Crop & Food Research Confidential Report No. 427

Coexistence of genetically modified and non-genetically modified crops

M Christey[1] & D Woodfield[2]

[1] Crop & Food Research

[2] AgResearch Ltd

Report prepared for the Ministry for the Environment

June 2001

Disclaimer

This report presents research done by M Christey at Crop & Food Research and D Woodfield at AgResearch Ltd, and should not be taken as representative of the views of the Ministry for the Environment.

1 Executive summary 1
2 Introduction 5
3 Deregulation of GM crops and coexistence overseas 7
3.1 Field trials and release of GM plants 7
3.2 Australia 7
3.3 UK large-scale GM farm trials 8
3.4 USA 10
3.5 Canada 11
3.6 Argentina 11
3.7 Summary 12
4 New Zealand situation 14
4.1 GM research 14
4.2 Range of crops grown and imported into New Zealand 16
4.2.1 Pasture and forage crops 16
4.2.2 Arable, vegetable, and fruit crops 16
4.2.3 Forestry 18
4.2.4 Cut flowers 18
5 Minimising GM contamination of non-GM crops 19
5.1 Pollen transfer 19
5.2 Seed dispersal 20
5.3 Barriers to gene flow 22
5.3.1 Physical barriers and seed certification 22
5.3.2 Genetic barriers 23
5.4 Consequences of contamination 24
5.5 Summary 25
6 Case studies for coexistence in New Zealand 26
6.1 GM Potato 26
6.2 GM Brassicas and herbicide resistance 27
6.3 GM White clover 28
6.4 Bt transgenics 29
7 Segregating GM and non-GM products 31
7.1 Imported seed and GM detection 31
7.2 Harvest/storage/transport of produce 32
7.3 Organic production 33
7.4 GM detection 34
7.4.1 Australia 34
7.4.2 USA 35
7.4.3 UK 35
7.4.4 Summary and recommendations 35
8 Regulation and regulatory agencies 37
8.1 ANZFA 37
8.2 HSNO Act 37
8.3 Resource Management Act 1991 38
8.4 Liability 38
9 Acknowledgements 40
10 References cited 41
10.1 Scientific papers 41
10.2 Web sites 44
11 Appendices 47
Appendix 1: Roundup Ready® Cotton Technical Manual 47
Appendix 2: Protocol for preventing GM contamination in sweet corn seed imports 51
Appendix 3: EPA assessment of glyphosate tolerant canola 58

Table 1: Separation distances applied to the spring 2001 FSEs *10*
Table 2: GM crops no longer regulated in the USA[a] *13*
Table 3: Summary of New Zealand GM research in economically important plants *14*
Table 4: Summary of field trials approved in New Zealand *16*
Table 5: Area sown in various crop types in New Zealand *17*
Table 6: Export value of cut flowers in New Zealand in 2000 ..... *18*
Table 7: Pollen detection at various distances[a] *20*
Table 8: Crop isolation distances and requirements for certified seed[a] *23*
Table 9: Crops undergoing ANZFA assessment *37*

1 Executive summary

In New Zealand, plants developed through the use of genetic engineering technology are defined as new organisms under the Hazardous Substances and New Organisms (HSNO) Act 1996. These genetically modified (GM) plants can only be developed and grown under containment after permission has been obtained from ERMA for their development or importation. However, the HSNO Act does also provide for release without controls but this has not yet occurred in New Zealand. The widespread adoption of GM plants overseas and the deregulation of numerous crop-trait combinations overseas means that New Zealand also needs to consider options for the wider scale development and planting of GM plants.

Experience gained from traditional breeding and crop production will help in defining appropriate management approaches for transgenic plants, although there is still much more to learn. It is not the genetic status of the new cultivar used in agriculture, but the way the plants are grown that leads to the anticipated problems. The same applies for both traditionally bred and GM cultivars. The crops for which approval will be sought to grow GM versions commercially in New Zealand are most likely to be the same as those already grown in their non-GM form for many years. Many problems associated with their production and isolation from related species or wild populations have been successfully dealt with. The proposed GM versions of these plants differ from the non-GM versions in the addition of one or two single-gene traits and often these genes are ones that already occur naturally in the environment.

Overseas 68 crop-trait combinations are deregulated with rare regulatory restrictions placed on the growing of such crops. In 1999 about 98M acres of GM crops were planted world wide with the USA by far the largest grower of transgenic crops. The situation in countries such as Australia, UK, USA, Canada and Argentina, which are among the largest producers of transgenic plants, is outlined. The current status of GM research, development and field testing in New Zealand is summarised with information provided on the range of crops imported into and grown in New Zealand.

The coexistence of GM and non-GM crops (including organic crops) is possible in New Zealand and is already the case for organic and non-organic produce. However, a series of guidelines and detailed protocols are required if this is to occur. In this report we provide ideas for guidelines for each stage of plant development and use, from seed importation to processing, as it is vital to know the GM status of plant material at all stages. These ideas are summarised below. Use of well established isolation distances designed to prevent cross-pollination and contamination of crops will minimise the occurrence of pollen contamination. Crop rotational and management practices and the use of certified identity preservation (IP) schemes will further prevent the mixing of GM and non-GM seed and produce.

Importation: New Zealand imports a large amount of plant material, especially seed, for planting. Current border inspections by MAF relate solely to the phytosanitary status of the imported plant material. This inspection needs to be extended to include the GM status of the imported plant material. Depending on the crop type and country of origin of the plant material, certification should be required detailing the GM status. The protocol outlined for detecting and preventing GM contamination in imported corn seed (Appendix 2) is an

excellent starting point. Currently there is no guarantee that any imported plant material is non-GM.

For seed that is produced in New Zealand, following ERMA approvals, use of the seed certification guidelines (section 5.3.1) will ensure that GM seed is labelled and segregated prior to planting.

Physical barriers: When a GM crop is planted under field situations the major concerns regarding contamination of non-GM crops relate to pollen transfer, volunteers and movement of viable plant material (particularly seeds) to other areas. Many of the systems required for coexistence of GM and non-GM crops are already in place in some sections of the cropping industry, e.g. seed certification, pathogen tested (PT) potato scheme, certified organic production and maize seed importing. These systems are designed to minimise the chances of contamination from accidental mixing and cross pollination at all stages, from planting to point of sale. The strict guidelines of the current seed certification scheme used in New Zealand should be adopted for the growing of GM crops as the isolation distances and time between crops are designed to ensure high rates of seed purity (above 98%) (section 5.3.1). These same strict guidelines could be used to minimise the risk of GM crops contaminating non-GM crops, including organic crops. In addition, the guidelines ensure correct identification of the seed source, thus seed from GM plants can be identified and segregated. As AgriQuality staff are already proficient in ensuring segregation and isolation of crops with regard to seed and pollen contamination, they would easily be able to include GM crops, although additional staffing may be required to meet the required quality assurance standards. Use of the pathogen tested potato scheme guidelines are of particular relevance for coexistence in potato crops. Inclusion of GM monitoring schemes (section 7.4) would ensure any system adopted was functioning correctly. In addition, any distinguishing morphological marker(s) that could be incorporated in transgenic cultivars would aid in identifying contaminants and eliminating them prior to flowering.

Genetic barriers: A number of genetic barriers are available that could be used to minimise cross contamination of GM and non-GM crops due to pollen and seed dispersal. Such barriers include male sterility, chloroplast transformation, apomixis, ploidy level and control of flowering.

