7 March 2001
13/01

DRAFT RISK ANALYSIS REPORT

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 lines Ms1, Ms8, Rf1, Rf2 and Rf3.

Note:

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

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

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EXECUTIVE SUMMARY

Background

An application was received from Aventis CropScience Pty Ltd[1] on 12 March 1999 seeking approval for oil derived from genetically modified (GM) canola lines Topas19/2, T45, Ms1, Ms2, Rf1, Rf2 and Rf3 under Standard A18 – Food Produced Using Gene Technology. All of the lines are tolerant to the herbicide glufosinate-ammonium and, in conjunction with the herbicide tolerance, the Ms and Rf lines contain a gene affecting pollination. The lines are known commercially in Australia and New Zealand as LibertyLink® open pollinated (Topas 19/2 and T45 only) and InVigor® hybrid canola. This report describes the scientific assessment of the application.

Issues addressed during assessment

i.   *Safety Evaluation*

The oil derived from glufosinate-ammonium and pollination controlled canola lines has been evaluated according to ANZFA's safety assessment guidelines. This involves an extensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the new GM food. This approach has been used to establish whether the food produced from the GM canola lines is as safe and nutritious as food produced from non-GM varieties of canola.

The sources of the new genetic elements present in the canola are either other plant species or non-pathogenic soil bacteria such as *Agrobacterium*. The detailed information available on the genetic modification indicates that either one, two or three new proteins may be expressed in the canola plants, depending on the specific line. The information also shows that the novel genetic material is stably inserted in the canola plant genome and is maintained over several generations and in different environments.

The dietary exposure to any of the new proteins present in the plants is expected to be virtually zero. This is because the oil extracted from canola seeds is the only fraction used as human food, and the technical information indicates that there is no detectable protein in the oil. In addition, canola oil is subject to stringent quality control measures which remove proteins and natural toxicants during processing. Nevertheless, data on the potential toxicity and allergenicity of the proteins encoded by the transferred genes have been reviewed and indicate that the new proteins expressed in the GM canola are non-toxic and would be unlikely to be allergenic to humans.

Compositional analyses demonstrate no significant differences in key constituents between the GM canola lines, the non-GM counterpart, or a range of commercial

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[1] Formerly AgrEvo Pty Ltd.

varieties, as measured in a number of different environmental locations and following treatment with the herbicide.

The potential impact on human health arising from the transfer of novel genetic material to cells in the human digestive tract was also considered. After evaluation of the information, the presence of novel genetic material, including an antibiotic resistance gene, in some of the GM canola lines is not considered to pose any additional health and safety concerns.

In assessing all of the above data, ANZFA concludes that consumption of the oil derived from the glufosinate-ammonium tolerant Liberty Link open pollinated canola and the InVigour lines for use in the hybrid program does not raise any public safety concerns.

ii. Labelling

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

When the amended Standard (A18 in the Australian *Food Standards Code*, 1.5.2 in the *Australia New Zealand Food Standards Code*) comes into effect on 7 December 2001, oil made 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.

iii. Public Submissions

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

Conclusions

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

- the introduced genes in canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 are not considered to produce any additional public health and safety risk;

- oil derived from the genetically modified canola lines is equivalent to other commercial non-genetically modified canola varieties in terms of its food safety and nutritional adequacy.

Recommendation

Based on the data submitted in the application, ANZFA concludes that food oil derived from canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 and

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

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

INVITATION FOR PUBLIC SUBMISSIONS

The Authority has completed a Draft Risk Analysis Report on this application, (referred to as the 'Full Assessment' in section 15 of the Act), which includes a draft Safety Assessment report and a draft variation to the Australian *Food Standards Code* (Volume 1) and the recently adopted *Australia New Zealand Food Standards Code* (Volume 2). The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the Food Standard *Codes,* and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' in section 16 of the Act).

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

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

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

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A372** at one of the following addresses:

Australia New Zealand Food Authority

PO Box 7186	PO Box 10559
Canberra Mail Centre ACT 2610	The Terrace WELLINGTON 6036
AUSTRALIA	NEW ZEALAND
Tel (02) 6271 2222 Fax (02) 6271 2278	Tel (04) 473 9942 Fax (04) 473 9855
Email info@anzfa.gov.au	Email nz.reception@anzfa.gov.au

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

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

BACKGROUND TO THE APPLICATION

ANZFA received an application from Aventis CropScience Pty Ltd on 12 March 1999 seeking amendment to the Australian *Food Standards Code* to include oil derived from seven glufosinate-ammonium tolerant canola lines (Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3) in the Table to clause 2 of Standard A18 – Food Produced Using Gene Technology. Two lines, Topas 19/2 and T45, are open pollinated canola lines while the Ms and Rf lines are designed as parental breeding lines for use in generating hybrid crosses by controlling pollen production.

