercial varieties of canola. The analyses were conducted on test material grown over multiple growing seasons and at different locations around the world and thus demonstrate that the genetically modified varieties perform to expectations and do not exhibit any significant variation in composition when compared to the controls grown under the same conditions, despite the known variations due to seasonal and environmental factors.

4. Conclusion

On the basis of the available evidence, oil derived from the genetically modified canola lines, and their crosses, is equivalent to oil from non-GM canola in terms of processing characteristics, composition and quality.

There is no evidence to indicate that consumption of the oil from these genetically modified canola lines (T45, Topas 19/2, Ms1, Ms8, Rf1, Rf2 and Rf3) represents any additional food safety risk when compared to conventionally modified canola oil, as the characteristics of the food are not altered by the genetic modification.

1. BACKGROUND

Aventis Crop Science Pty Ltd[4] have submitted an application to ANZFA to vary Standard A18 to include all food products derived from glufosinate-ammonium tolerant and pollination controlled canola. The lines encompassed by this application are known commercially in North America as LibertyLink® open pollinated and InVigor® hybrid canola.

Seven lines of canola (*Brassica napus*, *B. rapa* and crosses) have been genetically modified to confer tolerance to the broad-spectrum herbicide, glufosinate-ammonium. Five of these lines have been generated primarily for use in a hybrid seed production system by expressing one of two genes that enable control of pollen production, in conjunction with the herbicide tolerance trait. Two lines of open pollinated canola have been genetically modified with the herbicide tolerance trait only. Three traits may be contained within an individual transformed canola line, however not all lines contain all the traits. The genes coding for the new traits are the bacterial genes *bar* (or *pat*), *barnase, barstar* and *npt II*.

The *bar* and *pat* genes produce an enzyme, phosphinothricin acetyl transferase (PAT), that metabolises the herbicide phosphinothricin (PPT) into an inactive form. Phosphinothricin is the active ingredient of the commercial herbicide glufosinate-ammonium (OECD, 1999). Glufosinate-ammonium is currently registered in Australia under the commercial name of Basta® for non-selective uses, or Finale® for turf and home garden uses, and as Buster® in New Zealand.

The mode of action of glufosinate-ammonium (or phosphinothricin) is to inhibit the plant enzyme glutamine synthetase (GS), an essential enzyme in nitrogen metabolism and amino acid biosynthesis in plants. The result of GS inhibition is the over accumulation of inorganic ammonia leading to the death of plant cells.

In addition to the herbicide tolerance gene, five of the GM canola lines for use in hybrid production contain either of the genes, *barnase* and *barstar*. Expression of the *barnase* gene in specific plant cells induces male sterility (Ms) and when these plants are crossed with fertility restorer (Rf) canola plants expressing the *barstar* gene, fertility is restored in the hybrid offspring. Hybrids produced from conventional crosses between the Ms and Rf lines are reported to have significantly higher yields of oil-bearing seeds.

Canola oil and meal are the two major products produced from oilseed rape plants. Canola oil is used extensively in the food industry as vegetable oil and in products such as margarine, salad dressings, bakery products, low-fat foods and confectionery. It is also used in pharmaceuticals and nutritional supplements. Canola meal is used only as a protein supplement in animal feed.

[4] Formerly AgrEvo Pty Ltd before a merger in December 1999 with Rhone-Poulenc.

2. DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modifications

The new genes were introduced into canola plants (*Brassica napus*, AC Excel and Drakkar lines), by *Agrobacterium* mediated transformation (Zambryski, 1992). This is achieved using plasmid vectors, which allow specific genes, integrated into the *Agrobacterium* T-DNA between regions known as the left and right borders, to be transferred to the plant. In this application, six separate plasmids carrying the required genes were used to generate the seven new lines.

Agrobacterium mediated transformation involves incubation of the bacteria carrying the particular plasmid with plant cells for a few hours to days, during which time T-DNA transfer takes place. The cells are then washed and cultured in the presence of the selection agent, and transformed shoots are regenerated and characterised. In the case of one of the plasmids, two independent lines were derived from the original transformation event. As usually occurs, only one plant line was derived from transformation with each of the remaining plasmids.

2.2 Function and regulation of the introduced genes

Studies submitted:
Eckes, P. (1994) Comparison of the synthetic PAT gene and the PAT protein with other known nucleotide and protein sequences. Hoechst Biol. Research C, Plant Biochemistry, Frankfurt, Germany. Company file No. A53504.

2.2.1 *bar* and *pat* genes

The *bar* and *pat* genes conferring tolerance to glufosinate-ammonium were transferred to canola plants as markers both for use during *in vitro* selection and as a breeding selection tool in seed production. Both genes are of bacterial origin and code for the enzyme phosphinothricin acetyl transferase (PAT), which inactivates phosphinothricin (PPT), the active constituent of the non-selective herbicide glufosinate-ammonium. The *bar* gene was isolated from *Streptomyces hygroscopicus* and the *pat* gene was isolated from *S. viridochromogenes*. Both of these species are common soil bacteria that may also exist in water.

Phosphinothricin was initially characterised as an antibiotic (bialaphos), which is produced naturally by both species of bacteria, but was later shown to be effective as a broad-spectrum herbicide. By acetylating the free amino group of PPT, the PAT enzyme prevents autotoxicity in the bacterial organisms and generates complete resistance towards high doses of PPT, bialaphos or the synthetically produced glufosinate-ammonium.

The *pat* and *bar* genes are very similar, sharing 87% homology at the nucleotide sequence level (Wohlleben *et al*., 1988, 1992). The respective PAT enzymes encoded by these genes are also very similar, and share 85% homology at the amino acid level (Wohlleben *et al*., 1988, 1992). Further characterisation of these enzymes in 1996 concluded that they are so similar as to be functionally equivalent for the purpose of conferring tolerance to PPT (Wehrmann *et al*., 1996).

The native *pat* gene has been resynthesised to modify codon usage for improved protein expression in plant cells (Strauch *et al*., 1993). At the nucleotide sequence level, the synthetic gene demonstrates 70% homology with the native *pat* gene from *S. viridochromogenes*. The amino acid sequence of the PAT enzyme encoded by both the native and synthetic genes is identical.

In this application, either the *bar* or the *pat* gene is present in all of the canola lines to confer tolerance to the herbicide. The *bar* gene is under the control of a plant promoter (Pssu-Ara) which generates expression of PAT predominantly in the green tissues (leaves, stems) of the canola plant. Alternatively, in constructs involving the synthetic *pat* gene, a plant viral promoter (P35S) has been used for constitutive expression of the PAT protein in all tissues of the plant.

2.2.2 *barnase* and *barstar* genes

The lines of canola modified to facilitate hybrid seed production contain one or both of the *barstar* and *barnase* genes. Both of these genes are derived from the bacterium *Bacillus amyloliquefaciens* and each encodes a different small single-chain protein. Both of these proteins have been studied extensively as models for protein folding because of their small size, and there is an abundance of published scientific information relating to research work conducted since the early 1960s (Smeaton *et al*., 1967, Hartley, 1968, Mauguen *et al*., 1982).

The *barnase* gene encodes a ribonuclease that is naturally secreted by the bacterium. Ribonucleases are enzymes, which degrade and digest ribonucleic acid (RNA), the biochemical intermediate between a gene (DNA) and its encoded protein. Ribonucleases are ubiquitous in nature, and serve many biological functions. In this case, the secreted ribonuclease serves to protect the environment of the bacteria (Hartley *et al*. 1989).

Conversely, the *barstar* gene encodes a specific protein inhibitor of the ribonuclease encoded by *barnase*. In the *Bacillus* species from which the two proteins are derived, the function of the *barstar* protein is to protect the organism from the otherwise toxic effects of its own *barnase* activity. This naturally occurring system is well studied (Hartley *et al*., 1988 & 1989) and the interaction of the two proteins is known to be highly specific. In the GM canola lines, both genes have been placed under the control of a highly tissue-specific plant promoter, designated as TA29, that restricts their expression exclusively to the tapetal cell layer and only during anther development. The specificity of the interaction between the barnase and barstar proteins has enabled the bacterial system to be adapted for use in canola plants to allow the development of a breeding system to generate high yields of hybrid seed (Mariani *et al*. 1990 & 1992).

Hybrid design

One of the major goals of plant breeders is to create higher yielding varieties. Compared to the best open-pollinated varieties of canola, yields of seeds from F1 (first generation) hybrids can be increased by as much as 20-25%. In addition, the F1 hybrid seed is more uniform which facilitates both harvesting and marketing. Since canola is capable of both self-pollination (approximately 70%) and cross-pollination (30%), an effective pollination control system is required to enable production of high yielding 100% F1 hybrid seeds, containing all of the desired characteristics of both parental varieties.

One method of control that has been used widely in breeding programs of many different crops to ensure cross-pollination is the use of male sterile plants featuring abnormal pollen production. These plants are incapable of self-fertilisation but can be crossed with other plants to produce seed. Although naturally occurring male sterile canola plants have been used to a certain extent to develop hybrids, they have lacked appropriate features to allow commercial production.

In this application, a novel system is described whereby high yielding canola hybrids can be generated by crossing two different genetically modified parental lines. The basis of the system is that a male sterile line is unable to undergo self-pollination and this enables the production of 100% true hybrid seed only when crossed with a specific fertility restorer line.

The male sterile parental lines (Ms1, Ms8) contain the genes *bar* (herbicide tolerance) and *barnase* (with or without *nptII*), and the fertility restorer parental lines (Rf1, Rf2, Rf3) contain the genes *bar* and *barstar* (with or without *nptII*). Due to the presence of the *bar* gene, all parental lines and the subsequent hybrids exhibit tolerance to the herbicide glufosinate-ammonium.

The Ms lines do not produce pollen but are otherwise phenotypically unaffected by the genetic modification. The use of a plant promoter from an anther specific gene results in expression of the *barnase* gene only during flowering in the developing anthers or male tissue of the flower. Consequently, plants containing this gene have an altered anther shape and reduced pollen production (Mariani *et al*., 1990).

The Rf lines contain the *barstar* gene under the control of the same plant promoter that limits expression to the tapetum cells of the pollen sac and only when flowering during anther development. In contrast to the Ms lines, the Rf lines produce normal amounts of pollen, are fully fertile and in all respects are phenotypically normal.

The effects of the *barstar* gene activity are only apparent after crossing a male sterile line (Ms) with a fertility restorer line (Rf). When both introduced genes are expressed in combination in the same part of the flower, as occurs in a cross between Ms and Rf plants, the fertility of the resulting hybrid progeny is restored due to the inactivation of the barnase enzyme by the barstar protein, thereby ensuring full seed development. In this system, hybrid canola plants therefore contain the *bar*, *barnase* and *barstar* genes and some may also contain the *nptII* gene.

2.2.3 The *nptII* gene

The bacterial *nptII* gene is derived from *Escherichia coli* and codes for the enzyme neomycin phosphotransferase II (NPTII). Expression of this protein confers resistance to the aminoglycoside antibiotics kanamycin, geneticin (G418) and neomycin. The presence and expression of this gene, linked to the other genes of interest, allows for the early selection in tissue culture of transformed plant cells carrying the required genetic traits.

2.4 Gene constructs

The applicant has constructed a range of plasmids to deliver a specific number of gene expression cassettes to the plants. The genes together with the appropriate controlling

sequences were inserted between the left and right borders of the bacterial T-DNA, the segment that is integrated into the plant genome.

The applicant has provided detailed information relating to each plasmid, including a full description of the plant and bacterial genetic elements together with plasmid maps. The nucleotide sequence of the DNA segment between the left and right borders of each plasmid is completely identified. In addition, the genetic elements are all well described in the published literature in terms of their molecular size and their function in plants. However, following a request by the applicant, information relating to the exact combination of elements present in each of the plasmids is regarded as commercial-in-confidence, pursuant to section 3(1) of the *Australia New Zealand Food Authority Act* (1991). Therefore, only general descriptions of each of the plasmids are presented here.

Each plasmid contains a specific number of gene expression cassettes, which give rise to the new traits. For example, one expression cassette is used to confer glufosinate-ammonium tolerance to the plant. The expression cassettes used in the plasmid constructs consist of a promoter sequence for initiation of transcription in plants, sometimes in specific tissues only, the coding sequence of the gene of interest, followed by a 3' untranslated region providing the signals for termination of transcription and polyadenylation.

The two open pollinated lines of canola, T45 and Topas 19/2, were generated using a separate plasmid for each line. Open pollinated lines derived from these events do not contain the pollination control genes (*barnase*/*barstar*) used in the hybrid system. A total of four separate plasmids were used to generate multiple parental lines to be used for the production of hybrid canola seed. Both the open pollinated and pollination controlled lines are summarised in Table 1.

Table 1 Summary of transformed lines relevant to *Brassica napus*, open-pollinated canola and pollination controlled canola.

Line	Number of gene expression cassettes	Glufosinate-ammonium tolerance	Pollination control genes	*nptII* gene
T45	1	pat	N/A	-
Topas 19/2	2	pat	N/A	yes
Ms1	3	bar	barnase	yes
Ms8	2	bar	barnase	-
Rf1, Rf2	3	bar	barstar	yes
Rf3	2	bar	barstar	-

As described above, the Ms and Rf lines refer to plants that carry either the *barnase* or the *barstar* gene respectively, in conjunction with the *bar* gene. In addition, the *nptII* gene is present in the following lines only: Ms1, Rf1, Rf2 and Topas 19/2.