Identity preservation: Concerns regarding GM contamination mean that identity preservation practices need to be implemented at all stages from planting through to harvest and manufacturing site or point of sale. Once plant material has been harvested and removed from the field, segregation of GM material is required. This is already necessary for many crops, since under current ANZFA labelling regulations GM food must be labelled. Some industries, such as organic producers, already use certified identity preservation schemes to ensure their plant material remains segregated, from the field to the end user. These existing systems could be implemented for the labelling of GM or GM-free produce.

GM detection: The use of molecular techniques to identify the presence of transgenes in plant material in field and produce needs to be expanded (section 7.4). In particular, use of GM-free or non-GM on labels needs to be accompanied by a certified scheme to ensure the accuracy of such claims. The ideal GM detection system would include rapid, accurate, on-site tests at minimal cost. Such accredited testing facilities are needed in New Zealand not

only to satisfy New Zealand consumers and labelling requirements, but also for the assurance of some of our trade partners.

HSNO changes: We recommend a modification to the HSNO Act and ERMA regulatory processes be considered to provide a third category of field release which is intermediate between field trials under containment and general release. This level of containment might be called "Release with Controls". This intermediate level of release would be valuable in a number of situations, for example to conduct environmental risk assessment on an appropriate scale, as the final release level for GM plants genetically modified to produce bioactive compounds or to ensure appropriate controls are placed on some classes of GM plants such as Bt transgenics.

Overseas 68 crop-gene combinations have been deregulated and, therefore, those recommendations should be studied in detail as a starting point to determine if the same crop-gene combination could also be deregulated in New Zealand. Where a crop-gene combination has already been deregulated overseas then a streamlined process for deregulation in New Zealand should be considered. Factors unique to New Zealand must be included in a streamlined procedure. For example, relatives of the crop present in New Zealand that were not present in the country where deregulation occurred, and secondly, any increased likelihood of the crop-gene combination having greater propensity for invasiveness or weediness in New Zealand. This process will be on a case by case basis as different controls may be needed depending on the breeding system of the crop, the nature of the transgene, and the end use of the crop, e.g. seed vs. vegetable. In some cases, the specific gene will also need to be considered, e.g. a Bt refuge strategy may have to be adopted for Bt plants. Field trial applications submitted to ERMA cover in great detail concerns associated with field testing a particular plant. Any monitoring results from these existing and ongoing trials will provide vital information on the conditions to be placed on general release.

In addition, any regulations would need to ensure that the concerns of neighbouring properties are addressed. Organic and non-organic producers currently coexist and the use of the ideas contained in this report should enable the coexistence of GM and non-GM growers and producers.

Changes to the Resource Management Act (RMA) are not considered the appropriate policy instrument as the RMA relates mainly to local issues. The HSNO Act already deals with GM issues and it is, therefore, appropriate that law changes are made to this statute to enable release of GM crops. Such changes to section V of the HSNO Act need to also enable release of GM plants with controls on a case by case basis after sufficient scientific data and information are provided on the environmental safety of such plants.

Liability: While the above recommendations and guidelines may enable the safe co-existence of GM and non-GM crops, they cannot guarantee 100% purity. This is true of any segregation system and is why limits for contamination are set for organic production. GM detection systems are extremely sensitive and thus capable of detecting very low levels of contamination. Such levels of contamination could not be detected previously in organic crops concerned about contamination from non-organic sources. The organics industry is likely to have to adopt acceptable limits for accidental GM contamination. The area of liability for the consequences of any GM contamination problems is a complex legal issue

which will need to be addressed prior to deregulation of any GM crop in New Zealand. Co-operation between GM and non-GM producers will be required if co-existence is to occur.

2 Introduction

This report has been prepared for the Ministry for the Environment. It provides details about GM research being conducted in New Zealand and ideas as to how GM and non-GM crops (including organic) could coexist in New Zealand. In developing these ideas on coexistence of GM and non-GM crops (including organic), information is provided on the current status of GM research and release worldwide. Crops are classified according to their breeding system to provide a framework for risk assessment. This grouping has an effect on the management of coexistence and therefore on its likelihood of success. Case studies are provided that focus on the plant species and genes of commercial interest to New Zealand and on which GM research and development is being conducted in New Zealand.

Genetically modified (GM) organisms are classified as new organisms under section V of the Hazardous Substances and New Organisms (HSNO) Act 1996. The purpose of this Act is to protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of hazardous substances and new organisms. The Act prevents the development, importation, field-testing or release of any new organism without an approval from the Environmental Risk Management Authority (ERMA), which was established under section IV of the HSNO Act.

A vast array of transgenic crops has been produced, both internationally and in New Zealand, since the first transgenic plant was produced in 1983. By 1991, 40 different plant species had been transformed with foreign genes via *Agrobacterium,* including vegetable crops, arable crops, pasture crops and trees (Grant *et al*., 1991). These included only one monocotyledonous species, *Asparagus*, but recent advances have enabled most of the important cereal crops to be transformed via *Agrobacterium*-based methods. Now an OECD database lists 40 species that have been field tested, including monocots such as *Asparagus*, maize, rice and onions.

A variety of traits of agronomic importance have been introduced into plants through the use of genetic engineering technology. These altered traits include both input and output traits. Input traits are targeted at reducing production losses prior to harvest and during storage. Output traits cover areas such as feed quality, food quality, value-added traits and speciality chemical production. Several classes of input and output traits are listed below (based on Shoemaker *et al*., 2001).

Input traits:

1. Herbicide tolerance
2. Resistance to biotic stress, i.e. viruses, bacteria, fungi, nematodes and insects
3. Resistance to abiotic stress, i.e. tolerance to salt, heavy metals, drought, frost, “stacked traits”, e.g. herbicide tolerance and Bt resistance in one plant.

Output traits:

1. Improved animal feed quality through altered protein and oil levels, e.g. low phytate corn, high soluble carbohydrate forages
2. Improved food quality for human nutrition (nutraceuticals), e.g. increased ß-carotene, lycopene, iron, vitamin content, high stearate oil

3. Improved processing traits, e.g. altered cotton fibre, coloured cotton, high solids tomatoes, delayed ripening, improved starch quality, altered oil profile, altered nutritional value, increased shelf/vase life
4. Altered flower colour
5. Altered plant architecture/form
6. Altered flowering/breeding systems, e.g. male sterility, self incompatibility
7. Molecular pharming, i.e. production of vaccines, antibodies, pharmaceuticals, biopolymers, industrial enzymes, etc.

3 Deregulation of GM crops and coexistence overseas

3.1 Field trials and release of GM plants

The first field trial of transgenic plants was conducted in 1986. Over the past 15 years glasshouse and field trials with varying degrees of containment have been used to study and characterise potential environmental effects. Stewart (2001, Royal Commission) estimates that approximately 3.5 X 10^{12} transgenic plants have been grown in the USA in the last 12 years, with over two trillion being grown in 1999 and 2000 alone. The OECD Biotrack database lists 10,313 field trials of genetically modified organisms (GMOs) that have taken place in OECD member countries as of May 2001. Of these, 98.4% (or 10,148) were trials of GM plants, the remainder include trials of GM bacteria, viruses, fungi and animals. The vast majority of these trials has been conducted in the USA (71.1%), Canada (9%), France (5.3%), Italy (2.4%) and Australia (2.1%). New Zealand ranks 12th with 0.6% of GM field trials.

