ANZFA
Australia New Zealand Food Authority
TE MANA WHAKARITE KAI MŌ ĀHITEREIRIA ME AOTEAROA

10/02
26 June 2002

FINAL ASSESSMENT REPORT (INQUIRY - SECTION 17)

APPLICATION A388

OIL FROM BROMOXYNIL-TOLERANT CANOLA LINE WESTAR-OXY-235

THE AUSTRALIA NEW ZEALAND FOOD AUTHORITY

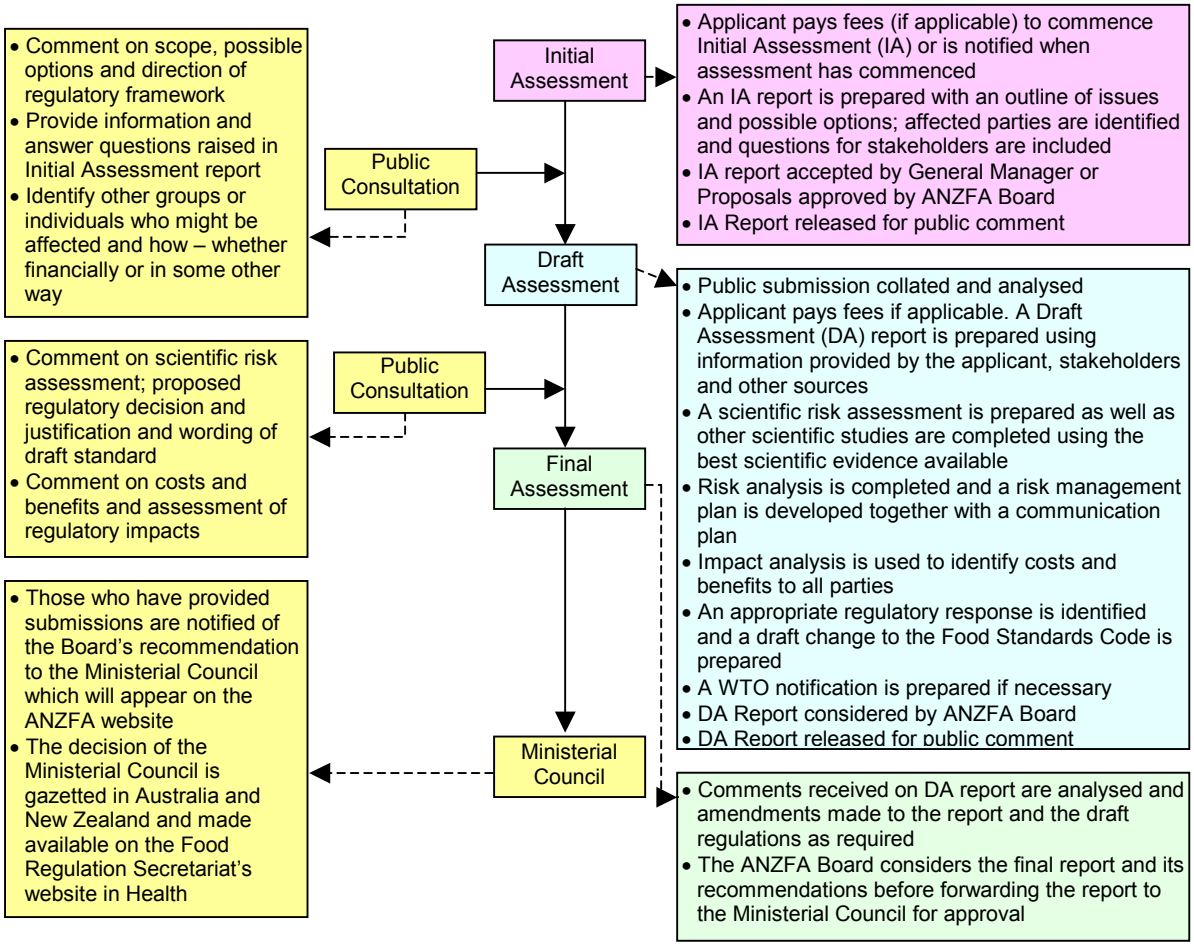
The Australia New Zealand Food Authority's (ANZFA) is a partnership between the Commonwealth Government, Australian State and Territory governments and the New Zealand Government. ANZFA is a bi-national, statutory body whose role, in association with others, is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply.

ANZFA seeks to achieve this goal by developing, varying and reviewing standards for food available for sale in Australia and New Zealand and through a range of other functions including national food surveillance and recall systems, conducting research, assessing policies about imported food and developing codes of practice with industry.

In developing and reviewing food standards for both Australia and New Zealand, ANZFA makes recommendations to change the food standards to the Australia New Zealand Food Standards Council, a Ministerial Council made up of Commonwealth, State and Territory and New Zealand Health Ministers. If the Council approves the recommendations made by ANZFA, the food standards are automatically adopted as regulations into the food laws of the Australian States and Territories and New Zealand.

STEPS IN DEVELOPING AND REVIEWING FOOD STANDARDS

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Australia New Zealand Food Authority Act 1991* (ANZFA Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



CONTENTS

EXECUTIVE SUMMARY AND STATEMENT OF REASONS

Regulatory problem

An Application was received from Rhone-Poulenc Rural Australia Ltd (now trading as Aventis CropScience Pty Ltd after its merger with AgrEvo) on 29 April 1999 for the approval of oil derived from bromoxynil-tolerant canola line Westar-Oxy-235 under the transitional arrangements of Standard A18 (clause 2A)/Standard 1.5.2 (clause 3). Food, containing oil derived from canola line Westar-Oxy-235, is thus already permitted in the food supply.

Objective

In addressing the issue of approving the sale and use of oil from bromoxynil-tolerant canola, the key objectives were the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, ANZFA also had regard for the need for standards to be based on risk analysis using the best available scientific evidence and the desirability of an efficient and internationally competitive food industry.

Options

Two regulatory options were considered. Option 1 involved the withholding of approval and Option 2 involved the granting of approval for the oil. Option 1 would result in the removal from sale of food containing oil from bromoxynil-tolerant canola whereas Option 2 would maintain the *status quo* and allow food containing oil from bromoxynil-tolerant canola to remain on the market.

Impact

The regulatory impact on all sectors of approving oil derived from bromoxynil tolerant canola is minimal because the food products are already permitted in the food supply under transitional arrangements, therefore the giving of approval merely serves to maintain the *status quo*.

Consultation

ANZFA undertook two rounds of public consultation in relation to this application. In response, 45 submissions were received during the first round, and 20 submissions were received in the second round. The majority of the submissions received were not supportive. Those opposing the application did so primarily on the basis of perceived health and environmental concerns. The food safety concerns raised in submissions have been addressed by the safety assessment report.

Conclusions and recommendation

Adoption of the draft variations, giving approval to the continued sale of oil derived from bromoxynil tolerant canola in Australia and New Zealand, is recommended for the following reasons:

- the genetic changes introduced into bromoxynil-tolerant canola line Westar-Oxy-235 are not considered to raise any additional public health and safety concerns;

- based on data supplied with the application and other available information, oil from bromoxynil-tolerant canola line Westar-Oxy-235 is as safe and wholesome as oil derived from conventional canola varieties;

- food products containing oil from bromoxynil-tolerant canola will be exempt from labelling unless it can be shown that novel DNA and/or protein is present in the final food; and

- the proposed amendment to the *Food Standards Code* is consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991; and

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

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

1. Introduction

An application was received from Rhone-Poulenc Rural Australia Ltd seeking approval of oil from bromoxynil-tolerant canola line Westar-Oxy-235.

The application was received on 29 April 1999 and was accepted under the transitional arrangements of Standard A18 (clause 2A)/Standard 1.5.2 (clause 3), which allow food, containing ingredients derived from canola line Westar-Oxy-235, to be on the market prior to finalisation of the assessment process. This arrangement is subject to certain conditions being met (see below). The assessment is at the final assessment stage.

2. Regulatory Problem

The provisions of Standards A18 / 1.5.2 require that genetically modified (GM) foods undergo a pre-market risk assessment before being offered for sale in Australia and New Zealand. For those foods that were already on the market prior to the Standard coming into effect, an exemption from pre-market approval applies under transitional arrangements provided that an application was accepted by ANZFA on or before 30 April 1999, that the food is lawfully permitted in a country other than Australia or New Zealand, and that the Ministerial Council has not become aware of evidence that the food poses a significant risk to public health and safety. Foods assessed under the transitional arrangements and confirmed as safe and subsequently approved by the Ministerial Council are listed in the Table to the Standard.

A new genetically modified variety of bromoxynil-tolerant canola (line Westar-Oxy-235, also known commercially as Navigator™ canola) was developed for commercialisation in Canada, where it is grown for both domestic use and for export. Although the current level of trade of canola and its commodities between Canada and New Zealand and Australia is

relatively small, some imported processed foods may contain oil derived from this genetically modified variety. Aventis CropScience Pty Ltd therefore made an application, under the transitional arrangements, to have Standard A18 – Food Produced Using Gene Technology (Standard 1.5.2 of Volume 2 of the *Food Standards Code*) amended to include oil from bromoxynil-tolerant canola line Westar-Oxy-235.

3. Objective

To determine whether food regulations can be changed to permit the sale of a GM food. Such an assessment needs to consistent with the section 10 objectives of the ANZFA Act.

ANZFA's objectives in developing and varying food standards are (in descending priority order):

(a) the protection of public health and safety; and
(b) the provision of adequate information relating to food to enable consumers to make informed choices; and
(c) the prevention of misleading or deceptive conduct.

In developing and varying food standards, ANZFA must also have regard to the following:

(a) the need for standards to be based on risk analysis using the best available scientific evidence;
(b) the promotion of consistency between domestic and international food standards;
(c) the desirability of an efficient and internationally competitive food industry;
(d) the promotion of fair trading in food.

In addressing the issue of approving the sale and use of food from bromoxynil-tolerant canola line Westar-Oxy-235, the key objectives were the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, ANZFA also had regard for the need for standards to be based on risk analysis using the best available scientific evidence and the desirability of an efficient and internationally competitive food industry.

4. Background

The herbicide-tolerant canola under consideration has been genetically modified to confer tolerance to bromoxynil, a herbicide used for the control of broad leaf weeds common in canola fields. The genetic change involved in the modification results in the transfer of one gene — the *oxy* gene from the soil bacterium *Klebsiella pneumoniae* subspecies *ozaenae*. The gene codes for nitrilase, an enzyme that breaks down bromoxynil and other related herbicides into non-phytotoxic compounds.

Navigator™ canola is not currently grown in either New Zealand or Australia, and is only imported as a highly processed product. Canola seeds are processed into two major products, oil and meal. The oil from the seeds is the only human food product being assessed as part of this application. Canola meal is principally used as an animal feed. Canola oil is a premium quality oil and is used in a variety of manufactured food products including salad and cooking oil, margarine, shortening, mayonnaise, sandwich spreads, creamers and coffee

whiteners. It can thus be imported as an ingredient of many processed foods.

5. Relevant Issues

5.1 Safety assessment

Oil from bromoxynil-tolerant canola line Westar-Oxy-235 has been evaluated according to the safety assessment guidelines prepared by ANZFA[1]. The assessment considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues. On the basis of the available information, ANZFA concluded that oil from bromoxynil-tolerant canola line Westar-Oxy-235 is as safe and wholesome as oil from other commercial canola varieties. The full safety assessment report can be found at **Attachment 2** to this document.

5.2 Labelling of oil derived from bromoxynil-tolerant canola

On 28 July 2000 the Ministerial 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 came into effect 12 months from the date of gazettal.

Under these new provisions, oil from bromoxynil-tolerant canola line Westar-Oxy-235 will require labelling if novel protein and/or novel DNA are present. However, refined oil from canola would not be expected to contain any plant protein or DNA, due to its high degree of refinement, therefore products containing such oil may be exempt from labelling.

5.3 Issues arising from public submissions

General issues

Of the 67 submissions received following two rounds of public consultation, 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 have been addressed in the safety assessment report. Of the general issues, the most commonly raised were:

- environmental concerns about the growing of GM crops;
- concerns about the use of antibiotic resistance genes;
- concerns about the use of agricultural chemicals;
- labelling of GM foods;
- reliance by ANZFA on industry-generated data for the safety assessment; and
- viral recombination.

[1] ANZFA (2001) Guidelines for the safety assessment of genetically modified foods. In: *Information for Applicants – Amending Standard A18/Standard 1.5.2 Food Produced using Gene Technology*. (www.anzfa.gov.au/_srcfiles/GM Guidelines Nov 01.pdf)

A discussion of these and other general issues raised in public submissions for this and other applications can be found in **Attachment 4**. This includes matters such as the publishing of the report of the New Zealand Royal Commission on Genetic Modification, the second OECD Conference on "New Biotechnology Food and Crops: Science, Safety and Society", the United Kingdom Royal Society's report on "Genetically modified plants for food use and human health – an update" and the deliberations of various international committees and taskforces including those of the Codex Alimentarius Commission, the OECD and FAO/WHO Expert Consultations.

Specific issues

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

(i) Toxicity of bromoxynil breakdown products

Both the New Zealand Ministry of Health (NZMoH) and the Public and Environmental Health Service in Australia raised the point that the ANZFA safety assessment should address the issue of whether residues of the herbicide degradation process are present, toxic and/or subject to a maximum residue limit (MRL).