The T-DNA region of the plasmids may contain the following genetic elements:

- the plant promoter from the atS1A ribulose-1,5-bisphosphate carboxylase small subunit gene (*ssu*), known as PssuAra, from *Arabidopsis thaliana*. The PSsuAra element comprises the 1.7 kb fragment upstream of the atS1A ATG codon and the transit peptide (tp) encoding sequence, for targeting to the chloroplasts (Krebbers *et al*. 1988). This promoter allows for expression predominantly in the green tissues of the plant;

- the promoter fragment from the anther specific gene TA 29 (PTA29) from the tobacco plant (*Nicotiana tabacum*);

- the promoter sequence (PNos) from the T-DNA nopaline synthase (nos) gene from *Agrobacterium tumefaciens*;

- the CaMV 35S promoter from the cauliflower mosaic virus. This promoter, denoted as P35S, gives rise to constitutive expression throughout the plant;

- the coding region of the *barstar* gene from *Bacillus amyloliquefaciens*;

- the coding sequence of the bialaphos resistance gene (*bar*), from *Streptomyces hygroscopicus* (Thompson *et al*. 1987);

- the synthetic *pat* gene, derived from *Streptomyces viridochromogenes* (Strauch *et al*. 1993);

- the coding region of the *neo* gene encoding neomycin phosphotransferase II from *Tn*5 of *Escherichia coli* (Beck *et al*., 1982);

- the coding region of the barnase gene from *Bacillus amyloliquefaciens* (Hartley, 1988);

- the 3' untranslated region of the TL-DNA gene 7 (3'g7) derived from the octopine Ti plasmid of *Agrobacterium tumefaciens* (Dhaese *et al*., 1983);

- the 3' untranslated region of the octopine synthase (3'ocs) gene from *Agrobacterium tumefaciens*;

- the 3' untranslated region of the CaMV 35S transcript; and

- the 3' untranslated region of the nopaline synthase gene (3'nos) from *Agrobacterium tumefaciens*, containing plant polyadenylation signals.

With respect to the food products derived from these transformed lines of canola, the seeds harvested directly from the two open pollinated lines are used for the production of canola oil. In contrast, the Ms and Rf lines are conventionally crossed by commercial seed producers to generate hybrid canola seed, which is marketed and subsequently used for cultivation. In this case, canola oil for human consumption is derived from the hybrid crop plants.

2.3 Characterisation of the genes in the plant

All of the lines were characterised using testing material from untransformed plants of the same cultivar, Drakkar, as a control. The transformed plants were characterised at the molecular and biochemical level using a range of laboratory techniques and procedures outlined below in Table 2.

Table 2 Outline of molecular and biochemical methods used for identification of glufosinate-ammonium tolerant male sterile and fertility restorer lines, with and without *nptII*.

Molecular or biochemical Methodology	Purpose
Southern Hybridisation analysis	- Detection of the gene cassettes in the canola plant genome - Quantification of the insertions in the plant genome - Verification of the physical linkage of the introduced genes - Verification that inserted DNA corresponds with plasmid DNA - Investigation of T-DNA borders - Identification of transgenic lines by their hybridisation pattern.
Polymerase Chain Reaction (PCR)	- Verification of the presence of the introduced genes - Characterisation of plant DNA sequence flanking the inserted DNA - Determination of target site deletion sequences - Development of primers to fingerprint specific male sterile or restorer alleles
Northern Blotting	- Analysis of the expression of the transgenes in different plant tissues (seeds, leaves, pollen)
NPTII assay	- Quantification of enzymatically active NPTII enzyme
PAT assay	- Quantification of enzymatically active PAT enzyme

Following transformation, shoots were regenerated on selective medium under tissue culture conditions. From these, all suitable plantlets identified for transfer to the glasshouse were first analysed for the presence of the inserted gene and the number of insertions by Southern blot hybridisation, using molecular probes specific for each gene expression cassette.

Using the *pat* gene as a probe, Southern analysis on the open pollinated line T45 showed that a single copy of the T-DNA was stably incorporated at a single locus in the plant genome. Further Southern hybridisation, using several probes to detect regions outside of the T-DNA border, indicated that there was no incorporation of any coding regions beyond the T-DNA border. This was confirmed by PCR analysis of T45 to verify integration of the *pat* gene and the absence of any unintended vector sequences.

The genetic analysis of the lines selected for hybrid production (Ms1, Ms8, Rf1, Rf2 and Rf3) indicated that a single insertion event had occurred. Further analysis of lines Ms1, Ms8, Rf1 and Rf2 using gene specific primers in a range of PCR based detection methods revealed that, as intended, only DNA sequences within the T-DNA borders were transferred to the plant. In the Rf3 line, detailed analysis of the site of integration of the introduced DNA revealed that one full copy and one truncated copy of the T-DNA gene expression cassette were present as one segment. The complete nucleotide sequence of the segment of introduced DNA, together with approximately 800 base pairs of flanking plant DNA, was provided and this revealed the presence of only a partial promoter sequence within the truncated gene cassette. The partial promoter lacks essential sequences necessary for it to function in the plant and therefore it is not transcriptionally active.

In addition to the above techniques, other test procedures were available to identify and detect the inserted selectable marker genes and their gene products. For example, to investigate the expression of the inserted *bar* gene in the transformed canola plants on a larger scale, glufosinate-ammonium dot or glufosinate-ammonium spray assays were performed at different stages of development. This involved applications of a commercial formulation of the herbicide either directly to the surface of a young leaf or by aerial spraying. Since the *bar* gene is genetically closely linked to the *barnase* or *barstar* gene in the plasmid constructs, this technique indirectly allowed the selection of plants carrying the male sterile (*barnase*) or fertility restorer (*barstar*) genes in larger populations.

Gene expression

In the lines created for hybrid production, RNA analyses were also carried out to further characterise the levels of expression of the transferred genes in specific plant tissues. The results obtained from these experiments were consistent with gene expression patterns expected from the specific plant promoters used in each case.

Bar/pat

For the Ms1, Ms8, Rf1, Rf2 and Rf3 transformants, messenger RNA (mRNA) corresponding to either the *bar* or *pat* gene could be detected at extremely low levels in the leaves and flower buds, but not in the seeds of the plants.

Barnase/ barstar

For the Rf1, Rf2 and Rf3 transformants, *barstar* mRNA was barely detected in flower buds only, but not in any other plant tissues, including the seeds. As expected, because expression results in cell death, *barnase* mRNA could not be detected in any tissues from the Ms1 and Ms8 transformants.

NptII

In a hybridisation system that could detect 0.1 pg/µg of total RNA, there was no detectable *nptII* mRNA in any tissues from the Rf1 and Ms1 transformants. This result indicates that the level of expression of this gene is extremely low in all parts of the plant. The *nptII* gene is present in the Ms1, Rf1, Rf2 and Topas19/2 lines only.

2.4 Stability of the genetic changes

The stability of the transferred genes was investigated for all lines to ascertain plant characteristics over multiple generations. For example, the open pollinated lines T45 and Topas 19/2 were monitored extensively in field trials in Canada during the 1994, 1995, 1996 and 1997 growing seasons. Mendelian analysis was applied to at least four generations derived from the original T45 transformant and demonstrates the stability of the inheritance pattern. Overall, the segregation patterns observed on analysis of the progeny of the original transformants, including hybrids, indicated the stable physical integration of the genes.

Genetic and agronomic performance of the Ms and Rf lines

The expected expression of the traits and the absence of unintended changes to agronomic characteristics were evaluated in a wide range of field conditions. Multiple crosses and backcrosses in more than 40 different spring or winter varieties have been performed in field experiments across Europe and Canada over a three year period (1991-1993) to generate segregation data on the glufosinate-ammonium trait as well as the hybrid production traits. These data indicate no loss of any of the new traits either by observation of the phenotype or in the molecular definition of the plants.

The extensive field experiments were carried out in a broad range of countries, including Canada, Sweden, UK, France, Belgium, Denmark, Spain, USA and Chile. Normal agricultural breeding practices were adopted in conducting these experiments to monitor the genetic and agronomic performance characteristics of the Ms and Rf lines in comparison with non-transformed canola, and to demonstrate the stability of gene expression in terms of the sustainability of the phenotype under different environmental conditions. Factors such as germination, crop establishment, plant vigour, flowering characteristics, seed yield and glufosinate-ammonium tolerance levels were monitored.

Under field conditions, transformed and non-transformed seedlings germinated at about the same time after sowing. Thereafter, both types developed evenly and uniform plant stands were established. Plant height and plant vigour of the Ms and Rf plants and their restored hybrid combinations were comparable to the control plants. No different susceptibility to temperature, humidity, desiccation, light or other environmental stress factor from those of other non-transformed canola cultivars was observed from planting to harvest.

Similarly, evaluations of the flowering characteristics of the Ms and Rf lines and their progeny, as well as their hybrid combinations, in the different environments revealed no major differences. Flower morphology was normal at all sites, nectaries in male sterile canola flowers developed normally and insect activity was also normal for both groups.

Spraying of mixed populations of plants (transformed and non-transformed) with variable rates of glufosinate-ammonium was carried out to determine field tolerance levels. Some non-transformed plant development was observed at sub-agronomic doses of the herbicide. The non-transformed plants did not survive a treatment at or above a rate of 750 g active ingredient due to competition with the glufosinate-ammonium tolerant plants. However, glufosinate-ammonium applications performed before planting and shortly after seeding showed no selectivity for the transformed plants.

Gene expression was scored from observation of the phenotype and subsequently confirmed by Northern blots and NPTII and PAT enzyme assays. These experiments demonstrate that the expression of the *nptII*, *bar*, *barnase* and *barstar* genes, when incorporated into the plant genome of the male sterile, the fertility restorer and subsequent hybrid lines, was stable throughout the growing season under varying conditions. The data therefore support the conclusion that once integrated into the different genetic backgrounds, the transferred genes were inherited as a single locus in a predicted manner according to standard Mendelian genetics.

5. Conclusion

All of the transformed canola, both the open pollinated and pollination control (Ms and Rf) lines, contain a bacterial gene conferring tolerance to the herbicide glufosinate-ammonium. In addition, the Ms and Rf lines contain up to two bacterial genes to generate plants that either do not produce pollen (male sterile) or are phenotypically normal (fertility restorer). A bacterial gene conferring resistance to kanamycin is present as a selectable marker in four lines only, that is Ms1, Rf1, Rf2 and Topas 19/2.

All lines were characterised at the molecular level and the analyses indicate that the genes of interest were transferred in a single T-DNA insertion event. Full nucleotide sequence information was provided for each line to demonstrate molecular events at the integration site. In one of the fertility restorer lines, Rf3, an additional truncated gene expression cassette was shown to be non-functional in the plants.

The conclusion from the many greenhouse and field experiments on the genetic stability of the traits is that the transferred genes remain structurally stable through meiosis and are transmitted in the seed. The organisation of the transferred DNA (as defined by Southern hybridisation) in the original transformant is preserved in all progeny under all environmental circumstances. The incorporated genes (*nptII*, *bar*/*pat*, *barnase*, *barstar*) are 100% linked and are inherited as a single locus according to Mendelian genetics and are expressed as dominant markers. Furthermore the timing, tissue specificity and levels of gene expression are preserved during propagation for several generations and under different environmental circumstances.

3. GENERAL SAFETY ISSUES

The genetically modified canola lines developed by the applicant have been assessed according to ANZFA's paper entitled 'Guidelines for the safety assessment of foods to be included in Standard A18 – Food Produced Using Gene Technology' (ANZFA, 1999).

3.1 History of use

Recipient organism

Since being developed as a vegetable oil for human consumption, canola oil has not been associated with any food safety concerns. The plant species *Brassica napus* L. oleifera Metzg. are more commonly known as oilseed rape, rape or rapeseed, with some cultivars referred to as canola. Two modifications introduced by classical breeding techniques have stimulated the development of this species as a commercial crop, namely the lowering of the erucic acid and glucosinolate content.

Presently, oilseed rape is grown primarily for its seeds, which yield about 40% oil and a high protein animal feed. World production of oilseed rape in 1996-1997, was the third most important of oilseed crops behind soybean and cottonseed, but above peanut, sunflower and palm. The main producers of the crop are China, India, Canada and countries of the European Union.

Using traditional plant breeding methods, *Brassica napus* can be crossed with a closely related species, *Brassica rapa*, to produce hybrids capable of producing canola quality oil. *B. rapa* has a similar life history to *B. napus*, but with a shorter growing season allowing the crop to be planted later in the canola season. Oil produced from *B. rapa* is required to exhibit the same qualities as that from *B. napus*, that is low erucic acid and glucosinolate content, for marketing as canola.

Canola oil is used in the manufacture of low-fat foods, pharmaceuticals, nutritional supplements, confectionery products, margarine and shortening, salad and cooking oil, mayonnaise, sandwich spreads, creamers and coffee whiteners. Canola meal is primarily used as a feed for livestock, but it is also used in poultry and fish feed, pet foods and fertilisers. In Australia, canola plant stubble may be grazed by livestock following harvest.