There has been rapid expansion in commercial release of transgenic crops. In 1996, only 10 years after the development of the first transgenic plant, 4M acres of transgenic crops was grown worldwide. By 1998, there had been a 17-fold increase with 70M acres grown worldwide, with soybean (52%), maize (30%), cotton (9%) and rape (9%) accounting for this. About 98M acres of GM crops were planted worldwide in 1999, a 43% increase over acreage in 1998 (James, 2000). The USA was by far the largest grower of transgenic crops worldwide at 51M acres (12% of the total arable acreage) or 74% of the transgenic crop area. Argentina with 15% (10M acres, 18% of the total arable acreage) of the transgenic crop area and Canada with 10% (7M acres) also had substantial areas. Australia and Mexico represent 1% each with 300,000 acres. In 2001, GM crops account for significant proportions of three major USA crops- soybean (63%), cotton (64%) and corn (24%).

Many developing countries (such as India, Philippines, Thailand, Iran) are extensively using genetic modification and GM organisms (see Agricultural Biotechnology and the Poor at www.cgiar.org/biotech/rep0100/contents.htm). Worldwide there are 68 trait-crop combinations that have non-regulated status covering 16 different plant types, 14 different crop types, one flower species and tobacco (as of June 2001, Agbios database).

3.2 Australia

In Australia, GM field tests have been conducted of 12 different plants including tomatoes, potatoes, sugarcane, cotton, canola, pea, apple root stock, lupins, hybrid tea rose and white clover. Greenhouse trials of GM carnation and chrysanthemum have also occurred. Florigene have released five commercial cultivars of carnation genetically engineered for improved vase life and violet flower colour. These cultivars, MoonDust, MoonShadow, MoonVista, MoonShade and Moonlight, are freely available to the Australian public with no labelling requirements. Cultivars are not available for the home garden and are only sold by the wholesale industry.

GM canola crops resistant to the herbicide Roundup will be grown in 2002 for sale in 2003 (GeneScan Newsletter, Autumn 2001).

Two types of GM cotton are commercially available in Australia. The area of Bt cotton grown has been limited by the National Regulatory Authority to about 30% of the total crop area, to

prevent build-up of Bt-resistant pests (Monsanto, pers. comm.). Currently approx. 165,000 ha of Bt cotton is grown in Australia. In addition, planting is restricted to areas in New South Wales and Queensland south of latitude 22 degrees due to the potential for gene transfer to wild relatives (BINAS website). These areas have relatively few native *Gossypium* species. A refuge system is in place to help prevent the development of Bt resistance in insects. After production, segregation of the product is not required.

In addition to the regulatory restrictions on growing herbicide resistant cotton, Monsanto has guidelines in place for the growing of its crops. Monsanto operates a stewardship programme that requires growers to be accredited before they are allowed to grow Roundup Ready® Cotton in Australia (Monsanto, pers. comm.). A summary of these guidelines is attached (Appendix 1).

In Tasmania substantial debate on the use of GM technology resulted in the parliament of Tasmania producing a joint select committee report on gene technology (http://www.parliament.tas.gov.au/CTEE/REPORTS/Gene2001.pdf). In Tasmania the possession and use of GMOs is restricted by the Plant Quarantine Act (Tasmania). In Australia, The Gene Technology Act 2000 allows for the creation of specific zones to be dedicated to GM, GM-free, or combined GM and GM-free production for marketing reasons. However, the ability of a State to declare a GM-free zone and have it recognised by the Gene Technology Regulator in the absence of a specific policy principle issued by the Ministerial Council is unresolved. Expert legal witnesses differed in their opinions as to whether a GM-free zone would be legally recognisable without a policy principle issued by the Gene Technology Ministerial Council.

3.3 UK large-scale GM farm trials

In the UK in 2001, GM field trials of sugar beet, fodder beet, oilseed rape, potato, barley and maize are being tested at 152 sites. Most of these GM crops include herbicide resistance genes but potatoes with nematode resistance, fungal resistance, and altered starch and sugar levels are also being tested. Of these, 105 are for government funded Farm Scale Evaluations (FSE), 14 are for National Seed Listing trials, 17 are intended to look at safety aspects and 16 are for research and development of new GM crop lines. One sugarbeet FSE will be used by Monsanto in a cow feeding study to determine if traces of the GM DNA can be found in the milk.

The FSE are of herbicide tolerant forage maize, oilseed rape, sugar and fodder beet. These trials are intended to research the impact of growing herbicide tolerant crops on agriculture, the environment and wildlife. They compare the effect of herbicide use in two halves of a field (one half planted with GM herbicide tolerant crop and the other with the conventional variety) on the diversity and abundance of plants and invertebrates under farm conditions. Gene flow from the crop will also be Monitored. The fields are up to 15 ha in size.

Nine of the trials being conducted in 2001 are the so-called BRIGHT (Botanical and Rotational Implications of Genetically modified Herbicide Tolerant crops) trials. They are intended to look at environmental and agricultural effects of GM herbicide tolerant crops and how farmers should manage them. The nine trials are a four-year MAFF funded research project that has small plots of GM herbicide tolerant oilseed rape, maize, sugar and fodder beet and one non-GM herbicide tolerant oilseed rape rotated with cereals. There is a “worst

case" scenario where herbicide tolerant oilseed rape was undersown in one of the rotations to examine the volunteer weed problem. Herbicide (phosphinothricin) tolerant GM maize is also being field-tested by Aventis. This crop has been given EU marketing consent through the Deliberate Release Directive which means Aventis no longer needs apply to an EU member state for consent each time it wants to grow it. However, it cannot be sold to UK farmers as it is not on the national seed list, but Aventis can grow it for other purposes (Source: GeneWatch).

The UK trials of GM crops are covered under a voluntary agreement between the Government and the Supply Chain Initiative on Modified Agricultural Crops (SCIMAC). SCIMAC, a formal grouping of industry organisations representing farmers, plant breeders, the seed trade and biotechnology companies, has voluntarily agreed not to grow GM crops commercially in the UK whilst environmental assessments take place. The farm-scale evaluations are due to be completed in 2002 for spring sown oil seed rape and maize, and 2003 for autumn sown rape. It is anticipated that at that time sufficient results will be available to determine the impact of these crops on the environment.

Separation distances are used to ensure product integrity by minimising the amount of cross-pollination that can occur between nearby crops of the same type. The industry body, SCIMAC, working with MAFF, has developed a code of practice and guidance for farmers on the growing and management of GM herbicide tolerant crops. This is designed to safeguard the GM crop and nearby conventional crops using good agricultural management, separation distances and volunteer control (removing plants that survive in the field into the next agricultural year). The distances used (Table 1) are based on the best evidence available that these will help ensure that any cross-pollination between the FSE and nearby compatible crops is below 1%. In the case of nearby organic crops of the same type these distances are increased, further minimising any cross-pollination to even lower levels.