The Consumers' Association of South Australia Inc. and the National Council of Women of Australia (NCWA) raised similar concerns, suggesting that the persistence and toxicity of bromoxynil had not been adequately assessed by the US FDA, and that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself. The NZMoH also stated that it would have been useful to include a consideration of the ioxynil metabolite, 3,4 diiodo-4-hydroxybenzoic acid, although they added that the toxicity and exposure of this compound is unlikely to be different from that of DBHA. A number of submitters also expressed concerns about herbicide residues, stating that it's not clear to what extent these residues persist in the oil.

Response

There is currently no MRL for either bromoxynil or DBHA residues in canola in Australia. The absence of an MRL means that no detectable residues are permitted in canola. Similarly, in New Zealand no MRL exists, although a level of 0.1 ppm is allowed under sub-regulations 257 (4) and (9) in the *Food Regulations 1984*.

Residue analysis for both bromoxynil and the metabolite DBHA was done on samples of canola from field trials in Canada. All were below the level of quantitation of the analytical method (0.05 ppm), and very close to the limit of detection (0.02 ppm), meaning that none of them can be considered significant. A further study on the processed fractions of canola seed (oil and meal) found no detectable residues, even with rates 10 times that of the commercial application rate, and no concentration of residues with processing. Overall, therefore, the residues expected to be present in refined canola oil are effectively zero.

This issue of toxicity of the bromoxynil breakdown product was fully addressed in the safety assessment report (**Attachment 2**). Briefly, the nitrilase enzyme (which confers tolerance to bromoxynil) hydrolyses bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) into 3,5-dibromo-

4-hydroxybenzoic acid (DBHA) and ammonia. As DBHA is a by-product specifically resulting from the activity of the introduced gene its potential toxicity was specifically considered in the safety assessment. The US EPA carried out a thorough toxicity assessment of bromoxynil, and produced a report in December 1998. They concluded that the risk from bromoxynil was "negligible". In addition they found that, based on an examination of the chemical structure of DBHA, that the toxicity of this metabolite would be similar to or lower than that of bromoxynil itself. The chemical structure of DBHA is such that it is less fat soluble than bromoxynil, and this is expected to reduce the amount of residue present in the oil. Although the nitrilase enzyme is capable of inactivating both bromoxynil and ioxynil herbicides, Navigator™ canola is primarily used with Buctril® which contains bromoxynil as the active ingredient, therefore the toxicity assessments have focussed on the bromoxynil metabolite, rather than the ioxynil metabolite. ANZFA agrees with the opinion of the NZMoH that the toxicity and persistence of the ioxynil metabolite is unlikely to be different to that of DBHA.

(ii) Allergenic effects of novel genes

Diane Davie suggested that the use of herbicide-resistance genes could increase allergies.

Response

The safety assessment carried out by ANZFA has addressed the issue of the potential allergenicity of nitrilase in some depth. Data was evaluated on a comparison of the amino acid sequence of nitrilase to that of known allergens, its resistance to acid and protease digestion, and its presence in the food as consumed. Nitrilase does not come from a source that is known to be allergenic and has none of the characteristics that are common to food allergens, nor does it have any significant amino acid sequence similarity to known allergens.

This, combined with the fact that refined oil contains no detectable nitrilase, means that in the case of bromoxynil-tolerant canola, nitrilase has very limited potential to become a food allergen.

(iii) Genetic stability

Groundswell have expressed concern about the genetic stability of the bromoxynil-tolerant canola, stating that a rigorous and independent analysis of the genetic stability has not been provided and that without this it cannot be proved as safe for human consumption.

Response

ANZFA routinely evaluates information about the genetic stability of a new GM plant in order to determine if the introduced genes are behaving in a predictable way when inherited from one generation to the next. Such an analysis is thus an important component of the safety assessment. Evidence of genetic stability can be gathered from a number of different pieces of information and typically is derived from data which shows the stable inheritance of the phenotype as well as the genotype. In other words, the trait conferred by the introduced genes, in this case bromoxynil tolerance, must be stably expressed over several plant generations and in different environments and the inserted DNA must also be stably maintained without undergoing any rearrangements. A number of different techniques are used to demonstrate this.

For the bromoxynil-tolerant canola, which is the subject of this application, genetic stability of the bromoxynil-tolerant trait was studied by backcrossing plants containing the *oxy* gene with elite canola varieties and by self-crossing followed by propagation. The bromoxynil-tolerant trait was found to segregate in a manner consistent with a single genetic locus and was also found to be stably inherited from one generation to the next. Additionally, molecular techniques were used to demonstrate that the *oxy* gene was stably maintained from one generation to the next and also in different genetic backgrounds.

The data provided by the Applicant was comprehensive and was derived from experiments which had been conducted according to sound scientific principles therefore ANZFA was satisfied that genetically stability had been established over several generations.

5.4 Risk analysis

Under the *Food Standards Code*, a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines.

On the basis of the safety assessment, together with a consideration of the public submissions, it is concluded there are no public health and safety concerns associated with the consumption of oil derived from bromoxynil-tolerant canola line Westar-Oxy-235. Oil from bromoxynil-tolerant canola line Westar-Oxy-235 will only require labelling if it can be established that novel DNA or protein is present in the final food.

In relation to the concerns raised in the public submissions with regard to gene technology and GM food, ANZFA has prepared a public discussion paper on the safety assessment process for GM food[2]. This is widely available and may assist in addressing some of the concerns raised by the public. In addition, in collaboration with Biotechnology Australia, ANZFA has produced an information pamphlet entitled *Genetically Modified Foods* that has been distributed throughout Australian supermarkets. Other government agencies such as the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand, and industry bodies are also addressing the broader concerns in relation to gene technology.

6. Regulatory Options

The following regulatory options were considered:

Option 1 – do not approve oil derived from bromoxynil-tolerant canola line Westar-Oxy-235. Standard A18/1.5.2 of the *Food Standards Code* would not be amended to include oil from bromoxynil-tolerant canola line Westar-Oxy-235. Food already in the food supply containing oil derived from bromoxynil-tolerant canola line Westar-Oxy-235 would need to be removed from sale.

Option 2 – approve oil derived from bromoxynil-tolerant canola line Westar-Oxy-235. Amend Standard A18/1.5.2 of the *Food Standards Code,* as sought by the Applicant, and include oil from bromoxynil-tolerant canola line Westar-Oxy-235 in the table to the Standard.

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

Food containing oil derived from bromoxynil-tolerant canola line Westar-Oxy-235 already in the food supply could continue being sold. Food containing ingredients derived from bromoxynil-tolerant canola line Westar-Oxy-235 would have to be labelled if novel DNA and/or protein are present in the final food.

7. Impact Analysis

Affected parties

1. Governments – State, Territory and New Zealand Health Departments, AQIS;
2. Consumers;
3. Manufacturers, producers and importers of food products

Impact of Option 1

Consumers:
- would mainly impact on those consumers who perceive GM food to be unsafe, and who may consider the prohibition of oil from bromoxynil-tolerant canola line Westar-Oxy-235, and its subsequent removal from the market, to provide a public health and safety benefit.
- there may be some market disruption from the removal from sale of products containing oil from bromoxynil tolerant canola.

Industry:
- there would be a cost to industry from the removal of products from sale and the sourcing of new suppliers.

Government:
- enforcement agencies would need to identify products that contain oil derived from bromoxynil-tolerant canola line Westar-Oxy-235 in order to remove them from the food supply or prevent their importation.
- prohibition is likely to affect a number of imported products, therefore this raises the possibility of a challenge by affected countries under the World Trade Organization .

Impact of Option 2

Consumers:
- no direct impact as *status quo* maintained.

Industry:
- no major impact as *status quo* maintained

Government:
- no direct impact other than minor costs associated with amending the *Food Standards Code*

The regulatory impact on all sectors of approving oil from bromoxynil tolerant canola is minimal because the food products are already permitted in the food supply under transitional arrangements, therefore the giving of approval merely serves to maintain the *status quo*. It is concluded that the amendment to the *Food Standards Code* is necessary, cost effective and of net benefit to both food producers and consumers.

8. Consultation

8.1 Public consultation

The Initial Assessment (Preliminary Assessment – section 13) of this application was released for public comment on 3 November 1999. A total of 45 submissions were received in response to the initial assessment report. A Draft Assessment (Full Assessment – section 15) of the application, including a comprehensive safety evaluation of the food and consideration of issues raised by public submissions, was subsequently released for public comment on 6 February 2002 for a period of six weeks. A total of 20 submissions were received.

This Final Assessment Report completes the assessment by ANZFA, again taking into account comments received from the public. ANZFA's recommendation will then be submitted to the Ministerial Council for consideration. **Attachment 3** contains a summary of all submissions received.

8.2 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). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

As there is significant international interest in the safety of GM foods, and the proposed amendments are likely to have a liberalizing effect on international trade, this application was notified to the WTO as a potential TBT or SPS matter.

9. Conclusion and Recommendation

Adoption of the draft variation (**Attachment 1**), giving approval to the continued sale of oil derived from bromoxynil tolerant canola in Australia and New Zealand, is recommended for the following reasons:

- the genetic changes introduced into bromoxynil-tolerant canola line Westar-Oxy-235 are not considered to raise any additional public health and safety concerns;

- based on data supplied with the application and other available information, oil from bromoxynil-tolerant canola line Westar-Oxy-235 is as safe and wholesome as oil derived from conventional canola varieties;

- food products containing oil from bromoxynil-tolerant canola will be exempt from labelling unless it can be shown that novel DNA and/or protein is present in the final food; and

- the proposed amendment to the *Food Standards Code* is consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991; and

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

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

Submissions

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

Further Information

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

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

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
email: nz.reception@anzfa.gov.au

Assessment reports are available for viewing and downloading from the ANZFA website www.anzfa.gov.au . People without access to internet facilities may request paper copies of reports from the Information Officer.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Safety assessment report
3. Summary of public comments
4. General issues raised in public comments

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

APPLICATION A388 – OIL DERIVED FROM BROMOXYNIL-TOLERANT CANOLA LINE WESTAR-OXY-235

To commence : On gazettal

[1] ***Standard A18*** *of Volume 1 of the Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2, *immediately after the last occurring entry -*

Oil derived from bromoxynil-tolerant canola line Westar-Oxy-235

[2] ***Standard 1.5.2*** *of Volume 2 of the Food Standards Code is varied by inserting into* Column 1 *of the* Table to clause 2*, immediately after the last occurring entry -*

Oil derived from bromoxynil-tolerant canola line Westar-Oxy-235

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

APPLICATION A388 – OIL DERIVED FROM BROMOXYNIL-TOLERANT CANOLA LINE WESTAR-OXY-235

SUMMARY AND CONCLUSIONS

Oil from bromoxynil-tolerant canola line Westar-Oxy-235 has been assessed by ANZFA to evaluate its safety for human consumption. A number of criteria are used in this assessment including a characterisation of the genes, their origin and function, the changes at the DNA, protein and whole food levels, stability of the introduced genes in the canola genome, compositional analyses, evaluation of intended and unintended changes and the potential allergenicity and toxicity of the newly expressed proteins.

Nature of the genetic modification

Bromoxynil-tolerant canola line Westar-Oxy-235 was generated by the transfer of the *oxy* gene from the soil bacterium *Klebsiella ozaenae*, using the *Agrobacterium*-mediated transformation system. The *oxy* gene codes for the enzyme nitrilase, which converts the herbicide bromoxynil (3,5-dibromo-4-hydrobenzonitrile) to its non-phytotoxic metabolite 3,5-dibromo-4-hydroxybenzoic acid (DBHA). No other genes were transferred and the transformed canola was shown to be phenotypically and genotypically stable by segregation and mapping studies.

General safety issues

Canola is a genetic variation of rapeseed developed by plant breeders specifically for its nutritional qualities, particularly its low levels of saturated fat and naturally occurring toxins. Oil is the only product of the canola plant that is being assessed for human consumption. Canola oil is routinely used in food and has a moderately long history of safe use.

The new protein, nitrilase, is an enzyme specific for oxynil herbicides. It was found to be easily detectable in leaf extracts from the modified plant, but was only present at very low levels in seeds. No detectable protein was found in refined oil.

The modification did not involve the transfer of any antibiotic resistance genes.

Toxicological issues

Analysis of naturally occurring toxins (erucic acid and glucosinolates) in bromoxynil-tolerant canola showed levels for both to be well below the respective mandatory and industry limits. There were no major differences between transgenic and control lines, indicating that the genetic modification process had not altered the levels of these compounds.

The potential toxicity and allergenicity of nitrilase was considered in the assessment. Proteins from the same family as nitrilase are ubiquitous throughout the animal and plant kingdoms, and are consumed by both animals and humans.

Nitrilase itself does not have any significant similarity to known protein toxins or allergens and is rapidly digested in conditions that mimic human digestion. The absence of toxicity of nitrilase has been confirmed through acute toxicity testing in mice.

Nitrilase, also cannot be detected in refined canola oil, therefore exposure to the protein, through consumption of refined oil from bromoxynil-tolerant canola, would be zero. There is thus no evidence to indicate that there is any potential for nitrilase to be either toxic or allergenic to humans.