Gene donor organisms

In this application, the genes introduced into the canola are derived from several species of bacteria. The *bar* or *pat* genes are derived from the common soil bacteria *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, which may also exist in water. These bacterial species are not used in the food industry.

The source of the *barnase* and *barstar* genes is *Bacillus amyloliquefaciens* which are aerobic, spore forming bacteria commonly found in the soil. *B. amyloliquefaciens* is used widely in the food industry as a source of enzymes.

The *nptII* gene is derived from transposon Tn5 from the bacterium *Escherichia coli* (Beck *et al.* 1982). Particular strains of *E. coli* are used in the food industry, also in the production of enzymes.

3.2 Nature of novel protein

3.2.1 PAT enzyme

The herbicide tolerant trait is conferred by the expression of either the introduced *bar* gene or the synthetic *pat* gene, as both code for the phosphinothricin-acetyl-transferase (PAT) protein, which detoxifies phosphinothricin (PPT). The mode of action of PPT is to inhibit the endogenous enzyme glutamine synthetase, an enzyme involved in amino acid biosynthesis in plant cells. By inhibiting this enzyme, PPT causes rapid accumulation of ammonia in the plant cell, leading to plant death. In transformed canola plants, the introduced PAT enzyme chemically inactivates the PPT by acetylation of the free ammonia group, giving rise to herbicide tolerance in the whole plant.

The PAT protein consists of 183 amino acids, has a molecular weight of 22 kDa, and exhibits a high degree of enzyme specificity, recognising only the one substrate L-glufosinate in the acetylation reaction.

This high substrate specificity was tested in the presence of each of 21 L-amino acids at substrate concentrations exceeding 50 times the K_M value for L-glufosinate. None of the tested amino acids substituted as an alternative substrate in the PAT catalysed reaction, but the enzyme reaction with L-glufosinate was not inhibited (Schulz, A., 1993. L-Phosphinothricin-N-Acetyltransferase – Biochemical Characterisation. Hoechst Biol. Research C., Company File No: A51230).

3.2.2 Barnase and barstar

The *barnase* gene, used to produce the male sterility trait in canola, encodes a ribonuclease, which degrades RNA in the tapetum at early stages of pollen formation. The eventual complete loss of RNA in the restricted cell layer leads to the death of these cells expressing the ribonuclease enzyme. In turn, this leads to the deposition of wound callose, which prevents nutrients reaching the tissues of the anther filament, thereby leading to wilting of the anther. Consequently, plants containing the *barnase* gene are phenotypically normal except that, during flowering, the shape of the anther is altered and pollen production is significantly reduced.

In contrast, transformed plants expressing the *barstar* gene are phenotypically normal and are fertile. The effects of the *barstar* gene activity in the hybrid canola are only visual after crossing with the male sterile line. Microscopic analysis of the anthers and the pollen grains of the restored plants show a complete resemblance to those of non-transformed plants.

The barnase enzyme is a small protein consisting of a single chain of 110 amino acids. The enzyme is characterised by no disulphide bonds, metal ion cofactors or other non-peptide components. The barstar enzyme is a small protein consisting of a single chain of 89 amino acids and includes some disulphide bonds.

3.2.3 Neomycin phosphotransferase II (NPTII)

Under the control of a plant promoter, four of the transgenic canola lines (Ms1, Rf1, Rf2 and Topas 19/2) express the NPTII protein allowing growth of transformed plants in the presence of kanamycin, neomycin or gentamicin (G418). NPTII is a commonly used marker protein that allows the selection of transformed plant cells early in the regeneration phase and can also be used in monitoring gene expression and genetic stability during later development of the plants (Kärenlampi 1996).

NPT 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).

Three of the lines, namely Ms8, Rf3 and T45, are not transformed with the *nptII* gene and therefore do not express the NPTII marker protein.

3.3 Protein expression

Generally, protein is considered to be a contaminant of processed canola oil, and causes cloudiness in the final product. The extraction process includes the use of high temperatures and solvent extraction, which denatures and removes the protein from the initial sample.

Therefore, due to the extensive processing methods applied during canola oil extraction and refinement, no protein, including any of the novel proteins, would be expected to be detected in canola oil products derived from the seeds. The applicant has provided extensive protein expression data for PAT as confirmation of the absence of protein in the oil.

3.3.1 PAT protein

Studies submitted:
Determination of Phosphinothricin Acetyltransferase (PAT) and NPTII content in glufosinate resistant canola (*Brassica napus*) cultivars HCN-10 and Innovator. B. Dang, Xenos Laboratories Inc., Ontario, Canada. Study Number 97AC26, 1997.

PAT enzyme content in glufosinate-tolerant canola seed and processed fractions. B. Dang, Analytical Testing Facility: Xenos Laboratories Inc., Ontario, Canada. Project Number XEN98-15, 1998.

Benchtop Processing of Oilseed Rape (SWO2631 Sprayed and Unsprayed). 1998 Technical Research Report to MacDonald, B., AgrEvo. POS Pilot Plant Corp., Saskatchewan, Canada. Project No. 98-690.

Measurement of PAT activity in leaves and seeds of the male sterile Ms8 transformant and the fertility restorer Rf3 transformant. A. van Vliet, Plant Genetic Systems (PGS), Belgium.

PAT ELISA on different oil fractions derived from Ms8/Rf3 *Brassica napus* seeds. A.van Vliet, Plant Genetic Systems, Belgium. Report ID PAT-ELISA oil Ms8/Rf3, completed June 1999.

Open-pollinated lines

Levels of PAT protein were measured in the seed harvested from a conventional cross between two glufosinate-ammonium tolerant lines, T45 and Topas 19/2. The processing and compositional characteristics of two seed lots from this resultant line (SWO2631) were compared, one lot harvested from a plot treated with the herbicide and one from an untreated plot.

Protein levels were analysed in whole raw seed, toasted meal and refined bleached and deodorised (RBD) oil. The processing of both seed lots (unsprayed 357g and sprayed 383 g) was performed by a contracted company using methods which emulate normal industrial practice in the preparation of oilseed rape fractions. There were no differences in processing characteristics of the seed from either the sprayed or unsprayed plants.

Data were provided on the amount of PAT enzyme present as determined by enzyme-linked immunosorbent assay (ELISA), which has a detection sensitivity limit of 2 ng/g in seed or meal and 0.4 ng/g in oil. The reference substance for the assay system was purified PAT protein. Negative controls fortified with PAT protein at 2.0 ng/g and 4.0 ng/g were included. The recoveries were 81.8% and 107% respectively, indicating that the assay system was optimised to detect PAT in the samples.

The results of the ELISA analysis indicated that PAT protein was found in the treated and untreated seed samples at approximately the same levels, whereas there was no PAT protein detectable in the toasted meal or refined bleached deodorised oil samples. A summary of the results is presented in Table 3.

Table 3: PAT content in canola seed and processed fractions from T45/Topas 19/2 cross.

Sample	**PAT content (ng/g)**
Raw seed – untreated	563
Raw seed – treated	669
Toasted canola meal – untreated	Not detected*
Toasted canola meal – treated	Not detected*
RBD oil – untreated	Not detected*
RBD oil - treated	Not detected*

* Below the limit of quantitation (2ng/g for seed and meal, 0.4 ng/g for oil).

The ELISA data support the conclusion that although the PAT protein is expressed in the seed at levels that are readily detectable, approximately 0.6μg/g for the T45/Topas 19/2 cross, the extensive processing which is required to produce the oil fractions effectively removes all traces of PAT protein from the oil. The PAT protein is present in the meal at approximately 0.005% of total protein (as determined by ELISA analysis of Topas 19/2). Processing affected the activity of the enzyme and the protein levels such that the levels of PAT in the toasted meal were approximately one-tenth of the levels in untoasted meal. The toasting process uses temperatures in excess of 90°C that denature the enzyme.

Using the same ELISA system, measurement of PAT protein in the seeds (pooled sample) of the T45 line was determined to be 295 ng/g, approximately half that of the cross. This result is consistent with the number of *bar* genes present in the plants – one copy of the gene in each parental line, and therefore two copies at different loci in the progeny. As expected, there was no PAT protein (below the limit of quantitation) found in the negative control sample (Excel).

Ms and Rf lines

Data were also provided on the amount of PAT protein in seeds obtained from a number of the Ms and Rf lines (and their crosses). In this instance, the amount of introduced PAT protein was calculated from a measurement of PAT enzyme activity detectable in a seed extract, and was not a direct measurement of the protein. These results are presented in Table 4 and show that the introduced PAT enzyme does not result in specific PAT activity above background acetyl-transferase activity in seeds.

Table 4 PAT content in seeds from Ms1, Rf1, Rf2 and crosses (Ms1xRf1, Ms1xRf2) and untransformed control variety (1995)

Sample	**Protein extract mg/ml**	**PAT protein U/ml**	**PAT protein in seed μg/g**	**PAT protein μg/mg protein**
Ms1xRf1	3.6	0.08 ± 0.04	4.6 ± 2.3	0.02 ±0.01
Rf1	3.4	0.14 ± 0.03	4.8 ± 1.9	0.04 ±0.01
Ms1xRf2	3.7	0.12 ± 0.03	7.4 ± 1.8	0.03 ±0.01
Rf2	3.5	0.19 ± 0.02	11.3 ± 1.2	0.05 ±0.01
Ms1	3.7	0.22 ± 0.02	13.2 ± 0.9	0.06 ±0.01
Drakkar (control)	3.2	0.22 ± 0.04	13.0 ± 2.2	0.06 ±0.01

- PAT U measured in seed extract concentrated 8.5 times, U refers to enzyme units (amount of enzyme to produce one micromole per minute).
- Protein concentration measured using Biorad assay (Lowry method) with BSA as standard.
- µg PAT is based on an estimated specific activity of 170 U/mg PAT.

Analysis of the seeds and leaves from the Ms8 and Rf3 lines confirm a similar pattern of expression of the PAT protein in these lines. Triplicate seed and six replicate leaf samples were assayed for PAT activity using a spectrophotometric assay system. Five replicate samples of leaves and seeds from a non-transformed control cultivar were also analysed. When expressed as a fraction of total protein, the levels of PAT protein in the seeds of the Ms8 and Rf3 lines were only marginally higher than in the seeds from the control cultivar. As expected with a herbicide tolerance trait where expression of the introduced gene is directed to the green tissues of the plant, the levels of PAT found in the leaves of the transformed lines were above those detected in the leaves from the non-transformed control cultivar.

In other experiments, the biochemical methods available for detecting the PAT enzyme in various plant tissues were applied to various oil fractions obtained from hybrid seeds produced from crossing the Ms8 and Rf3 lines. Ten kilograms each of non-transformed and transformed hybrid seeds were processed under simulated industrial processing conditions to produce crude oil, degummed oil, refined oil, washed oil and bleached oil. In addition, oil samples derived from crude seed pressing were obtained (POS Pilot Plant Corporation, Canada, 1998) for testing.

An ELISA system was used to determine the PAT content of the different oil fractions. The limit of detection of this assay system in crude oil and seed press oil was estimated to be 1µg/ml, while the limit of detection of the PAT protein in degummed, refined, washed and bleached oil fractions was estimated to be 3µg PAT/ml. As an additional measure, processed fractions from non-transformed seeds were fortified with purified PAT protein prior to assay in order to validate recovery of known, added amounts of PAT protein in the samples.

The PAT protein was not detected in any of the oil fractions tested, including the crude seed pressing, from either the transformed or the non-transformed samples. The validation analyses demonstrated that the PAT protein could be recovered using this assay system and therefore showed that the industrial processing effectively removes protein from the canola oil, with none detected after just the first stage of processing.

3.3.2 NPTII protein

Study submitted:
Determination of Neomycin Phosphotransferase II (NPTII) Levels by ELISA in Seeds of *Brassica napus* Hybrid Varieties PGS1, PHY14 and PHY35 (based on Ms1/Rf1), PGS2 and PHY23 (based on Ms1/Rf2). Xenos Laboratories Inc., Ottawa, Ontario, Canada, 1997.

Seed samples were collected from field trials conducted in 1995 in Canada. The seeds were shipped to Xenos Laboratories Inc. for determination of neomycin phosphotransferase II (NPTII) enzyme content using enzyme-linked immunosorbent assay (ELISA). Protein content was measured using the Bradford assay (Analytical Biochemistry, vol.72, pp248-254, 1976). Multiple samples of several hybrid varieties derived from the lines Ms1/Rf1 and Ms1/Rf2 were tested as well as a non-transformed control cultivar.

The results obtained showed that there was no detectable NPTII protein in the seeds derived from any of the hybrid lines tested. The limit of detection of this assay system was 350 pg/g seed, using this highly sensitive method of analysis. The results from additional control samples using laboratory fortified NPTII canola seeds indicated that the assay system was able to recover almost all of the NPTII spikes over a ten fold variation in concentration.

3.3.3 Barnase and barstar proteins

From a knowledge of its natural function as outlined in the scientific literature, it is known that expression of the *barnase* gene generates ribonuclease activity which is lethal to the cells in which it occurs. In the Ms lines, the expression of the *barnase* gene coupled to the plant promoter (PTA29), has been demonstrated to be specifically confined to the developing anthers where the enzyme causes the degeneration of a specific layer of cells known as the tapetal cell layer, resulting in a characteristic wilting of the anthers (Mariani *et al*., 1990).