Detailed results on the effects of the management of FSE of GM herbicide tolerant crops on the abundance and diversity of farmwife wildlife are published on the DEFRA website (www.environment.defra.gov.uk/fse) as soon as they are available. The latest report (dated 31 January 2001) indicated that neither for vegetation (i.e. weed and seedling density) nor invertebrates (i.e. number and diversity) were there any detectable difference in biodiversity in GM versus non-GM crops. The project still has a large backlog of unsorted invertebrate samples to analyse. Future reports will provide results from gene flow monitoring. Gene flow, both the flow of pollen and the extent of cross-pollination, from the GM half of rape and maize fields to the non-GM half is being monitored. Interim reports are published every 6 months on the DEFRA web site.

In the UK, when the Advisory Committee on Releases to the Environment (ACRE) considers an application to plant GM crops it makes the assumption that wind, bees and birds could carry pollen several miles. This is always taken into consideration as part of the detailed environmental risk assessment. They also assess how much of the pollen that travels significant distances would still be viable (i.e. able to pollinate); and how much of that would actually land on sexually compatible crops at a time when they are receptive to pollen; and what effect, if any, this would have on human health and the environment. ACRE has concluded that the amount of pollen transported any distance, by whatever means, is very small and poses negligible risks of cross-pollination. While this will be true for most GMOs

the pollen viability and potential for longer distance dispersal needs to be confirmed on a case by case basis.

In Wales, a voluntary 200 m buffer zone between GM and conventional or organic crops is likely to become a legal requirement. (Source: http://www.lifesciencenz.com/repository/external_news_material/0516_buffer.pdf). The legislation required to effect this change will involve issuing a prohibition notice under the Environment Protection Act 1990 which will affect any crops sown in Wales in the future.

Table 1: Separation distances applied to the spring 2001 FSEs

Crop	Seed crops (m)	Organic crops (m)	Non-seed/Non-organic crops (same species)
Oilseed rape	200	200	Conventional varieties and restored hybrids: 50 m Varietal Association and partially restored hybrids: 100m[a]
Sugar beet	600	600	all varieties 6 m[b]
Fodder beet	600	600	all varieties 6 m[b]
Maize	20	200	Sweetcorn 200 m, forage maize 80 m

[a] Varietal Associations have a proportion of male sterile plants, which means those plants do not self-pollinate as freely as conventional varieties and are therefore more susceptible to pollination by nearby rapeseed plants. The separation distance is greater than for conventional varieties to take account of this fact.
[b] As beet is not allowed to flower, cross-pollination will not affect the produce of non-seed crops. All plants that produce flowering stems must be removed from the Farm Scale trial crop so that pollen is not produced.

3.4 USA

In the USA a total of 52 different crop-gene combinations are no longer regulated. These include 13 different crops from a range of families, including one monocot, rice (Table 2). The first commercial GM cultivar was Flavr-Savr™ tomato, engineered to remain on the vine longer and ripen to full flavour before harvest. It was deregulated and therefore fully released in the USA in 1994. The first wide scale planting of GM crops in 1996 included Bt corn, Bt cotton and Roundup Ready® soybeans and in 1997 the first crop with multiple genes was released, Bt-Roundup Ready® cotton (Shoemaker *et al*., 2001).

Once an applicant has field tested a transgenic crop and accumulated enough scientific data and information to show that this crop is free from any risk under 7 CFR Part 340, the applicant can petition the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (UDSA), that a transgenic crop should no longer be considered a regulated article. Once this petition is successful, approval from APHIS will no longer be required for the planting or other environmental release, importation, or interstate movement of the plant or its progeny. Appendix 3 contains a copy of the Environmental Assessment prepared by APHIS in response to a petition from Monsanto for commercial deregulated release of canola transgenic for resistance to the herbicide glyphosate. The Environmental Assessment covered several environmental impacts in detail such as impacts based on increased weediness, outcrossing to wild relatives, potential impact on non-target organisms, biodiversity, and agricultural practices. Similar environmental assessments are available for all other crop-trait combinations deregulated in the USA.

Several different classes of traits are represented amongst the non-regulated crops including herbicide resistance, insect and virus resistance, altered fruit characters and altered oil profiles and pollination controls (Table 2). Note that in some cases combinations of genes are also

deregulated. Nearly all of these deregulated crops are for agronomic traits that affect crop production, e.g. herbicide tolerance, pest and disease resistance, delayed ripening.

While these crops are deregulated with no restrictions placed on growing or transporting them, restrictions on end use are imposed in some cases. For example, some GM products such as StarLink corn are only registered by the FDA for use for animal feed and not for use in human food.

3.5 Canada

In Canada, crops that require regulatory approval prior to release also include crops produced using more traditional methods such as chemical mutagenesis, wide hybrids and somaclonal variants. Canada is the only country to require such **P**lants with **N**ovel **T**raits (PNT) to be fully evaluated similar to GM crops prior to release. Under the Seeds Act, authorisations are issued, with or without conditions, only after environmental risk assessment has been conducted by the Plant Biosafety Office. Environmental release applications are considered for releases under either confined conditions, as in research field trials, or on an unconfined, unrestricted basis. When a developer wishes to release a PNT into the environment under unconfined conditions (i.e. towards marketing the PNT), information required to undertake a full environmental safety assessment must be provided to the Plant Biosafety Office. Detailed information about the PNT, the method used to introduce the novel trait into the plant and any risks of adverse environmental effects resulting from the release of the plant into the environment must be provided. Potentially adverse effects considered include the plant becoming an agricultural weed or becoming more invasive of natural habitats; novel traits passing to wild relatives through gene flow; effects on non-target organisms (including humans) of the plant or its gene products; and the plant's impact on biodiversity.

While non-regulated crops have no restrictions placed on them, seed companies do impose some restrictions. For example, a refuge system is used for Bt corn (section 5.5.4). Following the introduction of herbicide tolerant (Liberty Link) oilseed rape in Canada, Aventis voluntarily initiated a multi year and location monitoring study. The results confirmed the conclusion of the risk assessment in that the oilseed rape volunteers and related species were effectively managed by conventional agricultural practices and posed no greater risk to the environment than conventionally derived oilseed rape. The results of this study and others have been incorporated into a product stewardship information booklet that is targeted at further educating farmers at using best practices when cultivating glufosinate tolerant canola. This example demonstrates that a general release approval is not viewed as the end point for managing the release and environmental effect of a GM crop.

3.6 Argentina

The National Advisory Committee on Agricultural Biosafety, CONABIA, is responsible for the regulation of products of agricultural biotechnology in Argentina. The current rules developed by CONABIA for application in Argentina are based on the characteristics and risks posed by biotechnology products, and not on the process by which these products are produced, i.e. the rules that apply to the intended use of the transgenic organisms are concerned only with the potential risks to the environment, other farming activities or public health that may result from their release. Analysis of applications occurs on a case-by-case

basis. After at least one release into the environment has been approved, and the safety of the GMO has been demonstrated, the applicant may apply for a "flexibilization" permit. This permit allows the applicant to perform future releases by providing notification on the sown area and date, site of release and date of harvest. CONABIA will then perform inspections at the time of harvest and at the final disposal of the product.

3.7 Summary

The background information provided above on the extent of field testing and commercial release of GM crops overseas demonstrates that there are protocols in place in several countries to enable commercialisation of GM crops and, therefore, coexistence of GM and non-GM crops. However, the protocols used vary from country to country and from crop to crop. The larger scale FSE trials in the UK, particularly those on herbicide resistance, have shown no adverse environmental impact to date, are more informative and allow coexistence issues to be better addressed. The detailed results from these FSE trials are relevant to the issue of coexistence in New Zealand. Similarly, the USA experience demonstrates that GM and non-GM crops can effectively coexist despite highly publicised cases such as Starlink corn.