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. The presence of this 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 a hybrid plant. In the hybrid, expression of barstar counteracts the effects of the barnase and the plants are fully fertile, producing greater yields of seeds than either of the parental lines.

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 breeding lines for generating higher yielding commercial varieties of canola. More general benefits may also flow to the community as a result of reduced primary production costs.

PUBLIC CONSULTATION

ANZFA completed a Notice of Application (formally referred to as the Preliminary Assessment Report) upon receipt of the application and 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.

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). Further details on WTO are included in 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 genetically modified foods, the proposed changes to Standard A18 sought in this application are considered to raise potential Technical Barrier to Trade or Sanitary/Phytosanitary matters, and will therefore be notified to the WTO.

ISSUES ADDRESSED DURING ASSESSMENT

1.   Safety assessment (see Attachment 2)

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

Nature of the genetic modification

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.

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

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.

There are potentially four novel proteins that are expressed in the genetically modified canola lines: PAT, NPTII, barnase and barstar. 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 the Ms and 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.

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 any plant proteins, including the novel proteins, through consumption of canola oil. Notwithstanding the absence of protein in the oil, there is no evidence to suggest 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 extremely low levels, and when present (4 lines only) the NPTII protein is also at almost undetectable levels. 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.

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.

Conclusion

On the basis of the data submitted in the present application, oil derived from the glufosinate-ammonium tolerant and pollination controlled canola lines, and subsequent hybrid 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 open pollinated genetically modified canola lines T45, Topas 19/2 and pollination controlled lines 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.

2.   Labelling of oil derived from lines Topas 19/2,T45, Ms1, Ms8, Rf1, Rf2 and Rf3

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

Until the new labelling requirements take effect, the provisions in the original Standard A18 apply. Under these provisions, oil derived from 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.

3.   Issues arising from public submissions

3.1   General issues

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

3.2   Specific issues

This section of the report will address those issues raised in public submissions that are specific to an 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 detectible 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-ammonia 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[3] 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.

4.   Risk management

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

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

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

A public discussion paper on the safety assessment process for GM food[4] is widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies, for example OGTR and NZ government agencies, are also actively addressing broader issues concerning gene technology.

5.   Regulatory Impact Assessment

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

CONCLUSIONS

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

It is concluded that:

- the introduced genes in canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 are not considered to produce any additional public health and safety risk;

- based on the data submitted in the present application, oil derived from the genetically modified canola lines is equivalent to other commercial non-genetically modified canola varieties in terms of its food safety and nutritional adequacy.

RECOMMENDATION

Based on the data submitted in the application, ANZFA concludes that food oil derived from canola lines Topas 19/2, T45, Ms1, Ms8, Rf1, Rf2 and Rf3 and subsequent crosses is as safe for human consumption as food from other commercial canola varieties, and therefore recommends that the Australian *Food Standards Code* and the Australia New Zealand *Food Standards Code* be amended to give approval to the sale of such food in Australia and New Zealand. The proposed amendment to Standard A18 and Standard 1.5.2 is provided in Attachment 1.

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

ATTACHMENTS

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

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A372 - OIL DERIVED FROM GLUFOSINATE-AMMONIUM TOLERANT AND POLLINATION CONTROLLED CANOLA LINES TOPAS19/2, T45, MS1, MS8, RF1, RF2 AND RF3.

To commence : On gazettal

The Food Standards Code is varied by:

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

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.

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

DRAFT 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.

There are potentially four novel proteins that are expressed in the genetically modified canola lines: PAT, NPTII, barnase and barstar. 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 the Ms and 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[5] 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 Australia and New Zealand 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 the genetically modified canola, however not all lines contain all the traits. The genes coding for the new traits are the bacterial genes *bar* (or *pat*), *barnase* and *barstar*.

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 one or both 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 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.

2.   DESCRIPTION OF THE GENETIC MODIFICATION

2.1   Methods used in the genetic modifications

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

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.

2.2.4   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, dessication, 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. is 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 hybrid, 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 which 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 hybrid. 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 hybrid. 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[6]/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.