The potential toxicity of DBHA, the by-product of bromoxynil detoxification by nitrilase, was also considered. The evidence indicates that DBHA shows no potential to be toxic to humans at the predicted exposure levels.

Nutritional issues

Detailed compositional analyses did not reveal any consistent differences in key constituents between modified canola plants and control plants, or the oils produced from them. Treatment with bromoxynil also did not affect the levels of any of the key constituents measured. The results confirmed that the levels of key constituents in bromoxynil-tolerant canola are no different to those of non-modified canola varieties. An animal feeding study also confirmed that there is no difference between bromoxynil-tolerant and control varieties of canola in their ability to support typical growth and well being.

Conclusion

No potential public health and safety concerns have been identified in the assessment of canola line Westar-Oxy-235. On the basis of the data submitted with the present application, and other available information, oil derived from bromoxynil-tolerant canola line Westar-Oxy-235 is considered to be as safe and nutritious as refined oil derived from conventional canola varieties.

1. BACKGROUND

Aventis CropScience Pty Ltd has made an application to ANZFA to vary Standard A18 to include oil derived from canola, which has been genetically modified to be tolerant to the oxynil family of herbicides comprising bromoxynil and ioxynil. The genetically modified canola is marketed as Navigator™ canola.

The oxynil family of herbicides act by inhibiting electron transport in photosystem II in plants. Inhibition of electron transport causes super oxide production resulting in the destruction of cell membranes and an inhibition of chlorophyll formation, leading to plant death (Comai and Stalker 1986). Tolerance to either bromoxynil (3,5-dibromo-4-hydorxybenzonitrile) or ioxynil (3,5-di-iodo-4-hydroxybenzonitrile) is achieved through expression in the plant of a bacterial nitrilase enzyme that hydrolyses the herbicide to an inactive, non-phytotoxic compound. The nitrilase is derived from the bacterium *Klebsiella pneumoniae* subspecies *ozaenae* which is responsible for rapidly degrading bromoxynil in soil. The nitrilase enables the bacterium to utilise bromoxynil as a sole source of nitrogen (McBride *et al* 1986).

Bromoxynil is particularly effective on broadleaf weeds common in canola fields. The rationale for engineering canola to be bromoxynil-tolerant is to enable bromoxynil-containing herbicides to be used for the post-emergence control of broadleaf weeds in canola crops without crop injury. The modified canola was developed for commercialisation in Canada, where it is grown for both domestic use and for export. Although the current level of trade of canola and its commodities between Canada and New Zealand and Australia is relatively small, some imported processed foods may contain genetically modified canola oil.

Canola seeds are processed into two major products, oil and meal with the oil being the only human food product being considered in this assessment. Canola meal is used principally as an animal feed. Canola oil is a premium quality oil and is used in a variety of manufactured food products including salad and cooking oil, margarine, shortening, mayonnaise, sandwich spreads, creamers and coffee whiteners. It can thus be imported as an ingredient of many processed foods.

2. DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modification

Canola (*Brassica napus* L. oleifera Metzg.) line Westar was transformed with plasmid pRPA-BL-150a using the method of *Agrobacterium tumefaciens*-mediated transformation. A disarmed (i.e. non phytopathogenic) strain of *Agrobacterium tumifaciens*, EHA 101 was used (Hood *et al* 1986). The *Agrobacterium*-mediated transformation system is well understood, and is widely used in plant biotechnology (Zambryski 1992).

Regeneration of transformed plants was done in the presence of bromoxynil as the sole selective agent. The transformation resulted in the selection of a single transformation event – Westar-Oxy-235 – which was subsequently used in sexual crosses with elite canola lines to generate the Navigator™ canola varieties used in commercial production.

2.2 Function and regulation of the novel genes

The transformation of canola with plasmid pRPA-BL-150a resulted in the transfer of a single gene expression cassette. The genetic elements contained within the gene expression cassette are described in Table 1 below and their organisation is depicted in Figure 1.

Table 1: Description of the gene expression cassette contained within pRPA-BL-150a

Genetic element	Source	Function
35S promoter	The cauliflower mosaic virus (CaMV) 35S promoter region (Gardner *et al* 1981).	A promoter for high-level constitutive (occurring in all parts of the plant and at all stages of development) gene expression in plant tissues.
Enhancer	The non-translated leader of a RuBisCO small subunit gene derived from maize (Lebrun *et al* 1987).	The non-translated leader sequence helps to stabilise mRNA and improve translation.
oxy	Gene isolated from *Klebsiella pneumoniae* subspecies *ozaenae* encoding the enzyme nitrilase (Stalker *et al* 1988).	Inactivates the herbicide bromoxynil and confers bromoxynil tolerance when expressed in plants.
NOS 3'	The 3' non-translated region of the nopaline synthase gene isolated from *Agrobacterium tumefaciens* plasmid pTi37 (Bevan *et al* 1983).	Contains signals for termination of transcription and directs polyadenylation.

The oxy gene

The *oxy* gene was isolated from the soil bacterium *Klebsiella pneumoniae* subsp. *ozaenae* and encodes an enzyme that metabolises the herbicide bromoxynil (Stalker and McBride 1987). The 1150 base pair *oxy* gene has been fully sequenced and its encoded enzyme, nitrilase, has been fully characterised (Stalker *et al* 1988). When transferred into plants, the gene, through its encoded protein, confers tolerance to the oxynil family of herbicides including bromoxynil and ioxynil. The mechanism of tolerance involves the detoxification of the herbicide by the nitrilase enzyme. This degradation effectively inactivates the herbicide and enables the normally bromoxynil-sensitive plant to survive and grow when treated with applications of the herbicide.

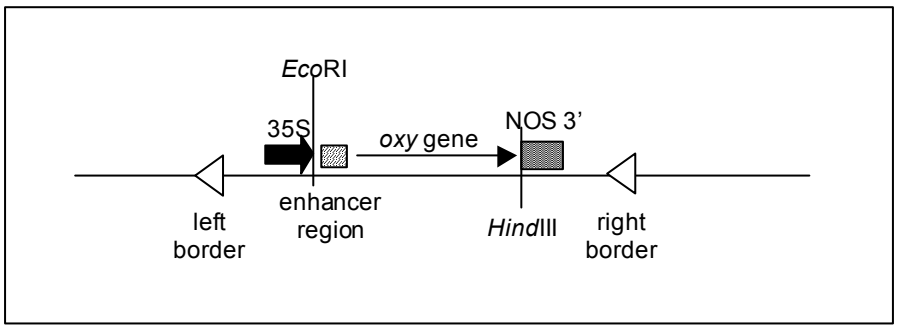
Other genetic elements

The plasmid pRPA-BL-150a is a double border binary plant transformation vector which contains well-characterised DNA segments required for the selection and replication of the plasmid in bacteria as well as the right and left borders delineating the region of DNA (T-DNA) which is transferred into the plant genomic DNA (Table 2). This is the region into which the gene expression cassette is inserted. DNA residing outside the T-DNA region does not normally get transferred into plant genomic DNA (Zambryski 1992). All DNA cloning and vector construction was carried out using the host bacterium *Escherichia coli* DH5α, a derivative of the common laboratory *E. coli* K-12 strain.

Table 2: Description of other genetic elements contained within pRPA-BL-150a

Genetic element	Source	Function
Left border	A DNA fragment of the pTiA6 plasmid containing the 24 bp nopaline-type T-DNA left border region from *A. tumefaciens* (Barker *et al* 1983).	Terminates the transfer of the T-DNA from *A. tumefaciens* to the plant genome.
Right border	A DNA fragment from the pTiA6 plasmid containing the 24 bp nopaline-type T-DNA right border region from *A. tumefaciens*. (Barker *et al* 1983).	The right border region is used to initiate T-DNA transfer from *A. tumefaciens* to the plant genome.
Genta	Gentamicin resistance gene from plasmid pH1J1 (Hirsch and Beringer 1984).	Confers resistance to the antibiotic gentamicin. Used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells.
ori-322	Origin of replication from *E. coli* plasmid pBR322 (Bolivar *et al* 1977).	Allows for autonomous replication of plasmids in *E. coli.*

Figure 1: Diagram of the T-DNA region transferred to Westar-Oxy-235.



2.3 Characterisation of the genes in the plant

Selection of plant lines

After the transformation of Westar with pRPA-BL-150a, regenerated plantlets were taken out of tissue culture and transferred to soil. The transformed plants were then assayed for herbicide tolerance, as well as other agronomic characteristics, in order to select the best transformation event. Line Westar-Oxy-235 was subsequently selected and used for all further studies, as well as for sexual crosses with elite lines.

Characterisation of inserted T-DNA

Southern blotting (Southern 1975) was used to characterise the inserted T-DNA in terms of insert number (number of integration events), insert integrity (gene size), and sequences outside the T-DNA borders (including the gentamicin resistance gene and the plasmid origin of replication).

Genomic DNA was isolated from leaf tissue of the non-transformed parental line, Westar and from the T_3 generation of the transformed canola line, Westar-Oxy-235. To determine the insert number of the T-DNA, genomic DNA was digested with either *EcoR*1 or *Hind*III, which reside at the 5' and 3' ends of the *oxy* gene, respectively (see diagram above). The number of hybridising bands detected will represent the number of copies of the *oxy* gene present in the plant genome, and hence serves as an indicator of the number of T-DNA insertions. With either restriction digestion, only a single hybridising band was detected, indicating that only a single copy of the *oxy* gene is present in Westar-Oxy-235. No hybridising bands were detected in genomic DNA isolated from the non-transformed control. Double digestion of the genomic DNA with both *EcoR*1 and *Hind*III resulted in a single hybridising band corresponding to the size of the coding region of the *oxy* gene (1150 bp). This indicates that the entire coding region has been transferred.

To determine if any sequences from outside the T-DNA borders had been transferred to the plant genome, genomic DNA from both Westar-Oxy-235 and the parental control were probed with a DNA fragment corresponding to the *ori-322* region of pBR322. No hybridising bands were detected, indicating that the bacterial origin of replication had not been transferred.

PCR analysis was used to determine if the gentamicin resistance gene had been transferred during the transformation process. DNA extracted from leaf tissue harvested from Westar-Oxy-235 and the parental control line was used in the analysis. Plasmid DNA, containing the gentamicin resistance gene, was used as the reference substance and positive control for the analysis. No gentamicin-specific DNA fragment could be amplified from DNA extracted from Westar-Oxy-235, indicating that the gentamicin resistance gene had not been transferred.

Conclusion

A single copy of T-DNA, containing the *oxy* gene, has been integrated at a single site in Westar-Oxy-235. No rearrangements of the T-DNA were apparent and no sequences residing outside the T-DNA region, including the gentamicin resistance gene, were transferred during the transformation.

2.4 Stability of the genetic changes

The genetic stability (i.e., inheritance) and segregation of the bromoxynil-tolerant trait was monitored using data obtained from herbicide-sprayed plants and Southern blotting.

Progeny derived from the original transformation event, Westar-Oxy-235, were sprayed with oxynil herbicides at the T_2 and T_3 generations.

By spraying seedlings with the herbicide and determining the Mendelian segregation ratios of the bromoxynil tolerant trait it is possible to determine the total number of functional (bromoxynil-tolerant) loci that have been integrated into an individual transformed plant. Ideally, a single genetic locus (i.e., a single insertion site) is preferred because, while not essential for the performance of the canola or the *oxy* gene, it simplifies the breeding of the trait into other elite commercial cultivars.

The segregation analysis done with the early generations derived from the original transformation event indicated the bromoxynil-tolerance trait is stably inherited by subsequent generations and that it segregates in a manner consistent with a single genetic locus.

Beyond the T_3 generation, lines homozygous for the bromoxynil-tolerant trait were selected. These lines no longer display segregation of the trait and oxynil spray screening is instead used to maintain and monitor seed purity. The maintenance of the tolerance trait over subsequent homozygous generations is thus a good measure of genetic stability. The bromoxynil-tolerant trait was found to be stably maintained over several generations produced from self-pollination, as well as in different genetic backgrounds produced through backcrossing with elite canola varieties. During the backcrossing program, the *oxy* gene was introgressed into a winter elite variety of canola called Samourai, producing Samourai-Oxy-235. Southern blotting was done on genomic DNA isolated from Samourai-Oxy-235 and compared to Westar-Oxy-235. The hybridisation patterns obtained were indistinguishable, confirming that the *oxy* gene is stably maintained in different genetic backgrounds.

Conclusion

Stability of the bromoxynil-tolerant trait was studied by backcrossing of plants containing transformation event Westar-Oxy-235 with elite canola varieties and by self-crossing followed by propagation. The bromoxynil-tolerant trait was found to segregate in a manner consistent with a single genetic locus and was also found to be stably inherited from one generation to the next. Additionally, Southern blotting demonstrated that the *oxy* gene was stably maintained in a different genetic background.

3. GENERAL SAFETY ISSUES

3.1 History of use

Donor organism

Klebsiella ozaenae is a member of the *Enterobacteriaceae*, a group of facultative gram-negative bacteria. The European Federation of Biotechnologies has classified *K. ozaenae* as a Class 2 microorganism. This class contains microorganisms that could potentially cause disease in humans, however no known pathogenicity exists for the subspecies *ozaenae*. Bacteria of the *Klebsiella* class are widely distributed in nature, occurring naturally in the soil, water and in grain and are normal inhabitants of the intestinal tract (Krieg and Holt 1984).