A detailed description of the anther and floral tissue development of male sterile canola plants has been obtained by histochemical analysis. These studies revealed that no cytological nor histochemical differences between transformed and non-transformed plants could be detected in other floral tissue for example, ovarium, style, sepals and the bottom of the developing flowers. The male sterile anther is therefore an observable characteristic (De Block *et al*., 1993).

Similarly, the plant promoter (PTA29) used in the fertility restorer lines limits expression of the *barstar* gene to the same specific sites within the plant (tapetum cells of the pollen sac) and to the same specific developmental stages (only when flowering, during anther development). Therefore, these proteins are coordinately expressed in the same specific cell types early in the flowering stage. They are not expressed in the parts of the plant that are used for human food.

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

In 1991, the World Health Organization (WHO) issued a report of a Joint FAO[5]/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 in the laboratory or in the field. It is generally accepted that there are no safety concerns with regard to the presence in the food of antibiotic resistance gene DNA *per se* (WHO 1993). There have been concerns expressed, however, that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of antibiotics.

[5] Food and Agriculture Organization.

The human health considerations in this regard depend on the nature of the genetic modification and the nature of the food products, and must be assessed on a case-by case basis. This section of the report will therefore concentrate on evaluating the human health impact of the potential transfer of an antibiotic resistance gene from transformed canola lines Ms1, Rf1, Rf2 and Topas 19/2, to microorganisms present in the human digestive tract.

The antibiotic resistant gene present in these lines is the *nptII* gene, discussed above. The bacterial *nptII* gene confers resistance to the aminoglycoside antibiotics neomycin, kanamycin, and geneticin (G418). 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).

One of the issues that must be considered in relation to the presence of the *nptII* gene in the transgenic canola is the likelihood that this gene could be successfully transferred to, and expressed in, microorganisms present in the human digestive tract. Canola oil undergoes extensive processing to remove protein and other cellular compounds such as nucleic acid, therefore the presence of any genetic material is extremely unlikely. The applicant undertook a number of studies to demonstrate the absence of novel DNA in oil from the transformed canola plants.

PCR analysis of oil from hybrid canola seeds

To determine whether recombinant DNA could be present in the oil or meal fractions of canola seeds, PCR analysis was performed on processed fractions of hybrid seed produced from the Ms8/Rf3 cross. Four different samples of processed canola material were subjected to DNA extraction and PCR analysis to test whether the introduced *bar* gene was detectable. The results showed that whereas the seed meal contains DNA detectable by the PCR method, no DNA could be detected in the bleached oil samples. This negative result was confirmed even when three additional different extraction protocols were applied. The negative PCR analysis on the oil fractions confirms that commercial processing of canola oil results in a product that is free of DNA, using the most sensitive analytical method available.

DNA digestibility study

Schneider, R., 1993. Fate of introduced DNA in gut: Degradation of phosphinothricin acetyl transferase gene from transgenic rape HCN 92 (*Brassica napus*) in stomach fluids from pig, chicken and cow. Hoechst AG Agricultural Division, Frankfurt am Main, Germany. Study No. BR 93/06

A study was conducted to determine whether the introduced DNA present in transformed canola line Topas 19/2 (containing *pat* and *nptII*) is sensitive to degradation by mammalian and avian digestive fluids. The study consisted of two separate experiments using leaf material from transformed plants incubated in digestive stomach fluids extracted from pig, chicken and cow.

In the first experiment, leaf samples were incubated at 37°C in pH step gradients of the digestive fluids over a range of time points up to 1 hour. DNA was extracted and analysed by PCR using primers specific for the detection of the *pat* gene and a labelled molecular probe. The PCR analysis indicated that the *pat* gene was readily degraded after *in vitro* incubation in any of the digestive fluids tested.

Degradation was somewhat pH dependent, being most efficient at low pH, which more closely mimics physiological conditions. The degradation was less complete at higher pH, well above the normal acidic environment of the human stomach.

The aim of the second experiment was to test whether the introduced DNA in the plant material could transfer to competent *E. coli* bacteria in a laboratory situation, using the *nptII* gene as a marker for transformation. The *E. coli* strain was converted from a disabled laboratory strain to a competent living strain for this experiment. Transformed bacteria were recovered by selection on medium containing the antibiotic kanamycin. Both plasmid DNA and leaf-extracted DNA from the transformed canola plants were exposed to the same range of digestive fluids, or to water as a control, prior to use in the transformation process.

The results obtained showed that, as expected, antibiotic resistant bacteria were recoverable at the beginning of the experiment, prior to incubation in digestive fluids, using the proprietary plasmid as the gene source. However, no colonies were recovered after the plasmid was incubated for 60 minutes in the various stomach fluid preparations. More significantly, when the transformed plant material itself was used as the gene source, no transformed colonies could be recovered either initially or after 1-hour incubation in the stomach fluids from any of the test animal species.

These results confirm that the transfer to intestinal bacteria of introduced DNA present in transformed plants, including the antibiotic resistance gene *nptII,* is extremely unlikely to occur.

Conclusion

The extensive processing that is used to produce canola oil from seeds, effectively removes all cellular material including DNA and protein. Under these circumstances, confirmed by the results of sensitive biochemical tests, there is virtually no possibility of horizontal DNA transfer from consumption of canola oil.

4. TOXICOLOGICAL ISSUES

Seeds from the original native oilseed rape plants naturally contain high levels of two toxins, erucic acid and glucosinolates, and prior to the mid 1950s, the extracted oil was used primarily for industrial purposes. Erucic acid, a long chain fatty acid, is a natural constituent of the seed oil, while glucosinolates are confined to the seed meal, along with the seed proteins.

In the early 1970s, the presence of erucic acid in rapeseed oil was reported to be associated with fat accumulation in the heart muscle of laboratory rats, resulting in cardiopathogenic effects. Located in the seed meal, glucosinolates were found to cause thymus enlargement in laboratory animals and therefore their presence also limited the nutritional value of the meal as feed for livestock.

In response to these findings, and subsequent detailed nutritional studies on erucic acid-free rapeseed oil, plant breeders systematically replaced the seedstock with varieties that were selected for a low erucic acid content (below 2%).

As a result of this deliberate plant breeding program, the present cultivars, now referred to as *canola*, are low in both erucic acid and glucosinolates and are used extensively for the production of vegetable oil for human consumption and meal for use as animal feed.

Consequently, canola is defined as seed, oil and meal specifically from *B. napus* or *B. rapa* cultivars that must meet specific quality standards in relation to the erucic acid content of the oil, and that also contain very low levels of glucosinolates in the meal. These so-called 00 varieties contain less than 2% of the total fatty acids as erucic acid and less than 30 micromoles of aliphatic glucosinolates per gram of oil-free meal (Codex 1993, 1999; Downey, 1995). Only oil meeting these specifications is processed and permitted for use in the food industry.

4.1 Levels of naturally occurring toxins

The applicant has submitted data in relation to the content of naturally occurring toxins present in canola seeds, both the seed meal and the oil. Although data were presented in relation to the meal, it has not been considered for the purposes of this safety assessment. Canola meal, whether from genetically modified or conventional plants, is not regarded as a food fraction suitable for humans due to the presence of glucosinolates, and the genetic modification in this application does not change this usual pattern of consumption.

4.1.1 Erucic acid

Erucic acid is a mono-unsaturated 22 carbon fatty acid (C22:1). Due to its previously described adverse effects in animal studies, the applicant has provided detailed fatty acid analyses of the seeds from the transformed plants, noting in particular the erucic acid content.

A comprehensive analysis of the oil derived from the canola seeds is presented in the nutritional assessment in section 5.1. A detailed analysis of the separate fatty acids showed that the level of erucic acid in the transformed lines Ms8 and Rf3, and the hybrid cross between these two lines, was equivalent to the commercial control varieties and the non-transformed counterpart (none detected in all lines tested). Furthermore, data on the fatty acid profile of several transformed lines, including the open pollinated Topas 19/2 line and Ms and Rf lines, showed that the levels of erucic acid were not above 1% and were generally less than 0.1%. These values were observed over different years of growth (1991-1995) in a number of different locations.

Because of considerable seasonal and locality variation, the transformed lines were compared to a significant number of non-transformed control varieties. The data indicate that the percentage of erucic acid in the transformed lines was always within the same narrow range as the control varieties, and that all lines tested (including non-transformed) were below the reported literature value for canola oil (below 1%).

4.1.2 Glucosinolates

Data were presented on the measured levels of glucosinolates in seeds and meal from transgenic lines T45, Topas 19/2, Ms1, Ms8, Rf1 and Rf3, together with a range of non-transformed varieties (at least fifteen control lines) when grown at locations in Canada, Belgium, Sweden and France over a number of seasons between 1991 and 1996.

In addition, some lines were tested following spraying with glufosinate-ammonium at variable rates from 2.5L/ha to 10L/ha (data not presented in this report).

The data show no differences in the level of glucosinolates in any of the transformed lines when compared to the control varieties. These results support the conclusion that neither the presence of the introduced genes, nor the application of glufosinate-ammonium affected the levels of glucosinolates in the seed or meal of the transformed plants. The variation was greater between locations than between transformed and non-transformed lines.

As the meal is not consumed by humans and is only used as animal feed, these data mainly serve to illustrate that there were no unexpected changes in the level of glucosinolates in the seeds of the genetically modified canola, when compared to a large number of commercial control varieties and the non-transformed counterpart.

4.2 Potential toxicity of novel protein

As canola oil from the various transformed lines has been shown to contain no traces of protein (see Section 3.3), humans are extremely unlikely to ever be exposed to the novel proteins through consumption of canola oil derived from these lines. Furthermore, the absence of toxicity of both PAT and NPTII is well documented in the scientific literature – both proteins are readily digested in conditions that mimic mammalian digestion (see Section 4.3 on potential allergenicity) and no adverse effects in various acute oral toxicity tests using laboratory animals have been documented.

4.2.1 PAT

The OECD (1999) states that there is no evidence available indicating that the PAT protein is toxic to either humans or other animals. In addition, data demonstrating the absence of acute oral toxicity of the PAT protein in mice have been evaluated previously by ANZFA in relation to another application (Application A380 - DBT-418 corn). In a 14-day feeding study using bacterially produced purified PAT enzyme, laboratory mice, which were administered high levels of the protein (5.05 g/Kg bodyweight) by gavage feeding, showed no significant treatment-related toxic effects (Merriman, 1996). From this study, the acute oral LD_{50} of PAT protein was concluded to be >2575 mg/kg bw.

In accordance with these results and other available evidence, an exemption from the requirement to establish a maximum permissible level for residues of PAT, and the genetic material necessary for its production, was granted by the United States Environmental Protection Agency in April 1997 (USEPA, 1997).

Furthermore, the metabolite that results from detoxification of the herbicide in glufosinate-ammonium tolerant canola, N-acetyl–L-glufosinate, is non-toxic to both plants and mammals, including humans (OECD official use document, 1999).

4.2.2 NPTII

The potential toxicity of NPTII has been evaluated by ANZFA for a number of different applications for GM foods[6] where acute oral toxicity studies in mice have been submitted for assessment (refer to Application A382 – New Leaf® Potatoes, safety assessment, section 4.2). The safety of this protein has also been considered on numerous occasions in the peer reviewed scientific literature (Flavell *et al.* 1992, Nap *et al.* 1992, Fuchs *et al.* 1993a, Fuchs *et al.* 1993b). In all instances it has been concluded that NPTII is non-toxic to humans. This conclusion also applies to NPTII expressed in the canola lines that are the subject of this application as the NPTII used is identical to the NPTII assessed for toxicity on previous occasions.

4.3 Potential allergenicity of new proteins

Studies submitted:

Van den Bulcke, M., 1997. Phosphinothricin acetyl transferase, neomycin phosphotransferase II, barnase, barstar allergenicity assessment: a common approach. Plant Genetic Systems Internal report 000463/ALLERMVDB/01.

Bremmer, J.N. & Leist, H. 1996. Statement on the lack of allergenic potential of PAT-protein and Glufosinate Tolerant crops containing PAT-protein. Report No. 96.0351.

Many foods have been reported to cause allergies in some people, and it is well established that this is primarily due to an immune reaction to a particular protein component of the food, whereas fats or oils are not generally associated with such reactions. However, the seed meal, containing the seed proteins, is used only for animal feed because of the presence of particular toxins (glucosinolates). The quality requirements of commercial canola oil production dictate the absence of protein in the final product. Consequently, due to the exclusive consumption of the oil component of canola seeds, humans are not exposed to any of the plant proteins including the novel proteins introduced through the genetic modification.

There are four novel proteins (PAT, barnase, barstar, NPTII) to be considered in this application. The protein expression analyses demonstrated that the introduced PAT protein is present in the leaves, stems and seed of all of the transformed lines. However, in the Ms1, Rf1, Rf2 and Topas 19/2 lines only, the NPTII protein is below the limit of detection in the seed using the most sensitive methods available to date. In addition, the barnase and barstar proteins are restricted to particular floral tissues only in the Ms and Rf lines (and hybrid crosses of these lines), and are not present in the seeds of the plants from which the oil is derived.