Table 2: GM crops no longer regulated in the USA[a]

Genus and Species	Common name	Approvals	Target trait	Transgene
Beta vulgaris	Beet	2	Herbicide resistance	Glyphosate, Phosphinothricin
Brassica napus	Canola/rapeseed	12	Altered oil profile	High lauric acid
			Herbicide resistance	Bromoxynil, Glyphosate
			Herbicide resistance	Imidazolinone, Phosphinothricin
			Herbicide resistance & pollination control	Phosphinothricin Barnase/barstar
Brassica rapa	Polish canola	1	Herbicide resistance	Glyphosate
Carica papaya	Papaya	1	Virus resistance	PRSV
Cichorium intybus	Chicory	1	Herbicide resistance & male sterility	Phosphinothricin Barnase
Cucumis melo	Melon	1	Reduced ethylene synthesis	SAM hydrolase
Cucurbita pepo	Squash	2	Virus resistance	WMV, ZYMV, CMV
Dianthus caryophyllus	Carnation	3	Altered flower colour & herbicide resistance	Mauve, Glyphosate
			Reduced ethylene synthesis	ACC synthase
Glycine max	Soybean	6	Herbicide resistance	Glyphosate, Phosphinothricin
			Altered oil profile	High oleic acid
Gossypium hirsutum	Cotton	5	Herbicide resistance	Bromoxynil, Glyphosate
			Herbicide resistance	sulfonylurea
			Insect resistant	Bt
			Herbicide & insect resistance	Bromoxynil and Bt
Linum usitatissimum	Flax/linseed	1	Herbicide resistance as soil residue	Sulfonylurea
Lycopersicon esculentum	Tomato	6	Altered fruit ripening	Decreased polygalacturonase
			Altered fruit ripening	ACC synthase, ACC deaminase
			Altered fruit ripening	SAM hydrolase
			Insect resistant	Bt
Nicotiana tabacum	Tobacco	1	Herbicide resistance	Bromoxynil
Oryza sativa	Rice	1	Herbicide resistance	Phosphinothricin
Solanum tuberosum	Potato	4	Insect resistant	Bt
			Insect & virus resistant	Bt and PLRV
			Insect & virus resistant	Bt and PVY
Zea mays	Corn/maize	17	Insect resistant	Bt
			Herbicide resistance	Glyphosate, Phosphinothricin
			Herbicide & insect resistance	Glyphosate and Bt
			Herbicide & insect resistance	Phosphinothricin and Bt
			Herbicide resistance & male sterility	Phosphinothricin, Adenine methylase
			Herbicide resistance & male sterility	Phosphinothricin barnase

[a] as of 21 June 2001. Source : Agbios database.

4 New Zealand situation

This section summarises the main GM research that is being conducted in New Zealand, both in the laboratory and in the field. In addition, this section summarises the main forage, arable, vegetable, and fruit crops grown in New Zealand as well as the range of seed-imported crops. This information is provided as it identifies the crops of importance to New Zealand on which this report focuses.

4.1 GM research

In New Zealand, GM development is conducted in containment by several Crown Research Institutes and private companies. Universities are also conducting contained GM research but mainly as a teaching and basic research tool. However, the majority of research on GM plants is being conducted at the CRIs and includes most of the economically important plants grown in New Zealand (Table 3). The current research deals mainly with input traits such as herbicide resistance, insect and disease resistance. However, there is a shift towards research in several areas of output traits, such as post harvest quality, speciality chemicals and nutraceuticals.

Table 3: Summary of New Zealand GM research in economically important plants

Plant	Trait	Organisation(s)
Forages		
White clover	Disease resistance	AgResearch/HortResearch
	Insect resistance	AgResearch/HortResearch
	Forage quality	AgResearch/Via Lactia
	Nutritive value	AgResearch
	Transposon tagging	AgResearch
	Controlled flowering	AgResearch/Via Lactia
Perennial ryegrass	Controlled flowering	AgResearch/Via Lactia
	Nutritive value	AgResearch
	Transposon tagging	AgResearch
	Herbicide resistance	HortResearch
Forage brassicas	Herbicide resistance	Crop & Food Research
Vegetables		
Vegetable brassicas	Reduced ethylene	Crop & Food Research
	Insect resistance	Crop & Food Research
	Disease resistance	Crop & Food Research
Pea	Disease resistance	Crop & Food Research
	Flowering control	Crop & Food Research
	Transposon tagging	Crop & Food Research
Onions	Herbicide resistance	Crop & Food Research
	Disease resistance	Crop & Food Research
	Altered flavour	Crop & Food Research
Leek	Herbicide resistance	Crop & Food Research
Garlic	Herbicide resistance	Crop & Food Research
Potato	Insect resistance	Crop & Food Research
	Herbicide resistance	Crop & Food Research
	Disease resistance	Crop & Food Research
	Altered colour	Crop & Food Research
	Nutritive value	Crop & Food Research
	Biochemical production	Crop & Food Research
Forestry		
Eucalyptus grandis	Altered wood quality	Genesis
Eucalyptus sp.	Altered wood quality	Trees and Technology Limited
Picea abies (spruce)	Herbicide resistance	Forest Research

Plant	Trait	Organisation(s)
Pinus radiata	Herbicide resistance	Forest Research
	Insect resistance	Forest Research/ C&F
	Altered flowering	Forest Research/C&F
	Altered wood quality	Crop & Food, Carter Holt Harvey
Fruit		
Apples	Reduced ethylene	HortResearch
	Post-harvest fruit quality	HortResearch
	Insect resistance	HortResearch
	Flower and fruit development	HortResearch
Kiwifruit	Reduced ethylene	HortResearch
	Post-harvest fruit quality	HortResearch
	Reduced spray use	HortResearch
Tamarillo	Virus resistance	HortResearch
Flowers		
Cyclamen persicum	Pigment alteration	Crop & Food Research
Eustoma grandiflorum	Pigment alteration	Crop & Food Research
Cymbidium	Pigment alteration	Crop & Food Research
Dendrobium	Pigment alteration	Crop & Food Research
Disa	Pigment alteration	Crop & Food Research
Pelargonium	Pigment alteration	Crop & Food Research
	Altered morphology	Crop & Food Research
Petunia	Pigment alteration	Crop & Food Research
Sandersonia aurantiaca	Pigment alteration	Crop & Food Research

In New Zealand, no commercial releases have been applied for yet. Monsanto was preparing to apply for a full release of Roundup Ready® canola but withdrew its application. A total of 48 field tests (38 under IAG and 10 under ERMA) involving transgenic plants have been approved for 15 different species. These include 20 different plant types, including one forestry species and one flower crop (Table 4). A range of different classes of genes have been field-tested including herbicide resistance, insect and disease resistance, reduced ethylene production and altered colour. Full details of the traits introduced and the conditions imposed on the trial are available from the ERMA website. In most cases, the conditions involved controls such as flower removal to minimise pollen transfer to other plants. Only with potatoes has gene transfer via pollen been investigated (section 5.5.1). All trials required monitoring to ensure no volunteers remained after completion of the trial. In addition, transgenic onions developed in New Zealand have been field tested overseas in the USA with no restrictions.