Host organism

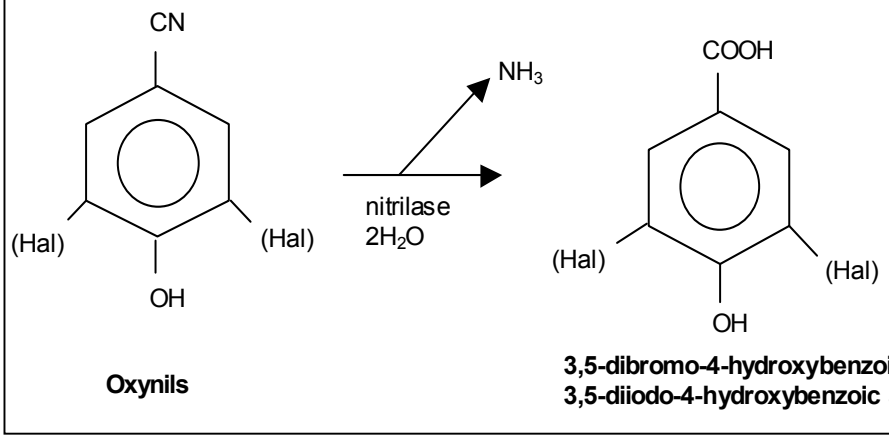
The plant species *Brassica napus* L. oleifera Metzg is more commonly known as oilseed rape, rape or rapeseed, with some cultivars referred to as canola. Rapeseed breeding began soon after the crop was introduced during the 1940s. Early rapeseed varieties were very high in the natural toxicants, erucic acid and glucosinolates, which made them unsuitable for consumption by either humans or animals. In the 1970s intensive breeding programs produced high quality varieties that were significantly lower in both erucic acid and glucosinolates. These varieties, largely *Brassica napus,* were called canola, the term denoting that these varieties contain an erucic acid level below 2% of total fatty acids and less than 30 micromoles of total glucosinolates. 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.

Presently, oilseed rape is grown primarily for its seeds, which yield about 40% oil and a high protein animal feed. Demand for canola has risen sharply, particularly the oil, which is used in margarine and other oil-based products. Canola oil-based products are routinely used in food and are considered to have a history of safe use.

3.2 Nature of the novel protein

The *oxy* gene was isolated from *Klebsiella pneumoniae* subspecies *ozaenae* (McBride *et al* 1986, Stalker and McBride 1987, Stalker *et al* 1988) and encodes a 37 kea nitrilase (EC. 3.5.5.6). This enzyme hydrolyses the oxynil herbicides into non-phytotoxic compounds: 3.5-dibromo-4-hydroxybenzoic acid or 3,5-diiodo-4-hydroxybenzoic acid and ammonia (Figure 2).

Figure 2: Oxynil degradation by the *oxy*-encoded nitrilase. (Hal is either Br or I)



Oxynils

3,5-dibromo-4-hydroxybenzoic acid
3,5-diiodo-4-hydroxybenzoic acid

Purified nitrilase has optimal activity at pH 9.2 and at a temperature of 35˚C. The pH optimum remains relatively constant at different substrate concentrations. Nitrilase activity declines to 15% at pH 7.0 and also in temperatures of 10 and 55˚C. The *oxy*-encoded nitrilase is highly specific for its substrates, exhibiting a K_m of 0.31 nM and a V_{max} of 15 µmole of NH_3 released/min/mg protein for bromoxynil.

3.3 Expression of the novel protein in the plant

Expression levels of the *oxy*-encoded nitrilase were determined by immuno-blotting techniques using a rabbit polyclonal antibody specific to nitrilase. Analyses were done on leaf and seed tissue extracts as well as processed fractions (oil and meal) from homozygous Westar-Oxy-235 and the non-transformed parental control. A positive nitrilase signal on the immunoblot consists of a single band at 37 kDa. The protein level was quantified by comparing the intensity of the signal in the protein extracts with known amounts of purified nitrilase. The detection limit for the assay was 20 ppb nitrilase. Nitrilase was not detected in any of the protein extracts from the non-transformed parental control line. The results are summarised in Table 3 below.

Table 3: Nitrilase expression levels in tissue from Westar-Oxy-235

	Nitrilase expression levels		
Sample	**ng/mg total protein**	**% tissue**	**Parts per million (ppm)**
Leaf	1000	0.002	20
Seed	<10	<0.0003	<3
Meal	5	0.0002	2
Refined Oil	Not detected		

The results show that the levels of nitrilase are highest in the leaf tissue, with only relatively low amounts of nitrilase able to be detected in the seeds. In refined oil, which is the only human food product derived from canola being assessed in this application, nitrilase could not be detected (detection limit of 20 ppb).

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

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

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

The major concern in relation to the transfer of novel genetic material to cells in the human digestive tract is with antibiotic resistance genes. Antibiotic resistance genes can be present in some transgenic plants as a result of their use as marker genes to select transformed cells. It is generally accepted that there are no safety concerns with regard to the presence in the food of antibiotic resistance gene DNA *per se* (WHO 1993).

[3] Food and Agriculture Organization.

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.

As no antibiotic resistance gene was transferred to Westar-Oxy-235 during the transformation process, this issue will not be considered further in the assessment.

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally occurring toxins

Canola contains two naturally occurring toxic compounds – erucic acid and the glucosinolates. High levels of erucic acid, a long-chain fatty acid, are considered to have cardiopathic potential based on laboratory studies with rats and the glucosinolates have been found to possess goitrogenic properties. Because of this, canola must meet specific standards on the levels of erucic acid and glucosinolates – these are less than 2% erucic acid in the oil and less than 30 μmoles of total glucosinolates in the meal.

The levels of erucic acid and the glucosinolates were measured in the oil and meal derived from seed samples taken from field trials with Westar-Oxy-235 canola and the non-transformed parental control grown at various sites in Canada and France in 1992-1995. Data was also obtained from field trials in France in 1994 – 1995 with French canola elite lines that had been crossed with Westar-Oxy-235. Data was obtained for both bromoxynil-sprayed and unsprayed plants. These data are presented in Tables 3 and 4 below.

Erucic acid

Table 3: Erucic acid content[1] of oil from Westar-Oxy-235 and elite crosses

Line	Bromoxynil treatment	Erucic acid level (% total fatty acids)
Control (n=21)	unsprayed	0.04 (0 – 2.2)
Westar-Oxy-235 (n=8)	unsprayed	0.01 (0 – 0.1)
Westar-Oxy-235 (n=5)	120 g/ha	0.0
Westar-Oxy-235 (n=5)	240 g/ha	0.0
Westar-Oxy-235 (n=5)	480 g/ha	0.0
French elite lines (n=8)	unsprayed	0.08 (0 – 0.5)
Westar-Oxy-235 x French elite lines (n=8)	480 g/ha	0.0
Literature range	-	0.0 – 2.0

[1] mean values with range in parentheses

Mean values of erucic acid in oil from Westar-Oxy-235 and elite lines expressing the bromoxynil tolerant trait were found to all be well below the limit specified for canola and comparable to that found in oil from the parental control lines. The application of bromoxynil to the plants did not result in any changes to the levels of erucic acid.

Glucosinolates

The glucosinolates are converted to more toxic compounds upon hydrolysis by myrosinase, an enzyme localised within the cells of Brassica seeds. When the seed is crushed, the enzyme acts upon the glucosinolate to produce isothiocyanates, thiocyanates and possibly nitriles depending on temperature and moisture conditions. However, during processing, a cooking step inactivates myrosinase, leaving glucosinolates intact. Some destruction and reduction of glucosinolates may occur in further processing steps. Nonetheless, breeders are encouraged to work towards the elimination of glucosinolates in canola.

There are over 100 known structural types of glucosinolates, nine of which have been monitored in canola because of the known potential toxicity of their metabolites. A group called the alkyl glucosinolates are monitored particularly closely – the sum of four of them must be less than a total of 30 µmoles/g seed for the seed to be classified as canola quality – this is an industry standard agreed by various canola associations worldwide. Of similar concentration but of less concern are the indol glucosinolates, two of which are monitored. Two types from a third group of glucosinolates, the thioalkyl glucosinolates are measured but are typically present in very low concentrations.

Table 4: Glucosinolate content[1] of canola meal from Westar-Oxy-235 and elite crosses

		Glucosinolates (µmol/g seed)		
Line	**Bromoxynil treatment**	**Alkyl**	**Indol**	**Total**
Control (n=21)	unsprayed	10.27 (5.88 – 20.47)	5.21 (1.57 – 8.04)	15.48 (11.58 – 25.05)
Westar-Oxy-235 (n=5)	unsprayed	8.12 (5.76 – 10.99)	6.51 (5.04 – 8.4)	14.63 (13.45 – 16.39)
Westar-Oxy-235 (n=5)	120 g/ha	8.79 (7.06 – 11.29)	6.15 (4.53 – 7.25)	14.94 (11.82 – 17.59)
Westar-Oxy-235 (n=5)	240 g/ha	9.19 (7.06 – 13.61)	6.29 (4.92 – 8.28)	15.48 (12.29 – 19.55)
Westar-Oxy-235 (n=5)	480 g/ha	8.22 (5.17 – 13.17)	5.73 (4.28 – 6.72)	13.95 (11.59 – 19.83)
French elite lines (n=8)	unsprayed	8.46 (7.1 – 11.5)	3.33 (2.8 – 4.1)	11.75 (10.2 – 15.4)
Westar-Oxy-235 x elite lines (n=8)	480 g/ha	8.59 (4.3 – 15.7)	3.44 (2.8 – 4.4)	12.03 (7.2 – 20.1)
Literature range		7.28 – 14.4	1.82 – 11.4	6.70 – 18.50

[1] mean values with range in parentheses

The levels of total glucosinolates in the bromoxynil-tolerant canola lines were found to be well below the 30 µmole maximum limit for oil-free meal and were also comparable to the levels found in the corresponding parental control lines. The application of bromoxynil to the plants did not result in any changes to the levels of glucosinolates in the meal.

Some differences were apparent in the levels of the different classes of glucosinolates. In particular, the bromoxynil-tolerant canola lines exhibit a pattern of slightly higher levels of the indol glucosinolates, compared to the parental control. This contrasts to the slightly reduced levels of the alkyl glucosinolates, compared to the parental control. Overall, this balances out to very little difference in the levels of total glucosinolates, which as stated above, are well below the industry standard of 30 μmoles. As the indol glucosinolates are much less of a concern than the alkyl glucosinolates, the slightly increased levels are not considered to pose a hazard, particularly as the meal is not intended for human consumption.

4.2 Potential toxicity of novel protein

The protein expression data demonstrates that Westar-Oxy-235 expresses a single novel protein – nitrilase. This section of the report will therefore assess the potential toxicity of nitrilase based on the following:

- the potential for human exposure to nitrilase;
- its amino acid sequence similarity to known toxins;
- an acute oral toxicity study in mice;
- prior history of human ingestion of similar enzymes; and
- potential toxicity of bromoxynil metabolites.

Potential for human exposure to nitrilase

Refined canola oil from Westar-Oxy-235 was analysed for the presence of nitrilase, which could not be detected down to a detection limit of 20 ppb (see Section 3.3). Therefore, it is highly unlikely that humans ingesting refined oil derived from bromoxynil-tolerant canola would be exposed to any appreciable amounts of nitrilase.

Similarity to known protein toxins

Astwood, J.D. (1997). *Klebsiella ozaenae* nitrilase (BXN) has no significant sequence similarity to known allergens or toxins. Monsanto Study Report No. MSL-15120 – submitted with Application A379 – Bromoxynil tolerant cotton.

A database of protein toxin amino acid sequences was assembled from the public domain genetic databases, which included GenPept ver. 92 (a protein database extracted from GenBank and EMBL), PIR ver. 45, and SwissProt ver. 31. Amino acid sequences were retrieved from the databases using the STRINGSEARCH program supplied with the GCG sequence analysis package version 7 (Devereux *et al* 1984). Using the DATASET program, the sequences of toxins were combined into a single database called TOXIN3.

The keyword "toxin" identified and retrieved 2662 amino acid sequences from the public domain genetic databases – this comprised the TOXIN3 database. There were no toxins in the TOXIN3 database that showed significant similarity to nitrilase.

Acute oral toxicity

Dange, M. (1996) Nitrilase: sub-acute oral toxicity study in the mouse. Rhône-Poulenc Study SA 96267 – submitted with Application A379 – Bromoxynil tolerant cotton.

To obtain sufficient quantities of nitrilase for toxicity testing, the enzyme was expressed in *Escherichia coli* BL21 and subsequently purified as an inclusion body pellet.

An acute oral toxicity study was planned to be performed using doses up to 2000 mg/kg body weight, using a suspension of nitrilase at 200 mg/ml. However, the consistency of the suspension once prepared did not allow the total dose to be administered at one time. Therefore, the suspension was administered over four consecutive days at 500 mg/kg body weight/day.

Four consecutive oral doses (500 mg/kg body weight) of nitrilase (Batch No. JHJ0001) were administered to groups of OF1 mice (5/sex) at a dose volume of 20 ml/kg. The purified nitrilase was suspended in 0.25% methylcellulose in distilled water.