Notwithstanding the absence of protein in the final food, the potential allergenicity of the new proteins introduced to the transformed canola lines has been evaluated by comparing certain molecular and biochemical properties of these new proteins to those of known allergens. The comparison includes a range of features to be considered using information available on food allergens already known and identified. Common physical characteristics of known allergens include a molecular weight ranging from 15-70 kDa, and usually poor digestibility.

[6] Applications A379 – Bromoxynil tolerant cotton, A382 – New Leaf® potatoes, A383 – New Leaf Y® potatoes, A384 – New Leaf Plus® potatoes.

Comparing the physical properties of the novel proteins with those of known allergens and considering other factors such as the relative abundance in the food and the presence of significant amino acid similarity to that of known allergens provides a range of criteria that are relevant to potential allergenicity.

The submitted data showed that the molecular weight of the introduced proteins PAT (approx. 22kD) and NPTII (approx. 29kD) are within the molecular weight range exhibited by known allergens, while the barnase and barstar proteins are below this range (12kD and 10kD respectively). As determined by ELISA, the levels of both PAT and NPTII proteins are <0.002% of total extractable protein in the seeds[7].

In addition, the amino acid sequence of the introduced proteins PAT, barnase and barstar was compared with amino acid sequences of known allergens (inhalation and food allergens) from both plant and animal origin available on three public protein databases - AA HIV, PIR and SwissProt. This comparison revealed that the novel sequences do not exhibit any significant amino acid homology with published sequences of toxins or allergens. The additional study by Van den Bulcke (1997, PGS Internal report), which included the NPTII protein, confirmed this finding.

Further evidence is available to indicate that the PAT protein in particular lacks any of the characteristics of known allergens. Common plant food allergens are usually glycosylated proteins and most are tolerant to heat denaturation, remaining stable during the high temperatures involved in cooking or processing (Taylor, 1995). However, the PAT protein lacks glycosylation sites and studies have determined that the enzyme is heat labile and is completely inactivated by temperatures above 75°C. Using Western blot analysis, experiments conducted by Shulz *et al.* in 1997 (Internal reports listed below) found that although the purified protein was not degraded by an experimental heat treatment at temperatures up to 100°C, a centrifugation experiment demonstrated that the protein is denatured at temperatures above 40°C.

4.3.1 Digestibility of PAT

Studies submitted:

Schulz, A. (1993). L-Phosphinothricin -N-Acetyltransferase, Inactivation by pig and cattle gastric juice. Biologische Forschung C, Biochemie der Pflanzen, Hoechst Aktiengesellschaft, Frankfurt. Hoechst Report 93.02.

Schulz, A. (1994). Digestion of the Phosphinothricin Acetyltransferase Enzyme in Human Gastric Fluid (Simulated). Hoechst Schering AgrEvo Ltd., Research Biochemistry, Frankfurt, Germany. Company Report No. AS 94.12E.

Schulz, A., Lutge, K. and Taggeselle, P. (1997). Stability of the Phosphinothricin Acetyltransferase Enzyme: Heat stability and digestion in Simulated Gastric Fluid and Simulated Intestinal Fluid. Hoechst AgrEvo, Frankfurt, Germany. Company File No. A58686.

Typically, most food allergens are resistant to digestion, proteolysis and other forms of hydrolysis (Bargman *et al*., 1992). The applicant conducted a number of studies to test whether the PAT protein, which is expressed in all transformed lines, is susceptible to proteolytic degradation.

[7] The study reports are based on pooled data from the lines Ms1 and Rf1/Rf2.

When tested in simulated human digestive fluids, the results of studies using Western blot analysis showed that PAT protein (purified from over-expressing *E. coli*) was readily degraded within seconds. The degradation of the protein was dependent on the presence of proteases, pepsin in simulated gastric fluid (SGF) and pancreatin in simulated intestinal fluid (SIF). The protein was also rapidly inactivated (within one minute) by acidic conditions in dog and pig gastric fluid and with bovine rennet-bag fluid (pH 1.3). Inactivation of PAT protein in bovine paunch fluid, which has a neutral pH (7.1), was slower but occurred within 30 minutes.

4.3.2 Digestibility of NPTII

The NPTII protein has been comprehensively assessed with respect to potential allergenicity in previously published work by Fuchs *et al*., in 1993 (a,b) and in other applications assessed by ANZFA (see section 4.2.2). In these studies, large quantities of recombinant protein were generated for extensive physical and biochemical analyses, and to provide sufficient material for a rodent feeding study. The results of the analyses support the food safety aspects of the NPTII protein present in four of the transformed canola lines in this application, by establishing that the protein underwent rapid inactivation and degradation in simulated digestive conditions and that it does not exhibit structural characteristics of known food allergens.

4.4 Conclusion

Of the four possible novel proteins introduced into canola plants, only the PAT and NPTII proteins are present in the seed. However, humans are extremely unlikely to be exposed to either of these proteins through the consumption of canola oil because of the stringency of the commercial processing in removing plant proteins from the final food product. Nevertheless, the scientific evidence indicates that both PAT and NPTII are non-toxic to humans and exhibit very limited potential as food allergens.

5. NUTRITIONAL ISSUES

Studies submitted:

MacDonald, R. (1997) A Comparison of Moisture, Oil, Protein, Ash, Carbohydrate, Gross Energy and Amino Acid Levels of Harvested Seed From Transgenic *Brassica napus* Line HCN-19 and a Standard Commercial Variety AC Excel. Analysis performed at: Smith Laboratory, NOVAMANN International, Toronto, Ontario, Canada. Report No. AC197-42.

MacDonald, R. (1997) Effect of Glufosinate Ammonium Treatment on the Composition of Glufosinate Tolerant Canola Meal and Oil. Report No. AC 197-07.

MacDonald, R. (1998) Seed Composition Characteristics of the Line SW02631 (T45/Topas 19/2). Report No: AC198-19.

Belyk, M. (1999) Comparison of HCN28 (pHoe4/AcII) Glufosinate Resistant Canola Fatty Acid Profile and Glucosinolate Content with Innovator (pOCA/Ac) Glufosinate Resistant Canola and Three Standard Commercial Varieties in 1994 and 1995. Report No: AC196-02/01.

Beriault, J.N. (1999) The Effect of Glufosinate Ammonium on the Seed Composition of T45 Glufosinate Tolerant Canola, POS Pilot Plant Corporation, Analytical Services Divisions, SK, Canada. Study Number: 98AC13.

Canola oil is a relatively recent inclusion in the human diet brought about through intensive plant breeding of oilseed rape during the past thirty years. This systematic modification by conventional breeders to improve nutritional and functional characteristics is supported by extensive research relating to seed composition, oil and meal quality and seed processing performance, which in turn provides a sound basis for analysis of the properties of new varieties of canola, including those generated using gene technology.

The purpose of this section of the safety assessment is to evaluate key nutrients in canola oil in order to compare equivalent data from the transformed lines, the non-transformed counterpart and published literature ranges obtained for conventional varieties of canola. This process includes a study of the major constituents that are characteristic of canola seeds, with particular reference to the oil as a human food. The process also may take into account natural variation in composition due to genetic variability and environmental factors, which are known to be major variables in determining the measured range obtained for most constituents.

The term canola has been registered and adopted in Canada to describe the oil (seeds and plants) obtained from the cultivars *B. napus* and *B. campestris*. In 1986 the definition of canola was amended to refer to *B. napus* and *B. campestris* lines containing <2% erucic acid in the oil and <30 µmol/g glucosinolates in the air-dried, oil-free meal (Codex, Downey, 1995). These varieties are referred to as double low (00) varieties. The applicant states that all of the genetically modified canola lines under assessment in this application, by definition, must comply with the above specifications to be permitted for use in commercial production of canola products.

The concerted breeding program to reduce or remove the presence of the natural toxicants in rapeseed oil has resulted in more extensive investigations, in both animal and chemical studies, than most other edible vegetable oils. Canola oil is characterised by a low level of saturated fatty acids, a relatively high level of monounsaturated fatty acids (oleic acid) and an intermediate level of polyunsaturated fatty acids (linoleic and linolenic acid).

Detailed compositional analyses were conducted on the seeds from transformed lines Ms1, Ms8, Rf1, Rf2, Rf3, T45 and Topas19/2. The analyses included measurements of glucosinolates, protein and oil content of the seeds and the fatty acid profile of the oil.

The data presented in Table 5 are a compilation of data showing that the percentage of oil in canola seeds harvested from transformed varieties is comparable to both the non-transformed counterpart and to commercial control varieties. These data demonstrate that the presence of the *bar* or *pat* genes, *barnase*, *barstar* and *nptII* (in some lines only) has not resulted in any change to the constituent levels of oil in the transformed seeds. The data were collected over a number of seasons from 1991 to 1995 and in a number (up to 9) of different locations in Canada.

Table 5: Oil content as a percentage of the seed from varieties of canola plants grown and tested in Canada. The values are the maximum and minimum measurements recorded over a number of seasons and at a number of different locations for any particular line. The groupings within the bolded lines represent concurrent analyses.

Canola varieties	Oil content (%seed)
Topas 19/2	40.1 – 48.0 (1991-1993)
Non-transformed (8 lines)	36.2 – 48.3 (1991-1993)
Male sterile (Ms1)	35.2 – 47.8 (1991-1993)
Fertility restorer (Rf1)	36.3 – 48.6 (1992-1993)
Cross (Ms1xRf1)	35.6 – 47.4 (1992-1993)
Drakkar control	35.1 – 49.0 (1991-1993)
Rf1	38.2 – 51.9 (1993-1994)
Rf2	38.7 – 51.7 (1993-1994)
Ms1xRf1	38.2 –51.4 (1993-1994)
Ms1xRf2	37.5 – 52.3 (1993-1994)
Drakkar control	39.0 – 53.0 (1993-1994)
Male sterile (Ms8)	37.5 – 44.1 (1995)
Fertility restorer (Rf3)	36.8 – 47.5 (1995)
Ms8 x Rf3	39.1 – 48.1 (1995)
Non-transformed counterpart	37.7 – 48.5 (1995)
Commercial varieties	37.0 – 45.6 (1995)

5.1.1 Fatty acid composition

The fatty acid composition of the oil derived from a number of transformed and non-transformed lines was analysed in detail. The measurements include 11 different key fatty acids, including in particular, the erucic acid (C22:1) content of the oil. As well as control varieties (eg. Drakkar), different generations of the male sterile lines (Ms1 and Ms8) were tested together with different generations of the fertility restorer lines (Rf1, Rf2 and Rf3), multiple backcrosses of Ms and Rf lines in different canola varieties and unrestored (Ms/control) and restored (Ms/Rf) hybrids. The seed samples were collected from plants grown at locations in Belgium, France, Sweden, Canada and the United Kingdom, and following treatment with different application rates of phosphinothricin up to 40 l/ha[8]. Seed samples were generally analysed by external laboratories[9] to determine % humidity, % oil, % protein, glucosinolate content and composition, as well as fatty acid composition.

Due to the amount of information provided, all of the data are not presented in this report. However, a representative set of data is presented in Table 6, which includes literature values for commercial non-transformed canola varieties. The profiling and quantification analyses clearly demonstrate that the 11 key fatty acid components are comparable in all of the oils tested from both a number of genetically modified canola varieties and a range of non-transformed control varieties. Variation across environmental conditions was greater than any variation between transformed and non-transformed canola plants.

[8] Within the hybrid canola program, standard selection level is determined at 5 l/ha.
[9] Laboratorium ECCA NV, Belgium; Plant Genetic Systems (PGS) Gent, Belgium; PGS, Canada; University of Guelph, Canada.