In general, trial sizes of GM plants in New Zealand have been small with no trials greater than 0.5ha. In addition, prevention of flowering in most cases has not enabled studies on gene flow to occur with transgenic traits. As a result these trials have not yet provided useful results relevant to the coexistence of GM and non-GM crops in New Zealand. Contained larger scale trials would enable this. However, it may be possible to extrapolate from some of the UK results with FSE trials to New Zealand crops. In addition, detailed environmental data is available from APHIS for each crop-trait combination that has been deregulated in the USA.

Table 4: Summary of field trials approved in New Zealand

Genus and Species	Common name	Transgenic Trait
Actinidia deliciosa	Kiwifruit	Marker and reporter genes
Asparagus officinalis	Asparagus	Marker and reporter genes
Beta vulgaris	Sugar beet	Herbicide resistance
Brassica oleracea var. *acephala*	Forage kale	Herbicide resistance
Brassica oleracea var. *italica*	Broccoli	Herbicide resistance Reduced ethylene
Brassica oleracea var. *gemmifera*	Brussels sprouts	Reduced ethylene
Brassica oleracea var. *capitata*	Cabbage	Marker and reporter genes
Brassica oleracea var. *botyris*	Cauliflower	Marker and reporter genes
Brassica napus	Canola (oil seed rape)	Herbicide resistance, male sterility
Brassica napus var. *biennis*	Forage rape	Herbicide resistance
Brassica rapa	Chinese cabbage	Reduced ethylene
Cyphomandra betacea	Tamarillo	Virus resistance
Eustoma grandiflorum	Lisianthus	Altered colour
Hordeum vulgare	Barley	Herbicide resistance
Malus domestica	Apple	Reduced ethylene
Petunia	Petunia	Altered colour, dwarf stature
Pinus radiata	Radiata pine	Altered flowering
Pisum sativum	Pea	Virus resistance
Solanum tuberosum	Potato	Herbicide resistance Marker and reporter genes Thaumatin production Bacterial resistance Insect resistance
Trifolium repens	White Clover	Virus resistance
Zea mays	Maize	Insect resistance

4.2 Range of crops grown and imported into New Zealand

4.2.1 Pasture and forage crops

Nearly 10M ha of pastures are sown in New Zealand. In addition approximately 28,000 ha of pasture species are grown annually for seed production. The six most common species are perennial ryegrass, annual ryegrass, hybrid ryegrass, tall fescue, white clover and red clover, accounting for 84% of certified seed production in New Zealand, while perennial ryegrass and white clover alone account for 64% (AgriQuality, 2001). Lucerne is also of importance with 87,000 ha grown in 2000,

GM research in New Zealand is conducted on perennial ryegrass and white clover but the only GM field trial to date was with white clover containing a virus resistance gene (Table 4). GM trials with white clover have also occurred in Australia, where GM research with a wider range of pasture plants is underway. No GM pasture plants have been commercialised to date worldwide, although herbicide-resistant lucerne is likely to be the first release.

4.2.2 Arable, vegetable, and fruit crops

Among the top five grain and arable crops grown in New Zealand (Table 5) GM research is only being conducted on peas. However, internationally GM wheat and barley are also being field tested, and GM maize is widely available and a scheme is in place to ensure that GM

maize does not contaminate seed sources imported for sowing in New Zealand (7.1, Appendix 2).

The main vegetable crops in New Zealand are potatoes, onions, squash and tomatoes (Table 5). GM research with potatoes is particularly advanced with over 13 years of field tests of GM potatoes for a variety of traits including insect and disease resistance. GM onions containing a marker and reporter gene have been developed in New Zealand and are currently being field tested in the USA. Research into squash transformation is only in the early stages with no transgenic plants produced to date. New Zealand has not conducted any tomato research but in the USA 10 lines of GM tomatoes are deregulated (Table 2).

The top five outdoor fruit crops in New Zealand are apples, grapes, kiwifruit, avocados and olives. GM research is being conducted on apples and kiwifruit in New Zealand (Table 3), and transgenic grapes have been field-tested overseas.

Table 5: Area sown in various crop types in New Zealand

Crop type *	Area sown (ha)
Pasture seed crops (Certified seed production 2000/2001)	
Perennial ryegrass	10,104
Annual ryegrass	2,103
Hybrid ryegrass	1,389
Tall fescue	1,245
White clover	7,583
Red clover	905
Other crops	4,353
Grain and arable crops	
Barley	55,792
Wheat	52,797
Maize	19,446
Field/Seed Peas	16,826
Oats	9,929
Other Crops	57,143
Vegetables	
Potatoes	11,816
Onions	7,044
Squash	6,713
Tomatoes-outdoor	723
Tomatoes-indoor	160
Fruit crops	
Apples	14,114
Grapes	12,665
Kiwifruit	12,184
Avocados	2,646
Olives	1,174
Pears	958
Mandarins	946
Apricots	759
Peaches	725
Nectarines	618

Sources: Statistics New Zealand, Agricultural Production Survey for the year ended 30 June 1999 (from web site); AgriQuality (2001).

4.2.3 Forestry

Forests cover about 29 percent or 8.1 million hectares of New Zealand's land area. Of this, about 1.7 million hectares in planted in production forests of which 91% is radiata pine (*Pinus radiata*), and 5% is Douglas fir. In New Zealand research on forestry tree species is mainly concentrated on *Pinus radiata* (Table 3). The first field trial of transgenic *P. radiata* was planted by Forest Research in 1998. Currently 2 trials are in progress with permission to last for up to 22 years. Plants in these trials are not allowed to flower. In their field trial applications (available at ERMA website), Forest Research have covered in detail the potential environmental risks that could be associated with the field testing of GM pine. As *P. radiata* is really a clonal crop, the use of male sterile lines for large scale field release is a feasible option to ensure contamination of non-GM plantations does not occur.

4.2.4 Cut flowers

Table 6 summarises the top 10 export flower crops grown in New Zealand. In addition, there is a large export market of flower foliage, seeds, bulbs, tubers, corms, etc. Genetic engineering of flower crops is well underway in New Zealand with researchers at Crop & Food Palmerston North, developing transformation systems for a number of flower crops, including lisianthus, petunia, chrysanthemums, carnations, orchids and sandersonia (Table 3). In most cases the eventual aim of the transformation research is the introduction of genes for altering flower colour. In New Zealand field testing of petunia and lisianthus has occurred (Table 4). Overseas researchers have produced transgenic roses, while GM carnations with altered flower colour (violet) and longer shelf life are commercially available in Australia (section 3.2).

Table 6: Export value of cut flowers in New Zealand in 2000
(Source: Statistics New Zealand)

Flower	Export Value (M$)*
Orchids	22.4
Other	7.8
Zantedeschia	7.7
Sandersonia	3.1
Lilium	1.9
Proteaceae	1.4
Nerines	0.6
Carnations	0.5
Paeonies	0.5
Roses	0.3

5 Minimising GM contamination of non-GM crops

The "escape" of viable plant material carrying foreign genes is considered to be a potential problem associated with the initial field-testing and release of transgenic plants (Tiedje *et al*., 1989). In most crops, the two main sources of transgene escape are pollen and seed. In this section we discuss the problems caused by pollen and seed escape and describe physical and genetic methods to minimise the consequences of these events. The use of such methods provides a means to enable the coexistence of GM and non-GM crops.