All animals were checked daily for clinical signs over a period of 15 days, and their body weight recorded weekly. At termination of the study period, all animals were killed and subject to necropsy. The necropsy included the macroscopic examination of abdominal and thoracic cavities, major organs and tissues.

No clinical signs were observed during the study and there were no unscheduled deaths. The body weight gain of the animals was unaffected by the treatment and no gross findings were recorded at necropsy. The LD_{50} was designated as >500 mg/kg body weight.

History of ingestion

Nitrilase enzymes, similar to that encoded by the *oxy* gene from *Klebsiella pneumonia,* have been found in a number of plant and microbial species. Although substrates and pathways differ, it appears as though nitrilases share common functions such as hydrolysis of nitriles to carboxylic acids. Plant nitrilases can also confer resistance to some of the nitrile containing herbicides. Nitrilases have been found in a number of important food crops such as wheat, cabbage, barley, and bananas (Buckland *et al* 1973, Thimann and Mahadevan 1964), therefore, humans have a history of exposure to similar types of proteins with no apparent ill effects ever being documented.

Potential toxicity of bromoxynil metabolites

Bromoxynil-tolerant canola plants inactivate bromoxynil by hydrolysing it to 3,5-dibromo-4-hydroxybenzoic acid (DBHA), a carboxylic acid. As this metabolite is a by-product resulting from the activity of an introduced enzyme it is important that a consideration of its safety be included in any evaluation of bromoxynil-tolerant canola. Two issues are relevant. Firstly, the actual toxicity of DBHA, and secondly, the residue levels of DBHA likely to be present in food derived from bromoxynil-tolerant canola varieties.

In relation to toxicity, the US Environment Protection Agency (EPA), in its evaluation of bromoxynil, also evaluated the toxicity of the DBHA metabolite and concluded "there was no concern that DBHA would exhibit significant toxicity over that of the parent bromoxynil" and that bromoxynil "poses negligible risk to human health at expected exposure levels" (US EPA 1998). Bromoxynil and DBHA are extremely similar in structure, varying only in that bromoxynil has a cyano (-CN) group that has been converted to a carboxyl (-COOH) group in the DBHA metabolite. Conversion to a carboxyl group is generally considered to decrease the toxicity of a molecule (US EPA 1998). The conversion to the carboxyl group should cause the DBHA to be more polar and therefore more soluble in water and less in fats. This increased water solubility, combined with the decreased fat solubility means that DBHA should be eliminated faster from the organism than its parent compound, bromoxynil. It is likely that these characteristics would also limit the amount of DBHA residue likely to be present in canola oil.

The Pest Management Regulatory Authority of Canada has recently agreed (February 2000) to the registration of bromoxynil for use on bromoxynil-tolerant canola varieties. To support this registration, a number of field trials were conducted on bromoxynil-tolerant canola between 1996 and 1997. The field trials monitored maximum residue levels of bromoxynil, as well as DBHA. The maximum residues of bromoxynil and DBHA in canola seeds, collected 71-119 days after the last application of bromoxynil, were less than 0.05 ppm each, that is, below the limit of quantitation for the method used. A further study, done with the processed fractions (oil and meal) of the seed, found no detectable residues, even with application rates 10 times that of the commercial rate, and therefore no concentration of residues with processing. Overall, the residues expected to be present in refined canola oil are effectively zero.

Conclusion

The evidence from the sub-acute toxicity study in mice does not indicate that there is any potential for nitrilase from *Klebsiella pneumoniae* subsp. *ozaenae* to be toxic to humans. Furthermore, humans are extremely unlikely to be exposed to this enzyme through the consumption of refined oil from bromoxynil-tolerant canola as the refined oil has been shown to be devoid of any detectable protein. The metabolite of bromoxynil, DBHA, also does not show any potential to be toxic to humans at the predicted exposure levels.

4.3 Potential allergenicity of novel proteins

The concerns regarding potential allergenicity of novel proteins are two fold. Firstly, there are concerns that the ability to express new or different proteins in food will result in the transfer of allergens from one food to another, thereby causing some individuals to develop allergic reactions to food they have not previously been allergic to. Secondly, there are concerns that the transfer of novel proteins to food will lead to the development of new allergies in certain individuals. The former is more easily addressed than the latter because if an allergen is already known it is possible, using human sera or human skin tests, to test if it has been transferred. There are no reliable tests or animal models, however, which enable the prediction of the allergenic potential of novel proteins.

Instead, potential allergenicity can only be indicated by examination of a number of characteristics of the novel protein, such as whether it is derived from a known allergenic source, its physical/chemical characteristics (resistance to acid and protease degradation, amino acid sequence similarity with known allergens) and whether it is likely to be present in large amounts in the food as consumed and therefore have potential for allergic sensitisation.

Notwithstanding the lack of any detectable nitrilase in refined oil, the allergenic potential of nitrilase has been assessed according to the following:

- potential for human exposure to nitrilase;
- similarity to known allergens; and
- digestibility in simulated mammalian digestion fluids

Potential for human exposure

Refined oil from bromoxynil-tolerant canola has been found to contain no detectible nitrilase therefore humans would be extremely unlikely to be exposed to nitrilase through consumption of the oil.

Similarity to known allergens

Astwood, J.D. (1997). *Klebsiella ozaenae* nitrilase (BXN) has no significant sequence similarity to known allergens and toxins. Monsanto Study Report No. MSL-15120 – submitted with Application A379 – Bromoxynil tolerant cotton.

A search for amino acid sequence similarity with known allergens and gliadins is a useful first approximation of potential allergenicity and potential association with coeliac disease (Fuchs and Astwood 1996, Metcalf *et al* 1996). Many protein allergens have been characterised and their amino acid sequences are known, and importantly, their IgE binding epitopes have been mapped (Elsayad and Apold 1983, Elsayad *et al* 1991, Zhang *et al* 1992). The binding epitopes are generally between 8 and 12 amino acids in length.

To undertake the amino acid sequence comparison between nitrilase and known protein allergens and gliadins, a database of allergen and gliadin sequences was assembled from the standard public domain databases containing protein sequences (GenPept ver. 86.0, PIR ver. 41, SwissProt ver. 30). In addition, DNA sequences were retrieved from GenBank/EMBL ver. 86 as some allergen sequence entries do not appear in the protein sequence databases. The amino acid sequences of the allergens retrieved from the GenBank/EMBL database were either obtained from the GenEMBL flat files or were obtained by translation of the open reading frames in the DNA sequences. Therefore the assembled database consisted of two parts: (1) a dataset of protein sequences and (2) a supplemental database of protein sequences initially retrieved as DNA sequences. Duplicates were deleted from the assembled database and irrelevant sequences were identified by examining complete flat files or by reference to the scientific literature. The resulting database of 219 allergens and gliadins has been published in the scientific literature (Astwood *et al* 1996).

The allergen and gliadin database was then searched for sequences similar to nitrilase. A significant sequence similarity was defined as a sequence identity of greater than seven contiguous amino acids. No significant similarity between nitrilase and any of the known allergens or gliadins was identified.

Digestibility

Aasen, E., *et al* (1997). Assessment of the digestibility of purified BXN nitrilase protein *in vitro* using mammalian digestive fate models. Monsanto Study Report No. MSL-15148 – submitted with Application A379 – Bromoxynil tolerant cotton

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

The digestibility of nitrilase was determined experimentally using *in vitro* mammalian digestion models. *In vitro* studies with simulated digestion solutions have been used as models for animal digestion for a number of years and have had wide application.

To obtain sufficient quantities of purified nitrilase for testing, the enzyme was expressed in *Escherichia coli* from a cloned *Klebsiella ozaenae* DNA fragment and purified to homogeneity (Stalker *et al* 1988). The coding region used to express nitrilase in *E. coli* was therefore identical to that transferred into Westar-Oxy-235. The molecular mass of nitrilase is approximately 37 kDa, however, the active form of the enzyme is as a dimer composed of two identical 37 kDa subunits.

Nitrilase was added to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) and incubated at 37ºC over a series of time points. The time points for SGF were 0 sec, 15 secs, 30 secs, 1 min, 5 mins, 10 mins, 30 mins, 1 hour and for SIF the time points were 0 sec, 1 min, 5 mins, 15 mins, 30 mins, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours.

Analysis of nitrilase after incubation in SGF showed that the protein is degraded to below the limit of detection within 15 seconds. Nitrilase was found to be stable in an inactive test system over the time period tested confirming that the degradation of nitrilase in the active test system is due to proteolytic activity, not to any molecular instability of nitrilase.

In SIF, nitrilase was degraded within 5 minutes of exposure. Once again, nitrilase was shown to be stable in an inactive SIF system.

The results of these studies demonstrate that nitrilase is rapidly degraded in conditions that mimic mammalian digestion, greatly minimising any potential for intact nitrilase to be absorbed by the intestinal mucosa.

Conclusion

Humans are highly unlikely to be exposed to nitrilase through the consumption of refined oil from bromoxynil-tolerant canola. Moreover, nitrilase does not possess any of the characteristics of known allergens. Therefore nitrilase has very limited potential to become a food allergen.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

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

Compositional analyses of key constituents were done on the seed, and its derived meal and oil fractions, harvested from field trials with Westar-Oxy-235 canola and its non-transformed parental control grown at various sites in Canada and France in 1992 – 1995. Data was also obtained from field trials in France in 1994 – 1995 with French canola elite lines that had been crossed with Westar-Oxy-235. Data was obtained for both bromoxynil-sprayed and unsprayed plants.

Proximate analysis

Proximate analysis was done on seed from Westar-Oxy-235, Tanto-Oxy (a hybrid between Westar-Oxy-235 and Tanto, a spring elite line used in France), and the respective parental control lines grown in field trials in Canada and France during 1995. All the bromoxynil-tolerant canola lines were treated with bromoxynil at 330 g/ha. The combined results are summarised in Table 5 below.

Table 5: Proximate analysis of seed harvested from Westar-Oxy-235 canola lines and control canola grown in Canada and France in 1995

Analysis	Parental control Lines Mean ± SE (n=6)	Westar-Oxy-235 canola lines Mean ± SE (n=6)
Dry matter (%)	92.85 ± 1.44	92.85 ± 1.76
Mineral content (% dry weight)	7.33 ± 1.66	8.18 ± 1.89
Nitrogen (2 reps)	4.35 ± 0.32	4.33 ± 0.20
Protein in seed (% D.W.)	27.03 ± 2.07	26.89 ± 1.26
Protein in meal (% D.W.)	45.91 ± 3.76	44.65 ± 2.84
Fat/oil (% D.W.)	41.10 ± 0.50	39.53 ± 1.76
Soluble sugars (% D.W.)	3.25 ± 0.95	2.90 ± 0.51
Total carbohydrates (% D.W.)	24.54 ± 3.76	25.40 ± 3.13
Gross energy (seed, Kcal/kg)	6491 ± 60	6494 ± 109
Gross energy (meal, Kcal/kg)	4894 ± 131	4802 ± 137

No significant differences were evident between the bromoxynil-tolerant canola lines and their parental controls in any of the major constituents.

Fatty acid analysis

New varieties of canola oil are analysed to ensure they meet certain specifications – this includes the fatty acid content. Canola oil has considerable natural variation in fatty acid composition and thus some variation in the composition of commercial canola oil is acceptable. The individual fatty acids measured were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 cis), linoleic acid (C18:2), and linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), and behenic acid (C22:0). Values for erucic acid (C22:1) are presented in Section 4.1, Table 3. The results are summarised in Tables 6 and 7.

Table 6: Fatty acid content (%total fatty acids) of seeds from Westar-Oxy-235 canola and control canola grown in Canada in 1992 – 1994. (see Table 7 for Codex ranges)

Fatty acid	Lines				
	Control (Unsprayed) (n =21)	Oxy-235 (Unsprayed) (n=5)	Oxy-235 (120g/ha) (n=5)	Oxy-235 (240g/ha) (n=5)	Oxy-235 (480g/ha) (n=5)
Palmitic acid	4.5 (3.9-5.9)[1]	4.4 (3.6-5.3)	4.2 (3.5-4.7)	4.1 (3.5-4.7)	4.1 (3.8-4.4)
Stearic acid	1.7 (1.2-2.5)	1.6 (1.3-1.9)	1.5 (1.4-1.6)	1.5 (1.3-1.7)	1.5 (1.3-1.7)
Oleic acid	61.9 (59.8-64.7)	63.8 (63.1-64.6)	64.0 (63.0-65.3)	63.7 (62.3-65.7)	64.4. (63.2-66.0)
Linoleic acid	19.5 (17.7-21.0)	18.4 (17.5-19.3)	17.9 (16.8-19.0)	18.3 (16.5-19.7)	17.8 (17.1-19.0)
Linolenic acid	9.0 (6.7-10.5)	9.1 (7.6-10.3)	9.6 (8.6-10.1)	9.4 (8.5-10.3)	9.5 (8.5-10.5)
Arachidic acid	0.7 (0.5-0.8)	0.7 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.7)
Eicosenoic acid	1.5 (1.2-2.4)	1.4 (1.2-1.6)	1.5 (1.3-1.6)	1.4 (1.3-1.6)	1.4 (1.3-1.6)
Behenic acid	0.4 (0.3-0.5)	0.4	0.4	0.4 (0.3-0.4)	0.4 (0.3-0.4)

[1] mean value, range in parentheses

Table 7: Fatty acid content (% total fatty acids) of seeds from French elite lines crossed with Westar-Oxy-235 grown in France in 1994 – 1995

Fatty acid	CODEX Ranges	Line	
		French elite lines (Unsprayed) (n=8)	Westar-Oxy-235 X French elite lines (480g/ha) (n=8)
Palmitic acid	2.5-7.0	5.7 (5.1-6.6)[1]	5.5 (4.8-6.2)
Palmitoleic acid	0.0-0.6	0.15 (0.1-0.2)	0.15 (0.0-0.2)
Stearic acid	0.8-3.0	1.55 (1.2-2.0)	1.5 (1.2-1.9)
Oleic acid	51.0-70.0	61.4 (58.1-65.3)	60.5 (50.6-64.4)
Linoleic acid	15.0-30.0	20.9 (19.0-23.0)	21.1 (19.0-26.0)

Linolenic acid	5.0-14.0	8.4 (7.6-9.5)	8.65 (7.3-11.8)
Arachidic acid	0.2-1.2	0.5 (0.2-0.8)	0.6 (0.5-0.8)
Eicosenoic acid	0.1-4.3	1.2 (0.8-1.9)	1.2 (0.9-1.7)
Behenic acid	0.0-0.6	0.3 (0.0-0.5)	0.3 (0.2-0.5)

[1] mean value, range in parentheses

All the fatty acids measured were within the ranges specified by Codex (Table 7) for canola quality oilseed rape (Codex Alinorm 99/7 Appendix II 3.1), and no major differences between modified and control crops were identified. Treatment with bromoxynil had no significant effect on the fatty acid content.