Table 6. **Minimum and maximum values of fatty acids (% of total) in canola oil (tested in Europe and North America in 1995).**

Entry	Oil composition (% of total)										
	C16:0 Palmitic acid	**C16:1 Palmitoleic acid**	**C18:0 Stearic acid**	**C18:1 Oleic acid**	**C18:2 Linoleic acid**	**C18:3 Linolenic acid**	**C20:0 Arachidic acid**	**C20:1 Gadoleic acid**	**C20:2 Eicosadienoic acid**	**C22:0 Behenic acid**	**C22:1 Erucic acid**
OSR literature	3 - 6	<0.5	1 - 3	50 - 66	18 - 28	6 - 14	<0.5	1	traces	<0.5	<1
Non-transgenic counterpart	3.9 – 5.2	0.0 - 0.4	1.6 - 2.1	60.8 - 68.4	16.3 -19.9	6.2 - 10.7	0.5 - 0.7	0.9 - 1.4	0.0 - 0.0	0.0 - 0.4	0.0 - 0.0
Ms8	3.9 - 4.8	0.3 - 0.4	1.5 - 1.8	60.1 - 67.6	16.4 -20.4	7.3 - 10.9	0.4 - 0.7	0.9 - 1.5	0.0 - 0.9	0.2 - 0.4	0.0 - 0.0
Rf3	3.9 - 5.1	0.3 - 0.4	1.5 - 1.7	58.2 - 67.4	17.4 -21.8	6.6 - 11.6	0.5 - 0.6	1.0 - 1.6	0.0 - 0.9	0.0 - 0.4	0.0 - 0.0
Ms8xRf3	3.9 - 4.5	0.2 - 0.3	1.6 - 1.8	60.9 – 67.4	17.4 -19.7	7.0 - 11.1	0.5 - 0.6	1.0 - 1.5	0.0 - 0.0	0.0 - 0.4	0.0 - 0.0
Other commercial control varieties	4.1 - 5.3	0.3 - 0.4	1.5 - 1.9	57.7 - 66.0	17.7 - 1.9	8.1 - 12.1	0.5 - 0.7	1.0 - 1.6	0.0 - 0.1	0.0 - 0.4	0.0 - 0.0
PGS hybrids based on Ms8 or Rf3	3.9 - 4.8	0.2 - 0.3	1.6 - 1.9	61.9 - 66.3	16.8 –19.2	7.9 - 10.6	0.3 - 0.7	1.0 - 1.5	0.0 - 0.3	0.0 - 0.4	0.0 - 0.0
PGS1 (Ms1xRf1)/ PGS2 (Ms1xRf2)	4.2 - 4.6	0.2 - 0.3	1.8 - 1.9	62.2 - 66.9	16.8 -17.8	7.4 - 10.5	0.5 - 0.7	1.1 - 1.9	0.0 - 0.0	0.0 - 0.3	0.0 - 0.0

5.1.2 Processing characteristics

The applicant provided a detailed analytical evaluation of seeds, processed oil and meal from transformed and non-transformed (isogenic) canola plants that were extracted using benchtop processing designed to emulate commercial processing. The study was carried out by POS Pilot Plant Corporation (Canada) and was undertaken to compare the minor constituent composition of canola fractions at particular stages of processing (seed cleaning, seed tempering, flaking, cooking, pressing, solvent extraction, desolventising, blending, degumming, refining, washing, bleaching, hydrogenation and deodorisation) through to completion of the final product. During the study, the processing characteristics and sample-stage composition of the transformed material from the Ms1, Ms8, Rf1, Rf2, Rf3, T45 and Topas 19/2 lines were compared to the processing characteristics and sample-stage composition of non-transformed canola varieties presently grown.

The quality of the oil samples in this study was comprehensively analysed in terms of both compositional and physical parameters. The compositional parameters measured included fatty acid composition, free fatty acid content, phosphorus, sterol, chlorophyll and tocopherol levels. In addition, some physical properties exhibited by the oil were determined including specific gravity, viscosity, smoke point, and a cold test. Finally, the oxidative stability of the oil of the transformed and non-transformed samples was determined via a number of analytical tests (peroxide value, p-anisidine value, AOM) carried out at different stages of the refining process.

The results of the processing analyses do not show any significant differences between the transformed canola seeds containing the male sterility and fertility restorer gene constructs (Ms1, Ms8, Rf1, Rf2, Rf3), T45 and Topas19/2 and non-transformed canola, in any of the parameters tested. The processing characteristics and the quality of the oil derived from the transformed seed and control seed were essentially identical throughout the processing stages. Furthermore, there were no compositional differences between the transformed and non-transformed samples and all of the seedlots produced measurements that were within a typical range for canola oil.

Although canola meal is not consumed by humans, this by-product of seed processing contains the seed proteins. Furthermore, the amounts of fibre, minerals and glucosinolates of the meal are nutritionally important in animal feed and also serve as additional biochemical indicators of any compositional differences brought about in the seed due to the genetic modification. As for most other commodity crops, the nutrient composition of canola seeds is known to vary considerably depending on environmental conditions and genetic factors and certain fluctuations in composition are considered to be normal. A detailed comparison of the meal derived from seed samples harvested from transformed and non-transformed hybrid canola was subsequently conducted on material obtained during the simulated industrial processing.

The analyses were sufficiently detailed to measure a number of individual glucosinolates in the whole seed (alkenyls, indols) and in the desolventised meal. The results of these analyses indicate that the protein and glucosinolate content of the transformed canola and/or the meal containing the bar/pat, and/or nptII, barnase and/or barstar genes corresponding to Ms1, Ms8, Rf1, Rf2, Rf3, T45 and Topas19/2 lines, were completely within the ranges observed for non-transformed canola varieties.

5.1.3 Proximate analysis following herbicide treatment

A study was conducted to directly compare the composition of seed derived from the open pollinated T45 line, untreated and treated with the herbicide glufosinate-ammonium. The plants were grown under normal agricultural conditions in field trials at two locations in Western Canada. Half of each plot was untreated and the remaining half was treated with Liberty® at a rate of 500 g active ingredient/ha, applied prior to bolting.

At harvest, a minimum of two 500 gram samples of canola seeds were taken from each treatment plot. In all cases, the untreated plots were sampled first, prior to sampling of the treated plots.

POS Pilot Plant Corporation was responsible for conducting a proximate analysis on the canola seed samples. The proximate analysis included moisture, oil, protein, ash and crude fibre expressed as a percentage of the seed. The analytical methods used were published, validated methods of the American Oil Chemists Society (5th Edition, 1998), and all results were statistically analysed. A summary of the results of these analyses are presented in Table 7, which represents the mean of 6 measurements for each treatment.

Table 7: Summary of Proximate Analysis on Canola Seed Comparing T45 Treated with T45 Untreated with Liberty®. Data from all sites combined.

VARIABLE	MEAN & STD T45 TREATED	MEAN & STD T45 UNTREATED	P-VALUE (T45 UNTREATED VS T45 TREATED)
% Moisture	5.09 ± 0.18	5.04 ± 0.19	0.646
% Oil	46.00 ± 1.50	46.80 ± 2.37	0.504
% Protein	22.54 ± 1.70	22.31 ± 2.28	0.851
% Ash	3.76 ± 0.15	3.59 ± 0.19	0.104
% Crude Fibre	10.99 ± 0.46	11.00 ± 0.29	0.953

The results demonstrate that there were no significant differences ($p>>0.05$) between the T45 canola seed samples from the untreated or treated plots for any of the proximate variables examined. In addition, the measured levels of protein and oil in both sets of seeds are consistent with similar proximate analyses for other canola varieties, including non-transformed varieties.

5.2 Levels of anti-nutrients

Consideration has been given to the use of canola meal in human nutrition as a source of food-grade protein. However, this has not been achieved so far due to the presence of components such as phytic acid and phenolic compounds. These compounds may not only add an astringent taste and flavour to the meal, but may also reduce the bioavailability of several minerals. In addition, the presence of glucosinolates has an effect on the quantity of digestible protein. As a consequence, the use of canola meal as a food product for human consumption cannot occur without improving the digestible utilization of the nutrients and limiting or destroying the anti-nutritional factors. The genetic modification to the Ms, Rf and open pollinated lines Topas 19/2 and T45 does not alter the food uses of the seeds.

There are no compounds present in canola oil that are known to exhibit anti-nutritional properties.

5.3 Ability to support typical growth and wellbeing

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

Animal feeding studies using the oil have not been conducted. The nutritional profile of the oil was determined by compositional analyses of the major components of the seed and these were found to be comparable to the conventional control lines. In addition, the level of dietary exposure to the novel proteins is expected to be zero, as effectively all contaminating plant protein is removed in the production of canola oil.

Where the human food in question is an oil, animal feeding studies are generally not considered feasible as the oil itself is unsuitable as a complete food for animals and may cause nutritional and biochemical imbalances if included in the diet in large quantities. Instead, in this application the applicant has provided two animal feeding studies using whole seed in support of the nutritional adequacy of particular glufosinate-ammonium tolerant lines.

Feeding study in chickens

Leeson, S. (1999). The Effect of Glufosinate Resistant Canola (Topas 19/2) on the Appearance and Growth of Male Broiler Chickens. AgrEvo report No. B002184.

As whole canola seeds can be utilised as a major component in the diet of broiler chickens, a study was conducted to compare the performance of broiler chickens fed glufosinate-ammonium tolerant canola (Topas19/2) with a standard commercially available canola cultivar. The applicant claims that these animals represent a very sensitive test species for a nutrient feeding study as a 15 fold increase in body weight occurs during the first 18 days of life and therefore any differences in nutrient availability are readily detectable in terms of the development of the chickens.

The study involved the use of 280 commercial strain male broiler chickens obtained at one day of age. The birds were weighed and allocated at random to 1 of 2 treatment groups, replicated 4 times, with 35 birds per replicate. The birds were maintained at temperatures and in environments that were consistent with normal brooding practice. They were cared for by agriculture assistants at the Arkell Poultry Research Station and according to required guidelines of the Canadian Council on Animal Care and with the approval of the University of Guelph Animal Care Committee, Animal Utilisation Protocol #96R072.

Birds were fed starter diets to 18 days of age at which time feed intake was measured and all birds were weighed individually. Grower diets were fed between 18 and 32 days, feed intake measured as before and all birds were again weighed individually. The finisher diets were fed between 32 and 42 days of age and the same protocol was followed. During the course of the experiment, which reared the birds on one of two diet treatments and varied only with the type of canola used in each diet, all occurrences of mortality were submitted to the Ontario Veterinary College, Department of Pathology for post-mortem examination.

The variables considered were initial body weight, 18, 32 and 42 day body weight, body weight gain in the different diet periods, feed intake and feed intake:body weight gain. The mortality rate was monitored and at the end of the study, various carcass characteristics were considered namely, chilled carcass weight and yield of deboned breast meat as a percent of carcass weight. For the statistical analysis, significance was accepted at $P<0.05$.

The results of this study showed that the source of the canola in the 3 diet types had no effect on body weight, feed intake, feed intake:body weight gain or percent mortality over the experimental period ($P>0.05$). The mortality rate was normal for this fast-growing strain of bird, where 5-8% is routinely expected. In all measured parameters, the birds were unaffected by the substitution of the genetically modified canola for the conventional form in the experimental diets.

Digestibility study in rabbits

Maertens, L. and Van Eeckhoutte, A. (1993). Digestibility of Transformed Oilseed Rape for Rabbits, Government Agricultural Research Centre, Belgium.

A study was conducted in rabbits to investigate the nutritive value of transformed canola compared to the control line, Drakkar, also used in the compositional studies. Drakkar is the elite variety that was used to generate the hybrid parental transformed lines and is a double low variety, containing little erucic acid and low glucosinolates (<15 µmoles/g). The hybrid line tested in this study was a cross between the Ms1 and Rf1 parental lines, and represents plants that are direct sources of canola oil for human consumption, rather than the parental lines themselves used in the hybrid breeding program.

Seed from the original variety (Drakkar) and the Ms1/Rf1 cross were offered to growing rabbits in order to study the digestibility of protein, fat, crude fibre and to compare bioavailable gross energy. Thirty 7-week old rabbits of both sexes were randomly assigned (10 animals per diet) to either a basal diet containing no canola, or to one of two experimental diets containing either transformed canola or unmodified control canola seed to a level of 30% in the basal diet.

It was noted in the study that due to the high fat content of canola seeds, the experimental diets were both very fat-rich (>16%) and, as a result, the quality of the feeding pellets was poor. In order to avoid deblending of the feed, the experimental diets were pelleted several times until satisfactory pellet quality was obtained comparable to the basal diet. In addition, a preliminary adaptation period of one week was allowed with the diets before measurements were commenced. This was necessary to overcome differences in palatability noted with the experimental diets containing both the control and transformed canola seeds.

The rabbits were fed *ad libitum* and faecal output was measured and recorded daily for the duration of the 4-day study. The individual faecal samples were analysed for dry matter, ash, nitrogen, fat and crude fibre following AOAC methods (Association of Official Analytical Chemists, 1990). In addition, gross energy was measured by an adiabatic bomb calorimeter. Apparent whole tract digestibility coefficients (DC) and digestible energy (DE) content of each diet were calculated from the respective dry matter intake and output, as well as their corresponding nutrient content.

Results and conclusion

Due to the high digestibility of both experimental canola seed diets, the DC was significantly higher than the basal diet ($p<0.01$). Furthermore, as both test diets containing the canola had higher energy content than the basal diet, some measurements were significantly higher for both test diets compared with the basal diet. For example, despite the allowed period of adaptation, the inclusion of 30% canola seed to the basal diet resulted in negative effects on the feed intake of the animals during the first days of the study. However, these effects diminished with time and the intake of feed was sufficient for the duration of the experiment, taking account of the increased dietary DE content of both experimental diets.

Of greater importance, the results demonstrate that there were no observed differences between the two experimental diets containing canola seeds, either transformed or non-transformed, indicating that the feeding value of the hybrid line (derived from transformed parental lines) is comparable to the original control variety. The conclusion therefore is that the seeds from the hybrid line (produced by a conventional cross between the Ms1 and Rf1 transformed lines) exhibited at least similar zootechnical performance as seeds from the original Drakkar variety.

6. Conclusion

The compositional analyses indicate that the genetic modifications in the various transformed lines of canola in this application have not produced any significant changes in the seeds of the plants with respect to processing characteristics, oil content, oil composition, oil quality (physical properties), protein content or glucosinolate content. The edible canola oil fraction derived from the transformed seeds is therefore indistinguishable from the oil fraction derived from unmodified seeds, when grown at a variety of locations representing different environments and following applications of the herbicide glufosinate-ammonium. On the basis of the submitted data, canola oil from the open pollinated lines T45 and Topas 19/2 and the pollination control lines Ms1, Ms8, Rf1, Rf2 and Rf3 (and crosses) is considered substantially equivalent to the oil from non-transformed canola.