5.1 Pollen transfer

The dispersal of transgenic pollen can either be to other crops of the same species or to related non-crop species (e.g. weeds). Pollen escape becomes a problem when this leads to development of a viable seed and a volunteer plant. Pollen escape is not a problem when the pollen is non-viable or it is transferred to another species with which crossing does not naturally occur. Even in cases of pollen transfer to related species viable progeny that can reproduce are often not produced. Possible outcomes of pollen escape include:

- **Plants unable to hybridise:** Transfer of introduced genes into other species is often limited by natural crossing barriers. The majority of crop plants do not cross naturally e.g. pine trees don't cross with wheat. Even in cases where species are more closely related, natural barriers often prevent crossing. Some of these mechanisms include incompatibility of pollen (pollen doesn't germinate or grow after landing on the stigma), style barriers or differences in timing of flowering, or of flowering events (e.g. in many self-pollinated species where pollination occurs before the flower opens, preventing crossing with other species).
- **Plants cross but no viable progeny are produced:** In these situations pollination occurs but either the embryo or seed aborts.
- **Plants hybridise and produce viable progeny:** Effective pollination has occurred and a viable seed is produced. In some crosses the progeny are sterile e.g. wild turnip X rape, but in other cases this represents the worst case scenario when a sexually reproducing plant develops in non-contained situations. In these situations physical or genetic barriers are required to minimise contamination of non-GM crops (section 5.3).

Gene flow from GM crops to non-GM crops and/or to related relatives has become an important issue with the development and release of transgenic crops. The amount of pollen transfer between cultivars is controlled by a number of factors. The physical isolation distance between the pollen donor and the recipient is the most important factor. However, the degree of out-crossing by each species, the overlap in flowering time, and the production area grown of the crop are also important.

The amount of out-crossing is lowest in inbred crops and is greatest in open-pollinated species. The production area of a GM crop is important because as the area increases there is greater potential for gene flow to non-GM crops. Most field studies with GM plants have focused on isolation distances and pollen dispersal over distance, but the duration of pollen viability is also important. Pollen viability of wheat is about 45 minutes while maize pollen viability ranges from 20 min to 2 hours (Dumas and Mogensen, 1993). The longevity of grass pollen may be as short as 30 minutes and even in insect-pollinated species with sticky pollen

it rarely exceeds 1 day (Richards, 1986; Moyes and Dale, 1999). Pollen concentration decreases rapidly from the source but low levels can be detected at much greater distances for both wind and insect pollinated species.

Pollen detection alone is not important as a viable plant must be produced to cause contamination. Moyes and Dale (1999) summarised pollen dispersal distances and contamination rates from a range of species (Table 7). While pollen was detected at distances in excess of 1.5 kilometres, the contamination rates were very low within 100 m in most crops. Dispersal of transgenic and non-transgenic pollen of the same cultivar did not differ (Hokanson *et al*., 1997), and therefore results of studies with non-transgenic pollen should hold for GM crops. While increases in isolation distance may reduce contamination levels in non-GM crops, the decreases will not be linear, e.g. increasing the isolation distance 3-fold may only reduce contamination by 10% (Moyes and Dale, 1999).

Table 7: Pollen detection at various distances[a]

Crop	Breeding System	Contamination rates at various distances	Maximum distance pollen detected
Apples	Clonal	6% at 15 m	56 m
Brassicas	Hybrid	0.4% at 12 m 6% at 137 m	1500 m
Grasses	Open-pollinated	5-17% at 250 m	1000 m
Forage legumes	Open-pollinated	<1% at 32 m[b] 0.3% at 400 m[b]	1609 m
Maize	Hybrid	<2% at 10 m	25 m
Onions	Hybrid	Not reported	4246 m
Potato	Clonal	0.14% at 10 m	80 m
Wheat	Inbred	10% at 3 m	20 m

[a] adapted from Moyes and Dale (1999) [b] Clifford *et al*. (1996).

Seed propagated, perennial crops represent a greater challenge than annual crops because pollen contamination can accumulate over several years. Contamination is currently identified based on variance from traits described during Plant Variety Rights on a particular cultivar. Transfer of a transgene into a non-GM crop may not give any observable change, and therefore DNA-based methods of detection that dramatically increase the chances of detection will form the basis of future testing systems (section 7.4).

Most of these factors have been taken into account by the Seed Certification Bureau, AgriQuality, in developing the requirements for minimum intervals between crops of the same species and minimum isolation requirements (section 5.3.1). However, such isolation distances may not always be appropriate for all clonal crops as pollen and seed are not used for propagation and can still travel long distances and effect gene transfer.

5.2 Seed dispersal

Seeds are produced and dispersed from crops in much lower numbers than pollen. However, seeds that are dispersed can go on to produce transgenic plants that are no longer physically isolated from non-GM crops. In these situations the impact can be greater than from pollen escape per se. Escape of seed can result in the appearance of volunteer plants from seed left following crop harvest or seed transport. The spread of vegetative material such as tubers, corms or bulbs can also result in volunteer GM plants. Escape provides an opportunity (not a

certainty) for contamination of non-GM crops, and options to minimise contamination of non-GM crops and enable coexistence need to be considered. The same sources of contamination must be overcome when producing seed of non-GM crops. Seed certification regulations stipulating the length of time between successive crops of the same or related species, acceptable levels of contamination and isolation distances were developed specifically to counter these problems.

There are several mechanisms by which seed can escape. In many plant species, animal and wind dispersal are important in long-distance dispersal (Thill and Mallory-Smith, 1997). Many crop plants have been selected for reduced pod, capsule, silique, etc, shatter thereby reducing the potential for seed dispersal prior to harvest. In New Zealand transfer by animals in wool or hair is a potential source of seed escape. Other avenues for seed dispersal are possible, for example white clover seed is dispersed to varying degrees through the digestive tracts of earthworms, birds, and particularly domestic livestock (Harris, 1987). Seed can also be dispersed between fields by sowing, cultivation or harvesting equipment which is not thoroughly cleaned between uses (Thill and Mallory-Smith, 1997). Longer distance dispersal can occur during seed transport when spillages occur. Other mechanisms include seed movement with manure, transport on motor vehicles and movement of topsoil (Moyes and Dale, 1999).

Seed dispersal over time can occur through delayed germination or dormancy of seed remaining in a field after loss during harvest. Crops that have little or no seed dormancy represent a lower risk since contaminants will germinate during normal intervals between crops and can be eliminated like other problematic weeds (i.e. by cultivation and spraying). Volunteers of high erucic acid oilseed rape were recorded up to 5 years after harvest (1 plant per 3 m^2). However, better management practices to prevent seed burial after harvest may decrease this further.

A key issue for successful coexistence is whether plants derived from crosses between GM and non-GM plants create super-weeds or will lead to plants that are more invasive. The concern that GM crops may transfer transgenes to wild populations of the same or related species has been evaluated for four crops over a 10-year period in the UK (Crawley *et al*., 2001). GM oilseed rape, potato, maize and sugar beet, containing Bt or herbicide resistance, were grown in 12 different habitats and monitored over a 10 year period to determine whether hybrid progeny containing introduced genes transferred by pollen to wild relatives became more weedy or invasive. They concluded that these hybrid plants were no more invasive than wild type plants. They also reported a general decrease in the number of individuals in the population that were transgenic over time.