Sterol and tocopherol analysis

The levels of sterols and tocopherols were measured in seed from control and Westar-Oxy-235 canola grown in France and Canada in 1995 as well as from an elite line (Samourai), also grown in France in 1995. The bromoxynil-tolerant canola lines were all treated with bromoxynil. The results are summarised in Table 8 below.

Table 8: Total sterol and tocopherol levels in bromoxynil tolerant canola and control canola grown in field trials in Canada and France in 1994 - 1995

Trial and crop		Bromoxynil treatment	Total Sterols (mg/100g oil)	Total Tocopherols (µg/g oil)
Canada (1995)	Control	-	777.3	876.0
(n=2)	Westar-Oxy-235	-	753.8	785.0
	Westar-Oxy-235	330 g/ha	760.8	808.5
France (1995)	Control	-	828.2	955.0
(n=2)	Westar-Oxy-235	-	851.2	1015.0
	Westar-Oxy-235	450 g/ha	840.2	1007.0
France (1995)	Control (Samourai)	-	922.3	739.88
(n=8)	Samourai-Oxy-235	450 g/ha	943.1	716.13
		Literature values:		424.0 – 1054.0

No major differences were evident in total sterol and tocopherol content between control and bromoxynil tolerant canola. Treatment with bromoxynil had no significant effect on either the total sterol or total tocopherol content.

Unsaponifiable matter

The Codex specification for unsaponifiable matter states that the level must be not higher than 1.5%. This component was measured for oils produced from the 1995 French and Canadian trial crops. All were below the specified level, and no significant differences were seen between control and modified crops.

5.2 Ability to support typical growth and well-being

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

In the case of oil derived from bromoxynil-tolerant canola there is adequate compositional data to demonstrate the nutritional adequacy of the oil. However, a feeding study on canola meal was provided as additional supporting information and is evaluated below.

Feeding study in rats

A 28-day feeding study was carried out in rats to compare the effects of ingestion of canola cake made from both non-transgenic (Westar and Tanto) and transgenic (untreated and that treated with bromoxynil) canola. The study was performed in accordance with the OECD Principles of Good Laboratory Practice (OECD, 1982):

From days 1 to 28, each type of cake was administered *ad libitum* at a 10% concentration to groups of five male and five female rats. Clinical signs were recorded at least once a day throughout the study. Additional detailed physical examination was performed weekly. Body weights were measured on days –1, 1, 8, 15, and 22 and at final sacrifice. The weight of food supplied to each animal and that remaining at the end of the food consumption period was recorded for each week throughout the treatment period. From these records, the mean weekly consumption was calculated for each rat. Food spillage was also noted. For clinical pathology studies, blood samples were collected before necropsy. At necropsy macroscopic examination of the external surfaces, all orifices and all major body cavities, organs and tissues was carried out. Any significant macroscopic findings were recorded and the tissues (adrenal gland, heart, kidney, liver and spleen) samples taken.

Results

There were no mortalities and no clinical signs of toxicity in any of the groups. Neither the mean body weight, mean daily intake, haematology nor clinical chemistry was affected by the type of canola administered. Likewise, no differences were seen in the macroscopic observations, or the microscopic examination of the organs sampled.

At a level of 10% inclusion of canola cake in feed, therefore, there was no difference between the control and the transgenic canola in their ability to support growth and well being of rats.

5.3 Conclusion

Analysis of the compositional data of the canola seed and processed fractions indicates that there were no significant differences in the levels of key nutrients between Westar-Oxy-235 line and control lines. This was true for both untreated plants and those treated with bromoxynil.

REFERENCES

Astwood, J.D., Fuchs, R.L. and Lavrik, P.B. (1996). Food biotechnology and genetic engineering. In: *Food Allergy, Second Edition*, Metcalfe, Sampson and Simon (eds). Blackwell Sci, New York, pp 65-92.

Barker, R.F., Idler, K.B., Thompson, D.V. and Kemp, J.D. (1983). Nucleotide sequence of the T-DNA region from the Agrobacterium tumefaciens octopine Ti plasmid pTi15955. *Plant Mol. Biol*. **2:** 335-350.

Bevan, M., Barnes, W.M. and Chilton, M.D. (1983). Structure and transcription of the nopaline synthase gene region of T-DNA. *Nuc. Acids Res*. **11:** 369-385.

Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L. and Boyer, H.W. (1977). Construction and characterisation of new cloning vehicles II. A multipurpose cloning system. *Gene* **2:** 95-113.

Buckland, J., Collins, R. and Pullin, E. (1973). Metabolism of bromoxynil octanoate in growing wheat. *Pestic. Sci*. **4:** 149-162.

Comai, L. and Stalker, D. (1986). Mechanism of action of herbicides and their molecular manipulation. In: *Oxford Surveys of Plant Molecular & Cell Biology*, Volume 3, B.J. Miflin, Ed. Oxford University Press, pp166-195.

Elsayad, S. and Apold, J. (1983). Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by native and synthetic peptides. *Allergy* **38:** 449-459.

Elsayad, S., Apold, J., Holen, E., Vik, H., Florvaag, E. and Dybendal, T. (1991). The structural requirements of epitopes with IgE binding capacity demonstrated by three major allergens from fish, egg and tree pollen. *Scandinavian Journal of Clinical Laboratory Investigation* **51:** 17-31.

Fuchs, R.L. and Astwood, J.D. (1996). Allergenicity assessment of foods derived from genetically modified plants. *Food Technology* **50:** 83-87.

Gardner, R.C., Howorth, A., Hahn, P., Brown-Luedi, M., Shepherd, R.J. and Messing, J. (1981). The complete nucleotide sequence of an infectious clone of cauliflower mosaic virus by M13mp7 shotgun sequencing. *Nuc. Acids Res.* **9:** 2871-2898.

Hammond, B.G. and Fuch,s R.L. (1998). Safety evaluation for new varieties of food crops developed through biotechnology. In: *Biotechnology and safety assessment*. Thomas JA (ed.), Taylor and Francis, Philadelphia.

Hirsh, P.R. and Beringer, J.E. (1984). A physical map of pPH1JI and pJB4JI. *Plasmid* **12:** 139-141.

Hood, E.H., Helmer, G.L., Fraley, R.T. and Chilton, M.D. (1986). The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside of T-DNA. *J. Bacteriol.* **168:** 1291-1301.

Krieg, N.R. and Holt, J.G. (1984). *Bergey's Manual of Systematic Bacteriology*, Volume 1. Williams and Wilkins (eds), Baltimore, MD.

Lebrun, M., Waksman, G. and Freyssinet, G. (1987). Nucleotide sequence of a gene encoding corn ribulose-1,5-bisphosphate carboxylase/oxygenase. *Nuc. Acids Res*. **15:** 4360.

McBride, K., Kenny, J. and Stalker, D. (1986) Metabolism of the herbicide bromoxynil by *Klebsiella pneumoniae* subsp. o*zaenae. Applied and Environmental Microbiology,* **52:** 325-330.

Metcalf, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit. Rev. Food Sci. Nut.* **S36:** S165-S186.

OECD (1982) OECD Principles of Good Laboratory Practice.

Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:** 503-517.

Stalker, D., Malyj, L. and McBride, K. (1988). Purification and properties of a nitrilase specific for the herbicide bromoxynil and corresponding nucleotide sequence analysis of the *bxn* gene. *J. Biol. Chem*. **263:** 6310-6314.

Stalker, D. & McBride, K. (1987) Cloning and expression in *Escherichia coli* of a *Klebsiella ozaenae* plasmid-borne gene encoding a nitrilase specific for the herbicide bromoxynil. *J. Bacteriol.*. **169:** 955-960.

Thimann, K. and Mahadevan, S. (1964). Nitrilase 1. Occurrence, preparation, and general properties of the enzyme. *Arch. Biochem. Biophys*. **105:** 133-141.

US EPA (1998). Re-*registration Eligibility Decision. Bromoxynil*. United States Environment Protection Agency. EPA738-R-98-013.

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

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

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

Zhang, L., Olsen, E., Kisil, F.T., Hill, R.D., Sehon, A.H. and Mohapatra, S.S. (1992). Mapping of antibody binding epitopes of a recombinant *Poa p* IX allergen. *Molecular Immunology* **29:** 1383-1389.

ATTACHMENT 3

SUMMARY OF PUBLIC SUBMISSIONS

A. First round submissions

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

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

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

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

5. **Public and Environmental Health Service (Australia)**
   - Believes that the data provided should cover both the intentional and unintentional effects of the genetic modification. The unintended consequences of random insertion of new genetic material into the host genome could include loss or change of function of gene or controlling element, disregulation or amended regulation of the gene or controlling element, or production of a novel hybrid protein which could occur in an unregulated manner. They should also cover any compositional changes e.g. nutrients, antinutritional factors, natural toxicants, and define when a change would be considered 'significant'

- Potential effect of introduced proteins on metabolic pathways should be addressed e.g. over-expression or inhibition of enzymes
- Data should include details of whether introduced proteins are detectable in whole commodities, processed products and highly processed derivatives
- Data should include details of toxicity and allergenicity tests to prove that food is safe, as well as address issues of specificity and potency of proteins. It should also address the ability to support typical growth and well-being
- Data for herbicide-tolerant plants should be derived from studies performed on plants treated with herbicide. They should address the human toxicity of the herbicide and whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.

6. David Grundy (Australia)

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

7. Leesa Daniels (Australia) Member of the Genetic Engineering Action Group

- Believes that:
  - Scientific research although limited, has brought concerns to light
  - Substantial equivalence is a subjective principal
  - Comprehensive and mandatory labelling must be urgently implemented
  - The cauliflower mosaic virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
  - Antibiotic marker genes could lead to increase in antibiotic resistance
  - Several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

8. Australian Food And Grocery Council

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

9. New Zealand Ministry of Health

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

10. Nestlé Australia Ltd.

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

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

- Believe that current testing procedure is inadequate and that human trials are the only adequate method, as with testing of new drugs. Also that physiological and neurological effects as well as the toxicological and allergenic effects should be looked at, and that an independent body should be responsible for testing
- Do not support the use of antibiotic markers, since they believe they may pose a threat to efficacy of antibiotics in humans

- State that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans confirming the inadequacy of the term 'substantial equivalence'
- Raise the point that although these crops have been approved elsewhere, this situation may change with consumer pressure
- Do not accept that it is impossible to source food to ascertain whether or not it contains GM ingredients. Believe that if McCain and Sanitarium can do it, then others should also be able to
- State general concern about the risk that MRLs will be raised as a result of herbicide-tolerant crops being developed, and feel that the calculations used are flawed and are not based on safety criteria
- Believe that the use of GM crops in animal feed should also be regulated. A378
- State concern over possible increase in glyphosate use (it is apparently confirmed in one reference that herbicide use increases with herbicide resistant crops), referring to studies that link the chemical to Hodgkin's lymphoma, and the possibility that Europe may ban it due to adverse effects on beneficial insects**.** They are particularly concerned that glyphosate is not looked at by the same regulatory body as that looking at GM foods

A379, A388

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

A372, A375, A380, A381, A386

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

A380, A382, A383, A384, A385, A386

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

A387

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

12. Health Department of Western Australia

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

13. Meat New Zealand

A379

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

14. BRI Australia

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

15. Food Technology Association of Victoria Inc.

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

16. Diane Davie (Australia)

- Believes all 13 applications should be rejected, since they have not undergone human safety testing here or overseas, and have not been assessed on their ethical merits
- Believes that risks include:
  - Bacterial and viral vectors which could affect human physiology

- Herbicide and insect-resistance genes, which could increase allergies and antibiotic resistance
- Environmental risks

- Also believes that ANZFA must heed the concerns of consumers opposed to GM foods.