The extensive compositional data are supported by the two feeding studies submitted by the applicant which both confirm that the introduced genes have not resulted in adverse effects on the nutritional adequacy of the transformed canola seeds. Both test species, rabbits and chickens, showed that the transformed canola seeds provided equivalent nutrition to control diets and adequately supported the growth of young animals. These studies do not raise any public health or safety concerns with respect to the overall nutritional characteristics of the oil from transformed canola.

References

Australia New Zealand Food Authority (1999). Guidelines for the safety assessment of foods to be included in Standard A18 – Food Produced Using Gene Technology.

Bargman, T.J., Taylor, S.L. and Rupnow, J.H. (1992). Food Allergies. In: Handbook of Natural Toxins Volume 7, Food Poisoning, 337-370. Published by: Marcel Dekker, New York, USA.

Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B. and Schaller, H. (1982). Nucleotide sequence and exact localisation of the neomycin phosphotransferase gene from transposon Tn 5. Gene, **19**, 327-336.

Codex Standard for Edible Rapeseed Oil (1993), Codex Stand 24 – 1981, Codex Alimentarius Commission, Rome.

Codex Standard for Named Vegetable Oils (1999), Codex-Stan 210 - 1999, Codex Alimentarius Commission , Rome.

Davies, J. *et al* (1986) Aminoglycoside-aminocyclitol antibiotics and their modifying enzymes In: *Antibiotics in laboratory medicine*, 2nd ed., Lorian, V., (ed) pp 790-809.

Davis, B.D., Dulbecco, R., Eisen, H.N. and Ginsberg, H.S. (1980). *Microbiology, 3rd Edition*. Harper and Row Publishers, USA, 1355 pp.

De Block, M. and De Bouwer, J. (1993) Engineered fertility control in transformed Brassica napus L.: Histochemical analysis of anther development. Planta, **189**, 218-225.

Downey, R.K. (1995). The Story of Canola/Rapeseed. Proceedings from the Gene Technology Workshop, LibertyLink. Nov 1-3, Saskatoon, SK, Canada.

Flavell, R.B., Dart, E., Fuchs, R.L. and Fraley, R.T. (1992). Selectable marker genes: safe for plants? *Bio/Technology* **10:** 141-144.

Fuchs, R.L., Heeren, R.A., Gustafson, M.E., Rogan, G.J., Bartnicki, D.E., Leimgruber, R.M., Finn, R.F., Hershman, A. and Berberich S.A. (1993a). Purification and Characterisation of Microbially Expressed Neomycin Phosphotransferase II (NPTII) Protein and its Equivalence to the Plant Expressed Protein. Bio/Technology **11**, 1537-1542.

Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W. and Berberich, S.A. (1993b). Safety of the neomycin phosphotransferase II (NPTII) protein. Bio/Technology 11.

Hartley, R.W. (1968). A reversible thermal transition of the extracellular ribonuclease of *Bacillus amyloliquefaciens*. Biochemistry, 7, 2401-2408.

Hartley, R.W. (1988). Barnase and barstar: expression of its cloned inhibitor permits expression of a cloned ribonuclease. Journal of Molecular Biology, 202, 161-168.

Hartley, R.W. (1989). Barnase and barstar, two small proteins to fold and fit together. Trends in biochemical Science, **14**, 450-454.

Kärenlampi, S. (1996). *Health effects of marker genes in genetically engineered food plants*. Nordic Council of Ministers, Copenhagen, Denmark, 66 pp.

Krebbers, E., Seurinck, J., Herdies, L., Cashmore, A.R. and Timko, M.P. (1988). Four genes in two diverged subfamilies encode the ribulose-1,5-bisphosphate carboxylase small subunit polypeptides of *Arabidopsis thaliana*. Plant Molecular Biology, 11, 745-759.

Mariani, C., De Beuckeleer, M., Truettner, J., Leemans, J. and Goldberg, R.B. (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature, **347**, 737-741.

Mariani, C., Gossele, V., De Beuckeleer, M., De Block, M., Goldberg, R.B., De Greef, W. and Leemans, J. (1992). A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. Nature, **357**, 387-394.

Mauguen, Y., Hartley, R.W., Dodson, E.J., Dodson, G.G., Bricogne, G., Cothia, C. and Jack, A. (1982). Molecular structure of a new family of ribonucleases. Nature, **297**, 162-164.

Merriman, T.N. (1996) An Acute Oral Toxicity Study in Mice with Phosphinothricin Acetyltransferase (PAT) Protein. DEKALB Genetics Corporation, 62 Maritime Drive, Mystic, CT 06355-1958. DEKALB Study No. DGC-95-A18.

Molecular Biotechnology for Plant Food Production, ed. O. Paredes-Lopez. Technomic Publishing Company, Inc., 1999.

Nap, J-P., Bijvoet, J. and Stiekema, W.J. (1992). Biosafety of kanamycin-resistant transgenic plants: an overview. *Transgenic Crops* **1:** 239.

Organisation for Economic Cooperation and Development (OECD) (1999). Consensus Document On General Information Concerning The Genes And Their Enzymes That Confer Tolerance To Phosphinothricin Herbicide. Series on Harmonisation of Regulatory Oversight in Biotechnology No. 11.

Shaw, K.J., Rather, P.N., Hare, R.S. and Miller, G.H. (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol. Rev*. **57**: 138-163.

Smeaton, J.R., Elliot, W.H. (1967). Isolation and properties of a specific bacterial ribonuclease inhibitor. Biochimica and Biophysica Acta, **145**, 547-560.

Strauch, E., Walter, A., Renare, A., Wohlleben, W., Puhler, A., Eckes, P., Gunter, D., Uhlmann, E., Hein, F. and Wengenmayer, F. (1993). Phosphinothricin-resistance gene active in plants, and its use. European patent 275957 B1.

Taylor, S.L. Lemanske, R.F. Jr., Bush, R.K. and Busse, W.W. (1987). Food allergens; structure and immunologic properties. *Ann. Allergy* **59:** 93-99.

Taylor, S.L. (1992). Chemistry and detection of food allergens. *Food Technol.* **39:** 146-152.

Taylor, S.L., Nordlee, J.A. and Bush, R.K. (1992). Food allergies. In: *Food Safety Assessment*, Finley, J.W., Robinson, S.F. and Armstrong, D.J. (eds). ACC Symposium Series 484, American Chemical Society, Washington, D.C.

Taylor, S. (1995). Evaluation of the allergenicity of foods developed through biotechnology. In: Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. Publisher: University of California, Division of Agriculture and Natural Resources, Oakland, California, USA.

Thompson, C.J., Movva Rao, N., Tizard, R., Crameri, R., Davies, J., Lauwereys, M. and Botterman, J. (1987). Characterisation of the herbicide resistance gene *bar* from *Streptomyces hygroscopicus*. The EMBO Journal, 6, 2519-2523.

The Lipid Handbook, 2nd edition (1994), Gunstone, F.D., Harwood, J.L. and Padley, F.B.. Published by Chapman & Hall, London. ISBN 0 412 43320 6.

United States Environment Protection Agency (USEPA), 1997. Phosphinothricin Acetyltransferase and the genetic material necessary for its production in all plants; exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register Volume 62, Number 70, pp17717-17720. http://www.epa.gov/fedrgstr/EPA-PEST/1997/April/Day-11/p9373.htm

Wehrman, A., Van Vliet, A., Opsomer,C., Botterman, J. and Shulz, A. (1996).The similarities of bar and pat gene products make them equally applicable for plant engineers. Nature Biotechnology **14**, 1274-1278.

WHO (1991). Strategies for assessing the safety of foods produced by biotechnology. Report of a joint FAO/WHO Consultation. World Health Organization, Geneva, 59 pp.

WHO (1993). Health aspects of marker genes in genetically modified plants. Report of a WHO Workshop. World Health Organization, Geneva, 32 pp.

Wohlleben, W., Arnold, W., Broer, I., Hillemann, D., Strauch, E. and Puhler, A. (1988). Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from Streptomyces Tu494 and its expression in *Nicotiana tabacum*. Gene **70**, 25-37.

Wohlleben, W., Alijah, R., Dorendorf, J., Hillemann, D., Nussbaumer, B. and Pelzer, S. (1992). Identification and characterisation of phosphinothricin-tripeptide biosynthetic genes in *Streptomyces viridochromogenes*. Gene **115**, 127-132.

Zambryski, P. (1992). Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 465-490.

ATTACHMENT 3

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[10]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[10] 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 PUBLIC SUBMISSIONS

A: First round submissions

1. National Genetic Awareness Alliance (Australia)

- 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 (Australia)

- 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 (Australia)

- 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 (Australia)

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

6. David Grundy (Australia)

- 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 (Australia) 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 (Australia)

- 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, Ungarie 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 Seymour, Richard and Sharon Moreham (see also below), Stuart Drury and Helen Murphy (All Australia), Brennan Henderson (New Zealand) – 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 (Australia)

▪ 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 (Australia)

▪ 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 (Australia)

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

22. Ian and Fran Fergusson (Australia)

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

23. Lyndal Vincent (Australia)

- 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 (Australia)

- 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 (Australia)

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

26. Diana Killen (Australia)

- 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 (Australia)

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

28. David and Edwina Ross (Australia)

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

29. Beth Schurr (Australia)

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

30. Beth Eager (Australia)

- 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 (Australia)

- 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 (Australia)

- 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 (Australia)

- 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)(Australia)

- 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 (Australia)

- 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 (Australia)

- 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 the increased use of chemicals and the 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 (Australia)

▪ 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 (New Zealand)

▪ 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 (New Zealand)

▪ 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 WTO agreement, but consider that the primary role of ANZFA is the assurance of health and safety.

41. Safe Food Campaign (New Zealand)

- 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 (New Zealand)

- 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 – New Zealand)

- 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 (New Zealand)

- 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 (New Zealand)

- 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

B. SECOND ROUND PUBLIC SUBMISSIONS

The Draft Risk Analysis Reports (formerly referred to as the Full Assessment Report) for A372, A375, A378 and A379 were released for a 6-week period of public comment on 1 March 2001. At the end of the public comment period (20 April 2000) a total of 23 submissions had been received. These are summarized below.

1. Australian Food and Grocery Council (AFGC)

- Supports the approval of the four applications:

  A372 -Oil derived from - glufosinate ammonium tolerant canola lines Topas 19/2 And T45 and;

  -Oil derived from glufosinate-ammonium tolerant and pollination controlled lines Ms1, Ms8, Rf1, Rf2 And Rf3;

  A375 Food derived from glufosinate ammonium tolerant corn line T35;

  A378 Food derived from glyphosate-tolerant sugarbeet line GTSB77; and

  A379 Oil and linters from bromoxynil-tolerant cotton transformation events 10211 and 10222.
- Submits that as ANZFA has concluded that foods encompassed by the four applications do not raise any public health and safety concerns, that there should be no reason for retaining the generic prohibition on their use merely because they are GM foods.
- Supports the application of the revised labelling requirements of Standard A18 to the products encompassed by these four GM applications.

2. Bentleigh-Bayside Gene Alert, Campaign for Safe Food

- Opposes all four of the GM food applications because of overwhelming concerns about the risks to health and the environment, particularly in the use of herbicides.
- Supports independent testing and questions the role and validity of overseas approvals of GM commodities in the Australian process.
- Contends that the safety assessments were questionable and scientifically unsound because of apparent inadequacies in the toxicity testing and in the conclusions drawn from the animal feeding studies.

- Considers that the assessment should include possible changes to the food product as it is metabolised by livestock that are bred for human consumption.
- Advises that the precautionary principle should be adopted in relation to the use of antibiotic resistance marker genes.

3. New Zealand Ministry of Health

- Supports the conclusions of the ANZFA Draft Risk Analysis Reports for all four applications, that the foods are safe for human consumption.
- Considers that the most important data are the molecular characterisation of the inserted DNA and compositional analyses, requiring presentation of as much raw data as possible, and that brief summaries of other issues are all that is required, especially where the same proteins have been previously assessed.

4. Anne FitzSimon (NZ)

- Opposes the approval of all four applications primarily for ethical reasons and concerns about safety.
- Demands detailed labelling of GM foods to enable consumer choice.

5. Nelson GE Awareness Group (Susie Lees)

- Do not support the approval of the four GM applications because they consider that GM foods pose unique public and environmental health risks.
- Submits that there has been no independent scientific testing of the products.
- Suggests complete removal of these foods from the market until safety testing and long term feeding studies of at least 12-18 years duration have been completed.
- Considers that the new labelling provisions do not capture all foods produced using gene technology.
- A372 – expresses grave concerns associated with the use of the *barnase*/*barstar* gene system (uses the term 'terminator technology'), and claims that whole canola seeds are used in certain bakery products.
- Opposes the use of antibiotic resistance genes in all of the applications.