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.

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[6] Food and Agriculture Organization.

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 phosphinotrhicin 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.

iv.   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 and 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 genetically modified or not, 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[7] 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.

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

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. 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[8].

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.

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

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.

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[9]. Seed samples were generally analysed by external laboratories[10] 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.

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[9] Within the hybrid canola program, standard selection level is determined at 5 l/ha.
[10] 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 contructs (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 well being

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

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 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 or 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 adaption 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 fecal output was measured and recorded daily for the duration of the 4 day study. The individual fecal 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.

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DRAFT REGULATORY IMPACT ASSESSMENT

Regulatory Impact Assessment

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

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

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

	Benefits	Costs
GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	• no benefits were identified.	• 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	• Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	• 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[11]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

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

CONSUMERS	Benefits	Costs
	• 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.	• 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.

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 phyto sanitary measure applied:

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

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

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

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

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

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

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

TBT Notifications

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

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

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

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

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

1. **National Genetic Awareness Alliance (Aus)**
    - believes that the patenting of life-forms and living processes represents a violation of human rights, threat to food security, impediment to medical research and a threat to animal welfare
    - believes that current GM techniques are inherently hazardous, and have been shown recently to offer no benefits
        - lower yields with high pesticide input
        - intensification of the corporate monopoly on food
        - spread of antibiotic resistance marker genes and promoter sequences
        - possible increase of allergenicity due to spread of transgenic pollen
    - urges governments to use precautionary principle and carry out research into sustainable agricultural methods
    - calls for suspension of trials and sale of GM products and public inquiry.

2. **Pola Lekstan and Anna Clements (Aus)**
    - are concerned that approval without long-term testing may pose a health threat, that more GM food means less choice for those wanting to avoid it, that Bt may affect non-target organisms, and that herbicide resistance may lead to overuse of chemicals.

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

4. **Australian GeneEthics Network**
    - believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
        - direct health effects of pesticide residues
        - possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
        - the possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
        - insertion of viral DNA could create new and virulent viruses
        - the possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
        - the threat to GE-free status export markets
    - believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

5. Public and Environmental Health Service (Aus)
- believes that the data provided should cover both the intentional and unintentional effects of the genetic modification. The unintended consequences of random insertion of new genetic material into the host genome could include loss or change of function of gene or controlling element, disregulation or amended regulation of the gene or controlling element, or production of a novel hybrid protein which could occur in an unregulated manner. They should also cover any compositional changes e.g. nutrients, 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 (Aus)
- considers that the expression of Bt toxins and other chemicals in plant tissues removes the choice of washing chemicals off fruit and vegetables. Believes that Roundup Ready crops have glyphosate or glufosinate molecules genetically attached
- believes that GM crops should not be used for feed given to animals bound for human consumption, that products encouraging antibiotic resistance should not be used, and that labelling should be mandatory for all products containing GM ingredients

7. Leesa Daniels (Aus) Member of the Genetic Engineering Action Group
- believes that:
    - scientific research although limited, has brought concerns to light
    - substantial equivalence is a subjective principal
    - comprehensive and mandatory labelling must be urgently implemented
    - the cauliflower mosaic virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
    - antibiotic marker genes could lead to increase in antibiotic resistance
    - several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

8. Australian Food and Grocery Council
- fully endorses the policy of minimum effective regulation, supports these applications, and considers that food manufacturers should make their own choice with regard to use of GM crops or products derived from them
- believes that since the growth of GM crops has been approved overseas, they would support their growth in Australia if approved through the GTAC/GMAC/OGTR process
- considers it unfortunate that ANZFA has not negotiated "equivalence" agreements for products already approved overseas to enable approval without having to carry

out its own safety assessment. In the absence of such an agreement it supports the ANZFA safety assessment process.
- believes that an appropriate information and labelling scheme would enable consumers to make an informed choice

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

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

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

A379, A388
- state concern over the persistence and toxicity of bromoxynil, and consider that these have not been adequately assessed by the US FDA. They understand that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself, and believe that a safety assessment needs to be done on this too. This is apparently the main residue, and they believe that this may appear in cotton oil and linters.

A372, A375, A380, A381, A386
- with respect to glufosinate ammonium, state concern about toxicity, neurotoxicity, teratogenicity and residues in food, soil and water. They believe that Monsanto is likely to apply for an increase in the MRL, and that such increases are likely to constitute a health hazard

A380, A382, A383, A384, A385, A386
- raise issues of adverse effects of Bt toxins on non-target insects and think that it needs more study.