17. Martin Hurley, David Hook, Ian Smillie, Margaret Dawson, Tee Rodgers-Hayden, David Lovell-Smith (Natural Law Party), Barbara Brown, 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 Australia), Brennan Henderson (New Zealand)

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

18. Richard and Sharon Moreham (see also above)

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

19. Vicky Solah (Australia)

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

20. Dr Rosemary Keighley (Australia)

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

21. Nicola Roil (Australia)

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

22. Ian and Fran Fergusson (Australia)

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

23. Lyndal Vincent (Australia)

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

24. Fay Andary (Australia)

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

25. John and Francesca Irving (Australia)

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

26. Diana Killen (Australia)

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

27. Sheila Annesley (Australia)

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

28. David and Edwina Ross (Australia)

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

29. Beth Schurr (Australia)

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

30. Beth Eager (Australia)

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

31. Bruce Pont and Ljiljiana Kuzic-Pont (Australia)

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

32. Chitta Mylvaganum (Australia)

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

33. John Stevens (Australia)

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

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

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

35. Jan Kingsbury (Australia)

- Believes that GM-foods could result in loss of economic advantage for Australia and New Zealand since they are known internationally for pure and safe products

- Believes that foods are being complicated and pushed by big internationals, and organic farmers are being contaminated by cross-pollination.

36. Teresa Sackett (Australia)

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

37. John and Sandy Price (Australia)

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

38. John Scott (New Zealand)

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

39. R A Randell (New Zealand)

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

40. National Council of Women of New Zealand

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

41. Safe Food Campaign (New Zealand)

- Believes that approval should be rejected, and a moratorium be put in place until after the Royal Commission of Inquiry, for various reasons:
  - Possible effects on non-target insects
  - Spread of GM pollen may cause contamination of non-GM (especially organic) crops, and may result in the spread of herbicide-tolerance genes and an increase in resistance development.

- Cross-pollination is considered a particular risk for canola (A372 & A388). Bt resistance development is noted as being a particular risk for A382, A383 & A384
- Lack of long-term testing means health risks are not known
- Use of broad-spectrum pesticides affects wild flowers and non-target insects.

42. Jocelyn Logan, Caroline Phillips (New Zealand)

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

43. Robert Anderson (member of Physicians and Scientists for Responsible Genetics – New Zealand)

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

44. Stephen Blackheath (New Zealand)

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

45. Claire Bleakley (New Zealand)

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

B. Second round submissions

1. Dr Kate Clinch-Jones (Australia)

- Notes that 41 of the 45 submissions received opposed the introduction of this GM food. States that this percentage would be higher if the 41 submitters using a generic email objection were counted individually rather than as one body.
- Notes that those opposed to the introduction of GM food represent a broad cross section of the community, and include individual doctors, scientists and the Health Department of Western Australia.

- States that ANZFA does not produce any new evidence to support its arguments against the points raised in submissions and instead, ANZFA's response is based on rhetoric, and designed to forward the political stance that GM foods are safe.
- ANZFA reiterates disputed statements from the original safety assessment and claims they provide additional reassurance that various aspects of the GM food are safe.
- States that the public does not want reassurance but demands scientific proof that GM food are safe for consumption.
- Scientific proof of safety simply does not exist.
- States that in particular, no human feeding studies have ever been done and that animal studies look at gross parameters and omit vital assessments such as blood analysis and histopathology and use a small number of animals, rendering any statistical analysis virtually meaningless.
- States it is time that those responsible for food safety assessments acknowledge that GM foods pose significant, unique health risks.
- Submits that in the interests of public health, all GM foods already on the market should be immediately recalled until satisfactory independent safety test data is available.

2. Ministry of Health (New Zealand)

- Agrees with ANZFA's assessment that oil from this variety of canola is as safe for human consumption as oil from other commercial cotton varieties.
- Notes that only the oil is intended for human consumption.
- The assessment report might have been improved by a discussion of any potential open reading frames around the insert/plant genome junction regions. However, this is unlikely to affect the outcome of the safety assessment, as the oil will not contain DNA or protein.
- It would have been useful to include a consideration of the ioxynil metabolite 3,4 diiodo-4-hydroxybenzoic acid, although the toxicity and exposure of this compound is unlikely to be different from that of 3,5 dibromo-4-hydroxybenzoic acid.

3. Food Technology Association of Victoria Inc (Australia)

- The Technical Sub Committee have reviewed the document and accepted the application without any further comment.

4. National Council of Women of Australia

- States that ANZFA shows no inclination to revisit their guidelines and update them in light of new knowledge.
- Council notes that the meal, used for animal feed, should be assessed by the Gene Technology Regulator however to their knowledge this has not been done yet humans eat animals which may be fed genetically modified meal.
- It's not clear from the Background information if both the oil and the meal are coming into Australia for further use in manufacture or whether it is just the possibility of this oil having been used in imported processed food that permission is being sought.
- There is no indication from the information that would indicate this line was one of the original products allowed to remain on the shelves before the standard came in.
- Council would have more faith in the studies submitted by the companies if they were peer reviewed and/or independently repeated, rather than just assessed, as is currently the situation.
- Evidence the Council has viewed suggests that bromoxynil has a moderately high acute toxicity, in addition this application does not include any chronic tests either on animals or humans.
- ANZFA have made a case for approval using the Pest Management Regulatory Authority of Canada's similar registration for bromoxynil, however there are reputable scientists in Canada who lobbied heavily, although unsuccessfully, against approval of this chemical. ANZFA states that the US EPA determined that there was no concern that DBHA would exhibit significant toxicity over the parent bromoxynil and that bromoxynil poses negligible risk to human health at expected exposure levels. Has ANZFA taken this on face value or evaluated the evidence the US EPA used to make this statement. How can such a statement be relied upon if chronic long term exposure has not been taken into account.
- Despite the majority of submissions being opposed to the GM applications, ANZFA inevitably approves them and the Regulatory Impact Statements shows that it is usually for reasons of industry and trade. The fact that consumers do not want this technology in their foods is never taken into consideration.

- States that that Regulatory Impact Statement is biased and that it is too broad and general and is not specific to the application, therefore many of the statements made are incorrect. To be valid, the RIS needs to be specific to the application. States that it is misleading to be non-specific when developing a RIS.
- The submission contained three enclosures – a copy of the ANZFA Media Release of 13 February 2002, an article by Barry Commoner entitled ' Unravelling the DNA Myth. The Spurious Foundation of Genetic Engineering" published in Harper's Magazine in February 2002, and an ISIS Report from 12 November 2001 on *Bt*.

5. Bruce Smith (New Zealand)

- Opposes the release of these products onto the market for growing and consumption, particularly for human consumptions.
- Finds the products unacceptable for the following reasons:
  - the right to personal choice – the majority of consumers in NZ do not want any GM products in their dietary intake. It makes no difference if ANZFA tells them they are safe as consumers consistently choose not to want to each GM products at all. The GM labelling that was supposed to come into force in December 2001 does not even give the public the information to reject GM products for themselves in all cases, e.g. oils.
  - food safety issues – ANZFA's statement that the food safety concerns raised in submissions have been addressed by the safety assessment is at best misleading, at worst a deliberate misrepresentation of the real situation for the purposes of trying to change or obviate actual negative public opinion. The safety assessment has not addressed food quality concerns that he would have raised had he known submissions were invited, including allergenicity, nutritional aspects and agricultural chemicals.
  - environmental issues – considers it incumbent on ANZFA to reject processes and foods the production of which diminishes or increases the risk of diminishing, the capacity of the environment to sustain life, especially human life, in both the short and long term. Where the likelihood of such an outcomes is unclear, ANZFA should invoke the precautionary principle and at least not allow propagation or introduction of any such materials into the human food chain until better processes and understandings are developed. Even if these GM products were considered acceptable for consumption (which they are not), but not for planting in Australasia, ANZFA's decision should have full regard for possible effects to the global environment, and again they should be rejected.
  - business ethics – it is unacceptable that profit and business interests should reign paramount in the important issues of food production in society. To truly represent the peoples of Australasia, ANZFA should at least be making an assessment of human and environmental outcomes in addition to profit margins.
  - the role of ANZFA – although ANZFA was set up to act in the protection of and on behalf of the people of Australia and New Zealand, ANZFA clearly works with and on behalf of the food industry as well. In the case of the release of GM material into the human food chain, these two masters are generally in direct conflict. It is clear from the ANZFA standardised reports that ANZFA effectively regards the public as its servant, or as buffoons to be told what is good for them. Consequently this means that political forces and the food industry, including the transnational food giants and the mechanisms of GATT actually act as ANZFA's master. There is no way ANZFA can try to represent the best interests of the people under such a regime. ANZFA clearly supports the release of GM products into the market for human consumption and just as enthusiastically supports a labelling regime that does not require full labelling of products.
- The public deserves the right to make their own fully informed choices about what they eat. Part of this process is recognition that a large majority of the people do not want the introduction of GM products, especially into the human food chain, and that therefore such products should not be released.

6. Consumers' Association of South Australia Inc. (Australia)

- Supports the submission of the National Council of Women of Australia.

7. Katrina Upperton for GE FREE Northland (New Zealand)

- Is concerned to hear that DBT418 corn is suitable for human consumption.

- Equivalent groups to ANZFA in the UK (the Royal Society) and France (AFSSA) have recently spoken out for the need for more robust testing and trialling of new foodstuffs before they are openly available for consumption. Believes their approach to be one of responsible caution and should be followed in this country while the verdict is still out on the potential effects in the population from consuming foodstuffs that have been genetically modified.
- New Zealand's government has given the lead for caution and the early release of insufficiently trialled food on the domestic market ignores these concerns. Once released the effects of genetic pollution, possible harmful effects and health concerns cannot be rectified.
- Strongly request that this and other genetically modified foods are not passed for released.

8. Mrs I.P. Hancox (New Zealand)

- Is very concerned about changing the make up of canola plants and will certainly look for a well-labelled oil, which has not been altered.
- Feels that the purpose of using science-based writing is to try to convince the majority of people to take no interest which does not seem to be listening to those who sincerely express concern.
- A very big concern is why is cancer increasing so drastically.
- What we eat affects our bodies and the effort the body makes to overcome diseases.
- Once steps are taken to alter the make up of canola plants it cannot be turned back.

9. Australian Food and Grocery Council (Australia)

- Supports approval of the application.
- As ANZFA has concluded that the introduced gene in bromoxynil tolerant canola line Westar-Oxy-235 is not considered to produce any additional public health and safety risks and that oil from bromoxynil tolerant canola line Westar-Oxy-235 is as safe and wholesome as oil from any other commercially available canola varieties the canola should be approved for use so that food manufacturers can make their own choice with regard to its use.
- Supports the application of the labelling requirements of Standard A18/1.5.2 to bromoxynil tolerant canola.

10. J. E. Brooks (New Zealand)

- Is opposed to the use of GM corn for human consumption in New Zealand.
- Notes that ANZFA believes the food to be safe but is not able to guarantee its safety.
- Will only consider the use of GM food when ANZFA can guarantee its safety.
- It is far to soon to say it is safe, it often takes years to see the results of some of these experiments on human health. In the meantime it should not be allowed into New Zealand.
- Asks that we wait and see some of the results from places like Canada for the next few years first.

11. David and Darcy Jones and Family (Australia)

- The US FDA recognises serious health concerns regarding bromoxynil resistant canola but have still allowed its passage.
- Wants to know where they can view or obtain the scientific information, field data, and test results along with the independent investigation of A388 that ANZFA used to conclude that these products are safe for human consumption and are in the best interests of the Australian and New Zealand consumer.

12. Alan Willoughby (New Zealand)

- Strenuously objects to the proposal to recommend the use of GM canola for human consumption. There appear to be few valid reasons for modification except for the convenience of the decreasing number of growers who spray food crops with poisons.
- His concerns include the following:
  - food sprayed with herbicides will contain residues which will be harmful to the health of some people. As a past sufferer of pesticide poisoning, some people are more susceptible to the effects of so called safe pesticides than others. Modifying crops to be resistant to herbicides simply increases the likelihood that people eating those crops will exhibit non-specific symptoms of pesticide poisoning, reducing their quality of life and ability to contribute to their communities and the nation while impoverishing them in their vain search for cause and remedies.

- The application of additional poisons to food crops has not been shown to increase yields. The percentage losses of crops to pests since the application of pesticides became routine has increased rather than showing the expected decrease. There is no evidence to indicate that there would be any difference in this case, the only winners being the chemical companies, which produce the pesticides.
- The growing of GM crops offers the danger of cross-pollination with non-GM crops, thereby reducing or eliminating the raw species, preventing any reversion to the original.

- Calls upon his representatives in government to strenuously fight the moves currently being made to introduce they genetically modified crops.

13. West Australian Department of Health (Australia)

- The West Australian Food Advisory Committee notes that canola oil is unlikely to have protein present therefore represents minimal potential risk.
- The meal that results from the crushing and extraction of the canola see will contain GM material and this meal may be used for animal feed. The Committee is concerned that the effects of feeding animals are largely unknown and that there should be appropriate controls over the use of such products grown in Australia.

14. John Forrest (Australia)

- Requests that ANZFA reconsider its decision to remove products containing GM canola from the list of products required to display labelling identifying its source. The assertion that these products contains no DNA is irrelevant as other substances may be produced which have not been identified and whose effects are unknown.
- The contention that the food is harmless is a hypothesis not a fact, presenting it as a fact does no service to ANZFA.
- ANZFA needs to act in the interests of consumers not industry.