6. Kate Clinch-Jones

- Opposes all of the applications on the basis that the respective Draft Risk Analysis Reports do not address the potential public health and safety issues associated with the genetic modifications.
- Claims that the safety assessments are not comprehensive, and lack adequate scientific evidence and peer review.
- Opposes the use of the herbicides glyphosate and glufosinate-ammonium because of concerns relating to potential toxicity in humans and the environment.
- Criticises the regulatory impact statement for each GM application. Contends that benefits of prohibiting the sale of GM foods include the protection of the integrity of the food chain, avoiding irreversible environmental damage, upholding the precautionary principle and meeting consumer demands.
- Disagrees with government obligations in relation to the WTO.
- Disagrees with ANZFA's assessment and discussion of the possibility for horizontal gene-transfer and refers to supporting scientific articles.
- Expresses concerns about food products derived from stock animals that consume GM crops.
- States that because of the confidentiality of some of the information, potential hazards may not be identified by independent reviewers.

- Suggests that ANZFA seek advice about antibiotic resistance genes from microbiology and infectious disease specialists.
- Supports full proteome analysis on all GM foods.
- Recommends that an expert team of advisors be established to design scientifically sound feeding studies that also consider ethical issues.

7. Food Technology Association of Victoria Inc.

- Supports approval of the four applications (A372, A375, A378 and A379) provided ANZFA is satisfied with their safety and that the foods will be appropriately labelled for the benefit of consumers.

8. Adrian Elliot (Aus)

- Supports the approval of the GM food applications and regards these as trailblazers.
- Claims that the new GM foods will assist in keeping Australian industry in step with developments made by the rest of the world.
- Considers that both industry and consumers benefit from the development of new varieties and new technology.
- Comments that the public would benefit from a national education campaign to provide greater awareness of the food supply and to promote public understanding of the technology, the safety and regulation of the products arising from this technology.

9. Aventis CropScience

- Suggests minor amendments and corrections to the Draft Risk Analysis Reports for each of the applications, which are addressed in the respective Final Risk Analysis Reports.

10. GeneEthics Network (Arlene Buchan and Bob Phelps)

- Opposes all four of the applications because of perceived adverse effects on the environment and public health.
- Opposes the use of the herbicides glyphosate, glufosinate ammonium and bromoxynil because of concerns about toxicity.
- States that ANZFA's regulatory impact assessment fails to acknowledge that primary production could be negatively affected by GM crops. ANZFA should consider the economic effects of its decisions.
- Considers that ANZFA's safety assessment process is too narrowly focussed and fails to consider environmental and animal health issues.
- Disagrees that ANZFA's assessments adopt a cautious approach.
- Considers that the safety assessment reports lack sufficient information to demonstrate food safety, and do not adequately consider the possibility of trace amounts of unintentional or unanticipated products.
- Expresses outrage that there is no post-market surveillance system in place to monitor any effects of crop release or GM food consumption.
- States that the new labelling regime is too lax and contravenes the rights of consumers to know whether foodstuffs have been genetically modified.

11. Public Health Association of Australia Inc (PHAA)

- Asserts that ANZFA does not respond to all issues raised in their previous submissions.

- Expresses concerns on the use by ANZFA of the concept of substantial equivalence.
- Raises concerns on the use of antibiotic resistance marker genes during GM crop development.
- Claims that ANZFA does not require data in support of applications that is generated by independent laboratories other than the applicant.
- Raises concerns regarding the lack of detail in reporting of the parameters investigated in the acute toxicity tests on CP4 EPSPS, GUS and protein 34550.

A375

- Raised concerns about the enzyme specificity of the PAT gene.
- Raised concerns about the adequacy of the toxicity studies.
- Commented on small compositional differences between GM and non-GM varieties of corn.
- Asserted that that there were no spray data submitted with the application.
- Commented on the adequacy of the feeding study submitted with the application.

A372

- Comments on the toxicity of glufosinate-ammonium.
- Expresses concerns relating to the use of the *barnase* gene in canola.
- Considers that the compositional analyses were insufficient to comprehensively assess the canola.
- Contends that nutritional studies would be useful.
- Considers that animal feeding studies using every line under assessment should be submitted.
- Objects to the commercial-in-confidence aspects of the application.

A378

- Raised concerns about the adequacy of the toxicity studies.

A379

- Raised concern about the adequacy of the toxicity studies.
- Raised concerns about ANZFA's assessment of the toxicity of bromoxynil and its break down products.
- Commented on the compositional differences between the GM versus control lines.

12. Consumers' Institute

- Provides comments on the GM applications as a group, not as individual foods, stating that the regulatory process should take into consideration new scientific information or data as, or when, it becomes available and react accordingly.
- Favours ongoing monitoring of any long term effects
- States that consumers are primarily concerned with the apparent lack of independent verification of testing carried out by developers of the products, as well as the failure to do long term testing and animal testing of the products.
- Expresses a lack of confidence in the assessment process and in the principle of 'substantial equivalence' because of concerns that unexpected changes may not be identified.
- Considers that the system of regulation applying to new medicines, which require random controlled trials, is rigorous and the same has not been applied to GM foods.

13. Claire Bleakley (NZ)

- States that the foods covered by applications A372, A375, A378, A379, A385 and A386 should not be allowed on the market until the New Zealand Royal Commission has reported and labelling of GM foods is in place.
- Expresses concerns about the safety of GM foods in general.
- Considers that the previous decisions do not reflect a "high degree of consumer confidence" in the regulations as per the ANZFA Act.
- Considers that not enough information is provided to consumers.
- States that long-term studies are required to show that the genetic constructs do not cause harm to the environment.

14. National Council of Women of Australia Inc

- Does not support the approval of any of the four applications due to concerns that GM foods have not been tested either adequately or appropriately.
- Provided comment on individual applications, which will be addressed within the specific issues section of the Final Risk Assessment Report.
- Raised concerns about the environmental impact as well as toxicity, neurotoxicity and teratogenicity of glufosinate ammonium and provided information about overdoses of glufosinate ammonium.
- Is concerned that GM applications for herbicide tolerant crops will result in the increasing use of herbicides.
- Considers that any health risk is not acceptable as the technology is not needed to feed the world or wanted by consumers.
- States that no further GM applications should be accepted until the Office of the Gene Technology Regulator has addressed the environmental, social and ethical issues, as ANZFA has no community consultative or ethics group to consider these issues.
- Considers that the benefits of the technology accrue to the applicant.
- Considers that ANZFA is not responding to objections raised previously and is repeating previous responses, leading to little desirable outcome from a community and public interest perspective.
- Believes that ANZFA is dismissing public opinion given that the majority of submissions are against approval of GM applications.
- States that the labelling laws are inadequate.

15. Consumers' Association of South Australia Inc

- Supports the submissions made by the National Council of Women.

16. Food Branch, South Australian Department of Human Services

- A372 – considers that data on tocopherol levels would enhance the compositional analyses; questions whether the proposed approval should refer to the hybrid lines rather than to the Ms and Rf parental lines.
- A375 – compositional analyses should relate to the line for which the proposed approval is sought; Vitamin A and carotene analyses were not provided for line T25.
- A378 – questions details in the drafting of the proposed variation to the *FSC*.

17. GE Free New Zealand (RAGE)

- Opposes all four of the applications, A372, A375, A378 and A379.
- Provides a list of health and medical concerns that are claimed to be attributable to gene technology.
- Expresses grave fears about the possible health consequences of GM foods in general.
- Application specific concerns include:
  A379 – the use of the CaMV 35S promoter and the presence of antibiotic resistance genes
  A372 – the use of antibiotic resistance genes.

18. Sandra Jacobs (NZ)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.
- Considers that GE foods are polluting other crops, particularly GE canola containing the *barnase* gene.

19. Brian Lister and Lorraine Leader (NZ)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.
- Considers that the safety of GE foods cannot be guaranteed.

20. Paul Elwell-Sutton (NZ)

- Opposes application A372, because of a lack of confidence in the independence of the laboratories that generated the assessment data.
- Expresses concerns about the possible presence of novel substances or proteins in the canola meal that may enter the food supply.
- Considers that the labelling provisions are not adequate to ensure that consumers will be able to know about GE foods in products.
- Considers that ANZFA has not addressed the issue of the possible transfer of antibiotic resistance marker genes to gut microorganisms of stock, as animals are fed on canola meal and stubble.
- ANZFA's reports do not address the precautionary principle.
- Considers that GE food could have effects on the ageing process in animals, including humans, which ANZFA failed to consider in the assessment.
- Expresses concern that food approval will lead to planting of GE canola in New Zealand that will then lead to inevitable contamination of other crops.
- ANZFA has not adequately considered consumers in the assessment process.
- Opposes the remaining GM applications A375, A378 and A379 for the same reasons.

21. Julian Yates (NZ)

- Opposes all four of the applications, A372, A375, A378 and A379 due to the lack of long term independent testing.

22. Oraina Jones (NZ)

- Opposes all four of the applications, A372, A375, A378 and A379 due to philosophical and ethical concerns relating to the environment and health.

23. Leila Huebner (NZ)

- Opposes application A372, because of concerns about the use of the *barnase* gene both from an environmental perspective (effect on neighbouring canola crops) and from a human and animal health perspective.

ATTACHMENT 6

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received in response to the Section 14 Gazette Notice, express general views against the use of gene technology and assert that food produced using this technology is unsafe for human consumption. A number of general issues were raised in these submissions that 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/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

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

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD). The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

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 laboratory animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

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

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

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

3. *Substantial equivalence*

A number of submitters express concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some reject 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. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

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

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for approximately ten years and has been an integral part of the safety assessment of some 40 products.

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

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

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

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

- *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 express 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 focus on this issue. Specifically, the submissions call for comprehensive labelling of foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters base their demands for full labelling on the presumption that all foods produced using gene technology are unsafe, even where no novel genes are present, and on consumer "right to know" arguments. It is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

- *Evaluation*

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard A18 (Volume 1) is now also known as Standard 1.5.2 in the joint Australia New Zealand Food Standards Code (Volume 2). To allow adequate time for compliance to the new provisions of the Standard, it will come into effect on 7 December 2001, twelve months after the date of gazettal.

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

Exempt from these requirements are:

- highly refined food, where the effect of the refining process is to remove novel genetic material and/or protein;
- processing aids and food additives, except where novel genetic material and/or protein is present in the final food;
- flavours which are present in a concentration less than or equal to 0.1 per cent in the final food; and
- food prepared at point of sale (e.g. restaurants, takeaway food outlets).

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

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

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

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

- *Evaluation*

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

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

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

11. *Public consultation and information about gene technology*

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

- *Evaluation*

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

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

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

In response to the concerns raised in public submissions with regard to gene technology and GM foods, ANZFA has prepared a public discussion paper on the safety assessment process for GM foods[11], available at no charge on request. Since completion, this document has been widely distributed 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 as part of the comprehensive assessment processes of the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand. Since June 2001, OGTR regulates all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

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

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

- the Australia New Zealand Food Authority (ANZFA)
- the Therapeutic Goods Administration (TGA)

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

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

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

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

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

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 Mandatory Food Standard 1999 (Maximum Residue Limits of Agricultural Compounds).

ATTACHMENT 7

STATEMENT OF REASONS

APPLICATION A372 - FOR RECOMMENDING A VARIATION TO STANDARD A18 OF VOLUME 1 AND STANDARD 1.5.2 OF VOLUME 2 OF THE *FOOD STANDARDS CODE* FOR THE APPROVAL OF OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT CANOLA LINES TOPAS 19/2 AND T45, AND FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA LINES Ms1, Ms8, Rf1, Rf2 and Rf3

The Australia New Zealand Food Authority (ANZFA) has before it an Application received on 12 March 1999 from Aventis CropScience Pty Ltd seeking amendment to Standard A18 of Volume 1 (Standard 1.5.2 of Volume 2) of the *Food Standards Code* for the approval of oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3.

ANZFA recommends the adoption of the draft variation for the following reasons:

- There are no public health and safety concerns associated with the genetic modifications introduced into the canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3;

- Oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 is as safe and wholesome as oil from other commercially available canola;

- On 7 December 2001, food products containing oil derived from canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 will require labelling if it can be shown that novel DNA and/or protein is present in the final food; and

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

The commencement date of the draft variation be the date of gazettal.

REGULATION IMPACT

The Authority has undertaken a regulation impact assessment process that also fulfils the requirement in New Zealand for an assessment of compliance costs. The process concluded that the amendment to the Code is necessary, cost effective and of benefit to both food producers and consumers.

WORLD TRADE ORGANIZATION (WTO) NOTIFICATION

Australia and New Zealand are members of the WTO and are bound as parties to WTO agreements. In Australia, an agreement developed by the Council of Australian Governments (COAG) requires States and Territories to be bound as parties to those WTO agreements to which the Commonwealth is a signatory.

Under the agreement between the Governments of Australia and New Zealand on Uniform Food Standards, ANZFA is required to ensure that food standards are consistent with the obligations of both countries as members of the WTO.

In certain 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. 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).

This matter was notified to the WTO because there is significant international interest in the safety of GM foods and the proposed amendments are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters.

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A372 - OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA LINES TOPAS 19/2, T45, MS1, MS8, RF1, RF2 AND RF3.

To commence: on gazettal

[1] *Standard A18 of Volume 1 and Standard 1.5.2 of Volume 2 are varied by inserting in Column 1 of the Table to clause 2 -*

Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and glufosinate-ammonium tolerant and pollination controlled canola lines Ms1, Ms8, Rf1, Rf2 and Rf3.