A387
- believe that raising the amount of a nutrient in a food may have unknown drawbacks e.g. affecting the efficacy of other nutrients

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

13. Meat New Zealand
A379
- concerned at how labelling regulations will apply to sausage casings that may contain cotton linters even if they are not to be eaten, i.e. are effectively a processing aid. Think that labelling should only be used to advise the sausage manufacturer not consumers.

14. BRI Australia
- supports the approval of all 13 applications provided ANZFA is satisfied with their safety

15. Food Technology Association of Victoria Inc.
- supports the approval of all 13 applications provided ANZFA is satisfied with their safety

16. Diane Davie (Aus)
- believes all 13 applications should be rejected, since they have not undergone human safety testing here or overseas, and have not been assessed on their ethical merits
- believes that risks include:
  - bacterial and viral vectors which could affect human physiology
  - herbicide and insect-resistance genes, which could increase allergies and antibiotic resistance
  - environmental risks
- also believes that ANZFA must heed the concerns of consumers opposed to GM foods

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

- believe that most Australians and New Zealanders do not want GM foods, there are no benefits, and deferral would not be disadvantageous. Approval should be delayed until they are proven safe.
- feel that there is insufficient time to assess these applications thoroughly, and there are so many products under development that there is a high overall risk of a major disaster
- believe that GM foods encourage pesticide use, and applications have made for commercial purposes only, and also that here could be commercial benefit to Australia and New Zealand in remaining GM-free.

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

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

20. Dr Rosemary Keighley (Aus)
- will not purchase foods unless they are certified GM-free. Believes that Australian producers who do not actually use GM products, but who fail to label them as such, will suffer.

21. Nicola Roil (Aus)
- believes that GM foods pose health threats and may contaminate non-modified crops

22. Ian and Fran Fergusson (Aus)
- believe there has been inadequate testing, and are concerned about possible side-effects

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

24. Fay Andary (Aus)
- does not want any of the 13 products covered by the applications to be approved for inclusion in the food supply

25. John and Francesca Irving (Aus)
- thinks that no GE foods should be approved for inclusion in the food chain

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

27. Sheila Annesley (Aus)
- does not want any of the 13 foods included in the food supply.

28. David and Edwina Ross (Aus)
- state concern for the future food supplies and well-being of their grandchildren.

29. Beth Schurr (Aus)
- wishes to protest against the threat of GM foods, the possible future detrimental effects and the further endangering of the planet.

30. Beth Eager (Aus)
- as a parent is concerned that neither the long-term effects on health nor the environment are being considered.

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

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

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

34. Tim Carr (Convenor of the Emergency Committee against GE Foods)
- believes that GM-foods are produced using a radical and unpredictable new technology so should be subject to more rigorous testing
- states that it is unknown how the introduced gene will interact with and influence genetic expression in the host genome, and could change the chemical nature of the food

- considers that health risks could result from the increased use of pesticides, and also that ANZFA should consider wider environmental, ethical and socio-economic issues.

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

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

37. John and Sandy Price (Aus)
- approval of GM foods and seeds should not be allowed, as it is an affront to the sovereignty of Australia and the dignity of the Australian people. The results of the experiment cannot be reversed.

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

39. R A Randell (NZ)
- believes that all GM products should be placed under a moratorium until the Royal Commission of Inquiry has considered the issue, and until all scientific, philosophical, ethical and moral issues have been looked at.

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

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

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

41. Safe Food Campaign (NZ)

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

42. Jocelyn Logan, Caroline Phillips (NZ)

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

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

- considers that the GM issue should be reconsidered in the light of the release of internal FDA documents made available for a recent lawsuit aimed at amending their policy. Attached document (presentation given by Steven Druker, Alliance for Bio-integrity) suggests that:
  - scientist's warnings have been ignored
  - FDA policy may be illegal, violating the Food, Drugs and Cosmetic Act – Mr Druker believes that the term generally-regarded-as-safe (GRAS) cannot apply to foreign DNA