15. Carl D. Kneipp (Australia)

- Wishes ANZFA to advise how he as a food consumer is being protected from potential side effects from the GM industrial production process, considering we have chosen not to enforce the disclosure of GM food to the consumer.
- If ANZFA have failed to identify how GM food could affect the society and the consumer, then ANZFA is not producing responsible public policy.
- Would prefer not to consume GM food and by ANZFA's lack of specifying that GM foods must be declared have reduced that choice for him.
- Demands his right to make the choice as to the source of food he consumes by making that decision when purchasing food for consumption, or else to hold ANZFA responsible for improperly protecting the consumers of this food from reasonable mis-consumption.
- By backing the pro-GM lobby, ANZFA is simply collapsing in the face of a strong and profitable pro-GM lobbying to preserve the mass food producers' market share and profitability and limit natural food producers' and natural food consumers' rights.
- Suggests ANZFA is shirking its responsibilities as a public regulatory as ANZFA does not know the absolute and long term effects of GM food.

16. Dougal Crockett (New Zealand)

- Expresses deep concern about the granting of consent to farm bromoxynil tolerant canola. Has concerns about what benefit they will bring to consumers and the nation as a whole.
- The only benefit he can see is that they can withstand continual exposure to the company's own herbicide.
- Is concerned that the herbicide will get into the human food chain directly through the canola itself.
- His real concern is that such continued usage of herbicides will have long-term destructive effects upon the soil and local waterways.
- Knowing that countries such as the UK have supermarkets that are committed to being 1005 GE free, with aims of being 100% organic by 2008, he wonders what possible benefit the introduction of such GE crops can have to New Zealand. Thinks that we would be better placed investing our farming future in organics, as this is what our export markets are demanding.
- The GE industry appears to be trying to introduce its products by stealth and he does not trust them to have the public's interest at heart.

- Hopes that ANZFA does not see fit to be bullied into accepting these crops and considers the benefit for us all in declining further GE crop licences in Australia and New Zealand.

17. J. Reeve (Australia)

- Is against any form of genetically engineered food.
- Is disturbed that modified foods have already been introduced into the open market.
- Objects most to the following:
  - lack of detailed information on packaging, which is absolutely essential to give the public the choice to eat GM foods or not;
  - the problem arising from large companies suing farmers of non-GM crops because of cross contamination;
  - the monopoly arising with large companies on the distribution of seeds.

18. Mark Neilson (Australia)

- Wants to express his deep regret and anger that ANZFA persists in forcing GM foods on Australians without requiring they be clearly labelled.
- Notes that Monsanto is being sued for their actions in the US over the contamination of a waterway in Anniston.
- Asks what effects the higher residual quantities of pesticides/herbicides have on humans.
- Wants to lodge an official objection to the approval of any GM foods until the following have been done:
  - full independent testing over a minimum of 5 years to allow any short term negative effects to be observed;
  - full independent study on the possible long term effects of cross-pollination;
  - full investigation of all unauthorised and accidental GM seed releases, with the guilty releasers receiving life bans on their product in Australia/New Zealand.
  - Knows that the makers of GM seeds will put the sale of their product before the well being of the public.
- States ANZFA must put the people of Australia and New Zealand first and must not be influenced by government or corporations in doing its job.

19. Groundswell Canterbury (Spirit of Living Trust) (New Zealand)

- Would like to see the Aventis GE canola not approved for the following reasons:
  - the simplistic assumption that when a totally unrelated species gene can be put into a new host DNA structure it will only do one thing it is expected to do has been proven completely false.
  - Aventis does not provide a rigorous and independent analysis that the GE canola is genetically stable and thus cannot prove it is safe for human consumption therefore it should be rejected from being allowed in our food chain.
  - The 28 day tests with rats was completely inadequate and scientifically indefensible to test for health risks to humans. Far greater testing needs to be carried out by independent scientists before this GE canola can be approved safe for human consumption.
  - Believes that the risk from CaMV alone is sufficient grounds to not approve the Aventis GE canola application.
  - The risk of having another GE food introduced into our diet that is glyphosate resistant puts another food into our diets with increased glyphosate residues which increases the risk of cancer and serious illness.

20. GE Free New Zealand In Food and Environment (RAGE) Inc (New Zealand)

- Wishes to state its opposition to the proposed amendment giving approval to these crops to be used for human food.
- In light of recent reports around the world on the need for more adequate regimes to determine nutritional differences, allergen and toxin identification and post sales monitoring, it is felt that no more GE foods should be approved particularly while they are still unlabelled as a result of more tardiness by manufacturers and the willingness of ANZFA to support this view.
- The foods should not be passed for the followed reasons:

- pesticide residues – the additional burden on health from increased herbicide residues consistent with the use of pesticide resistant crops is one that most people do not want to be exposed to. With regard to bromoxynil residues the ANZFA report states that "significant residues can be present on BXN cotton; although it is not clear to what extent these residues persist in refined oils and linters." And yet without clear evidence of levels of contamination ANZFA is prepared to approve the product. This is irresponsible, particularly since it goes on to say that the absence of a CODEX or Aus or NZ MRL maximum residue limit means that it is not permitted in food in New Zealand at above 0.1 ppm, therefore it may well be responsible for aiding and abetting lawbreaking on food standards in New Zealand if levels are found to be above this. It appears however that sufficient tests have not yet been carried out or any standard set.
- General safety issues - the use in application A388 of *Agrobacterium* is not well described in the summary, merely referred to as the transformation system. Until quite recently, the genetic engineering community has assumed that *Agrobacterium* does not infect animal cells, and certainly would not transfer genes into them. But this has been proved wrong. A paper published earlier this year reports that T-DNA can be transferred to the chromosomes of human cancer cells.
- toxicology issues – insufficient testing has been done to prove beyond doubt that this GE food is safe.
- conclusions of the safety assessment – safety assessments are merely an assessment, they are subjective and involve value judgements being made by the assessor. It is not sufficient to cynically point out that microbial food sources have been a component of the human diet over several thousand years and this statement does not satisfactorily take the place of real evidence to prove the safety of GE foods. No evidence has been given that has been peer reviewed of any stringent and rigorous testing having taken place and this has been so with all previous approvals prior to this.
- labelling – a sham of a labelling system has been implemented that will undoubtedly allow much of these products to remain unlabelled and unseen.
- public submissions – the blanket statement that describes how opposition to the foods was from those who perceived GM foods to be unsafe is a very dismissive way of treating justified concerns by members of the public. This statement demonstrates the condescending attitude held by ANZFA with regard to public submissions.

- It appears that despite the use of a science in its infancy ANZFA appears to disregard the fact that continuing evidence demonstrates that there are problems with GE foods and that consumers do not wish to eat them. Unbiased external panels may well be able to identify issues that ANZFA representatives are unable to see due to their somewhat blinkered approach to safety assessment.
- It would not have expected any other conclusion from ANZFA, who have proved them to be at the behest of corporate and government interests.

ATTACHMENT 4

GENERAL ISSUES RAISED IN PUBLIC SUBMISSIONS

The majority of submissions received in relation to GM foods express general views opposed to the use of gene technology and assert that food produced using this technology is unsafe for human consumption. The general issues, which are not necessarily specific to the application, are addressed below.

1. ANZFA's processes

ANZFA's general processes for the risk assessment of GM foods have been criticised by several submitters from Australia and New Zealand.

Response

The processes used by ANZFA for safety assessment and labelling of GM foods were subject to an independent assessment by the New Zealand Royal Commission on Genetic Modification which was conducted during the first quarter of 2001. In its deliberations, the Royal Commission considered that both the New Zealand Environmental Risk Management Authority (ERMA) and ANZFA provided a robust regulatory environment and stated that the authorities acted conscientiously and soundly in carrying out their duties. The Commission expressed confidence in the ANZFA safety assessment process, stating that it considered it unlikely that food that has satisfied the food standard will have harmful effects. The Commission also considered that ANZFA carries out its functions with an appropriate degree of independence not only from political influence but also from the influence of commercial interests. In reaching this view, it should be noted that the Commission examined the criticisms levelled at ANZFA's processes and the detailed rebuttal of those criticisms supplied to the Commission by ANZFA, including issues such as adequacy of the toxicological studies, use of substantial equivalence, sources and independence of data, and the use of antibiotic resistance marker genes.

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

2. Sources of data

The use of company data from the Applicant during the assessment is seen by some submitters to compromise the independence and validity of the safety evaluation.

Response

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

ANZFA submitted a full data package (15 volumes of raw data on Roundup Ready Soybeans) to the Commission for inspection. The Commission states that it looked closely at the quality of this data and came to the view that ANZFA did receive and assess raw data and that the processes were valid in this regard.

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

3. Imported GM foods versus GM crops

Some submitters have argued that approvals for GM foods or commodities as imports to Australia and New Zealand is a tacit approval for the GM crop to be grown in either country.

Response

The regulatory framework for approval by ANZFA of safety of GM foods (imported foods and derived from GM crops grown in Australia) is separate from that of the Office of the Gene Technology Regulator (OGTR) and the Environmental Risk Management Authority (ERMA), which have responsibility for approving the environmental release of GM crops in Australia and New Zealand respectively. ANZFA's responsibilities are to ensure the safety of the food supply and protect public health. Approval of GM food under Standard A18 of the *Food Standards Code* (Standard 1.5.2 in Volume 2) cannot be regarded as tacit approval for the environmental release of the crop in Australia since the environmental issues are completely separate and entirely different to food safety issues.

4. Compositional studies

The compositional analysis occasionally reveals that some of the components of the genetically modified plant line under assessment are statistically different to the control line. Some submitters therefore claim that the GM line is not comparable to the control line.

Response

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

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

5. The safety of genetically modified foods for human consumption

Many submitters raise the issue of public health and safety in relation to food produced using gene technology. In particular, it is often 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.

Response

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

Because the use of gene technology in food production is relatively new, and a long history of safe use of these foods has yet to be established, it is appropriate that a cautious approach is taken to the introduction of these foods onto the market. The purpose of the pre–market assessment of a food produced using gene technology under Standard A18/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

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

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organization (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD).

ANZFA has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

6. The need for long-term feeding studies

Concerns are often expressed in relation to the lack of long-term toxicity studies on genetically modified foods.

Response

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 would 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.

7. Substantial equivalence

Some submitters express concern regarding the use of the concept of substantial equivalence as part of the assessment process and reject the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

Response

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

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

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

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organization (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for over ten years and has been an integral part of the safety assessment of some 50 products.

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

8. The nutritional value of food produced using gene technology

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

Response

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.

9. Potential toxins and allergens

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

Response

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.

10. Antibiotic resistance

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

Response

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 Organization 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 microorganisms 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 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 potentially unnecessary given the existence of adequate alternatives, and therefore should be phased out.

The recent JETACAR (Joint Expert Technical Advisory Committee on Antibiotic Resistance) Report states (page 117, referring to a specific gene, *nptII*) that the use of antibiotic resistance genes in GM foods is unlikely to contribute in any significant way to the spread of antibiotic resistance in human pathogens. The issue of the use of antibiotic resistance marker genes in GM foods was discussed at the Ministerial Council meeting held in late July 2000. At that meeting, Professor John Turnidge, former Chair of JETACAR and now Chair of the NHMRC Expert Advisory Group on Antibiotic Resistance (EAGAR), appeared at the Council meeting as expert adviser on this matter in support of ANZFA's assessment on this issue.

11. Transfer of novel genes to humans

Some submitters have expressed the view that the transfer of any novel gene within the human digestive tract may be a health concern.

Response

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.

12. Viral recombination

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

Response

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 is considered by the scientific technical committee of the Office of the Gene Technology Regulator (OGTR) on a case–by–case basis when assessing such projects.

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. This was also the conclusion of the UK Royal Society, which recently considered this issue[4]. Plant viruses are also biologically incapable of naturally infecting human or animal cells.

13. Labelling of foods produced using gene technology

Submissions generally call for comprehensive labelling of foods produced using gene technology, based on perceptions that the foods are potentially not as safe as conventional foods, even where no novel genes are present. Based on consumer "right to know" arguments, it is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

[4] The Royal Society (2002). Genetically modified plants for food use and human health – an update.

Response

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard A18 (Volume 1) / 1.5.2 (Volume 2) in the *Food Standards Code* came into effect on 7 December 2001, allowing 12 months implementation period for compliance to the new provisions.

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

Exempt from these requirements are:

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

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

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

14. 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.

Response

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.

15. 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.

Response

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 for GM foods 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, 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[5], available at no charge on request. Since completion, this document has been widely distributed and may assist in addressing some of the safety concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

16. Maori beliefs and values

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

Response

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.

17. 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.

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

Response

These issues are considered as part of the comprehensive assessment processes of the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand. Since June 2001, OGTR regulates all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

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

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

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

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

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

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

18. Maximum residue levels of agricultural/veterinary chemicals

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

Response

Residues of these chemicals can only legally be present if the chemical has been registered for use in Australia and/or New Zealand, and it has been demonstrated that the residue at specified levels does not lead to adverse health impacts.

The concentration of a chemical residue that may be present in a food is regulated through maximum residue limits (MRLs). The MRL is the highest residue concentration that is legally permitted in the food. Food products have to meet the MRL, whether or not they are derived from genetically modified organisms. The MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

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