44. Stephen Blackheath (NZ)

- argues that ANZFA's approach to safety assessments is scientifically unsound:
  - antibiotic resistance marker genes have been cited as being potentially dangerous by groups other than ANZFA e.g. the Royal Society
  - unanticipated toxins and allergens are a concern, and it is suggested that the ANZFA process does not adequately consider these possibilities
  - doesn't address the question of whether risks exist that are unique to the GM process
  - it relies on data from the manufacturers themselves, with little sway given to evidence from public submissions. Companies have vested interests the

results and cannot be trusted (also gives evidence of Monsanto's past dishonesty)
- believes that ANZFA is subject to undue influence through the directors, and is biased towards being pro-GM
- suggests that RoundUp Ready soybeans are not substantially equivalent as the stems have been found to be more brittle than traditional lines, and may be lower in phytoestrogen content
- also cites the lawsuit being brought by the Alliance for Bio-integrity, and the internal FDA documents that suggest concern from FDA scientists, as evidence of the FDA ignoring important evidence.

45. Claire Bleakley (NZ)
- believes that approval should be rejected for various reasons:
  - they may be against Maori views
  - further long-term trials are needed and should be carried out by ANZFA themselves - certain trials have apparently shown effects on immune system, allergies and rare syndromes
  - health concerns of pesticide overuse
  - the possibility of horizontal gene transfer with respect to antibiotic resistance transfer
  - lack of labelling and the use of the unsatisfactory 'substantial equivalence' concept, which makes hazard difficult to assess
  - there is no substantial gain to consumers

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, expressed general views against the use of gene technology and asserted that food produced using this technology is unsafe for human 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 is to establish that the new food is at least as safe as existing foods. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

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

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

2. *The need for long-term feeding studies*

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

- *Evaluation*

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to 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 expressed concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some rejected the premise of substantial

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

- *Evaluation*

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

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

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

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

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

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

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

- *Evaluation*

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

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

5. *Potential toxins and allergens*

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

- *Evaluation*

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

6. *Antibiotic resistance*

Some submitters raised concerns about an increase in antibiotic resistance resulting from the use of gene technology. Some felt 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 expressed concern about the long term effects of transferring viral sequences to plants.

- *Evaluation*

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

environment if viral genes are transferred to plants because it may lead to the generation of new plant virus variants capable of infecting a broader range of plants. This is a matter that will be addressed by the Genetic Manipulation Advisory Committee (GMAC) on a case–by–case basis when it assesses such plants.

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

9. *Labelling of foods produced using gene technology*

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

- *Evaluation*

As early as August 1999, the Health Ministers comprising ANZFSC decided in-principle to require labelling of all genetically modified foods. However, due to the complexity of this issue, it was agreed that there was a need for a whole of government approach requiring input from all sectors of the community. To achieve this, the respective Cabinets of the Commonwealth, States, Territories and New Zealand established a Task Force to review the requirements for genetically modified food labelling.

On 28 July 2000, the ANZFSC met again to consider the outcomes of reports from the Task Force and other consultants, and 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 will be incorporated in to the new joint Australia New Zealand Food Standards Code. 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. Guidelines, to assist with compliance with the amended labelling provisions of the Standard, were released for public consultation on 7 December in conjunction with gazettal of the Standard. The period for public comment closes on 26 February 2001.

The new Standard will require 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.

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[12], 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*

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

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

- *Evaluation*

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

13. *Environmental concerns and the broader regulatory framework*

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

- *Evaluation*

These issues are considered in the assessment processes of GMAC in Australia and the Environmental Risk Management Authority (ERMA) in New Zealand. The Authority does not have the mandate to assess matters relating to environmental risks resulting from the release of food produced using gene technology into the environment. However, links exist between ANZFA and other regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs. In relation to genetically modified crops actually cultivated in Australia or New Zealand, ANZFA would not recommend the approval of a food derived from such a crop unless the appropriate clearance for general release from either GMAC or ERMA had been obtained, following environmental assessment.

In Australia, the current regulatory system includes a number of 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)
- 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).

In addition, the Office of the Gene Technology Regulator (OGTR) has been established to complement the existing regulatory framework. OGTR will supersede the existing arrangements now within the Genetic Manipulation Advisory Committee (GMAC), which advises on research and environmental release of GMOs. OGTR will regulate all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

All GM food will continue to be assessed and regulated by the Australia New Zealand Food Authority (ANZFA) under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as Australia New Zealand Food Standards Council (ANZFSC). However, there will be an interface between ANZFA and OGTR. Consequential amendments proposed to the ANZFA Act arising from the draft Gene Technology Bill 2000 will establish a statutory interface between OGTR and ANZFA. This will involve amendments to the ANZFA Act requiring the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (currently Standard A18).

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