Adoption of the seed certification regulations for the number of years between crops of the same type should minimise contamination from seed escape (Clifford *et al*., 1996; section 5.3.1). However, Young and Youngberg (1996) found that 2-year rotations between perennial ryegrass seed crops were insufficient to meet minimum contamination standards for certified seed crops. Similarly in New Zealand, 3-year rotations between white clover seed crops did not meet the minimum contamination standards (1 contaminant per 10 m^2) for seed certification (Clifford *et al*., 1996). However, 5-year rotations in white clover are adequate.

5.3 Barriers to gene flow

Mechanisms for minimising pollen and seed transfer between crops are based on either physical barriers, such as isolation distance, or genetic barriers, such as the inability to transmit genes following pollination or to produce viable seeds following fertilisation. These barriers are discussed below to illustrate mechanisms that might be adopted to enable the coexistence of GM and non-GM crops.

5.3.1 Physical barriers and seed certification

Isolation distance is the main physical barrier to gene flow between GM and non-GM crops. In New Zealand, isolation distances between different crops of the same or related species are controlled by AgriQuality under the seed certification system.

Seed certification operates to ensure that cultivars maintain their identity through successive generations of multiplication, for the ultimate benefit of end users. The New Zealand scheme operates on a voluntary basis and is operated by staff of AgriQuality New Zealand Ltd. A detailed series of rules and standards are issued covering all aspects of seed production, including previous paddock history to ensure crops are not contaminated, field inspection, freedom from weeds, etc. New Zealand guidelines are among the most stringent in the world and maintain the genetic purity of any given cultivar by minimising contamination from both volunteer plants, e.g. derived from buried seed, and foreign pollen (Clifford *et al.*, 1996). New Zealand is a member of the Association of Official Seed Certifying Agencies (AOSCA), the principal members of which are the USA and Canada. Cooperation among member agencies assures uniformity and consistency of field inspection and laboratory services. Many AOSCA agencies provide customised field inspection services, independent third-party record keeping and verification.

The following criteria are of particular relevance to co-existence of GM and non-GM crops:

1. paddock history/previous cropping history: minimum time intervals must be observed between seed of different species and different cultivars of the same species,
2. minimum isolation distances must be observed between cross pollinating species,
3. identity preservation of seed after harvest: this involves labelling and segregation of seed.

These criteria cover the areas of pollen and seed contamination recognised as concerns for co-existence of GM and non-GM crops. The paddock history and minimum isolation distances set out for crops in New Zealand are outlined in Table 8. In general, these isolation distances are only for cross-pollinating species but peas (self-pollinated) also have isolation requirements. These requirements and isolation distances are designed to achieve purity levels of 98 to 99%.

In addition to seed production, isolation distances are also used as a management strategy to maintain quality attributes during crop production. For example, isolation is important in oilseed rape crops because it enhances uniformity in oil quality by minimising mixed oil components arising from cross-pollination with neighbouring crops such as forage rape or weedy species. Likewise, isolation between maize and sweet corn is important to maintain the quality attributes in both crops.

Table 8: Crop isolation distances and requirements for certified seed[a]

Crop	Previous cropping history[b]	Areas less than 2 ha (m)	Areas more than 2 ha (m)	Generation	Purity level for basic seed (%)
White clover	5	200	100	Basic seed	99.0
Lucerne	3	200	100		98.0
Ryegrass, perennial	2	200	100		99.0
Kale	5	700			99.0
Maize[c]	0	400			99.5
Pea	2	100			98.0
Rape	5	400			99.0
Swede	5	400			99.0
Turnip	5	400			99.0
Wheat[d]	1				98.0
Barley[d]	1				98.0

[a] From AgriQuality (2000-2001). Basic seed isolation distances were used as these are the strictest requirements.
[b] Refers to the number of previous harvest seasons the area must be free of crops of the same crop.
[c] For hybrid seed, isolation by time is also taken into account. Isolation distance can be reduced by sowing additional border rows of the pollen parent adjacent to the seed crop.
[d] For wheat and barley the area must not have been grown with any other cereal the previous season. Isolation distances require only a space sufficient to prevent mixing during harvest. But for some wheat cultivars distances of 50-100 m are required.

5.3.2 Genetic barriers

Genetic barriers can also be used to prevent cross contamination of GM and non-GM crops. There are several genetic mechanisms that can control pollen transfer and thus reduce contamination of neighbouring non-GM crops. Transfer of introduced genes into other species is prevented when there are no related (cultivated or wild) species present in New Zealand with which these plants can successfully cross. Other genetic barriers that could be implemented to minimise gene flow between GM and non-GM crops include chloroplast transformation, male sterility, apomixis, ploidy level and control of flowering.

Chloroplast transformation has been demonstrated for herbicide resistance in tobacco (Daniell *et al*., 1998). As chloroplasts are generally maternally inherited except in conifers, chloroplast transformation reduces the likelihood of contamination through transfer of pollen. However, paternal transmission of chloroplasts does occur rarely in some species (Moyes and Dale, 1999).

Male sterility is the main pollination control mechanism used in the development of conventional hybrid varieties, but would also be useful for deployment of transgenic open-pollinated varieties harvested for vegetative organs to prevent gene transfer via pollen. Male-sterility systems have been identified in many crops including alfalfa, rose clover, birdsfoot trefoil (Hill *et al*., 1988; Molina-Freaner and Jain, 1992; Negri and Rosselini, 1996), maize, brassicas, carrot and onions but have not always been effective. For example, development of hybrid alfalfa varieties has not been commercially viable due to reduced pollination of male-sterile rows by bees (Hill *et al*., 1988). Male sterility can also be introduced via genetic engineering technology. The deployment of male sterile crops could be particularly useful in perennial forage crops where the potential for gene transfer is high.

Apomixis occurs naturally in some plant species such as *Hieracium*, dandelions, *Citrus*, blackberry and mango (Ross Bicknell, pers. comm.). In apomixis, as in vegetative propagation, the daughter plants are genetically identical to the mother plant. The

introduction of apomixis into crops traditionally propagated through seed could lead to the development of hybrid varieties in crops that have the greatest potential for pollen dispersal. In some cases the use of apomixis in combination with male sterility could reduce the potential for contamination of non-GM crops by GM crops. Commercial application of apomixis in any major crop is probably 10-15 years away.

Ploidy level Many open-pollinated species are polyploid, for example white clover is tetraploid (2n=4x=32), tall fescue is hexaploid and perennial ryegrass has both diploid and tetraploid cultivars. Deploying transgenes at a different ploidy level is a potential genetic barrier to transgene transfer to wild populations as hybrids between different ploidy levels are sterile. For example, gene flow from a hexaploid (2n=6x=48) white clover to wild tetraploid populations of white clover would be severely reduced or eliminated.

Controlled flowering may be the most effective means of containing transgenic plants by preventing or retarding flower production. In some clonal crops flowering is rare, e.g. garlic. Identification of naturally occurring genetic variation for flowering (e.g. differences in flowering time) or use of GM techniques that modify floral initiation or induce male sterility may offer a longer-term solution in some crops not dependent on flowering for production, e.g. forestry.

5.4 Consequences of contamination

The consequences of a transgene entering a non-GM crop are affected by the end use of the contaminated crop and nature of the transgene (Moyes and Dale, 1999), as this will determine where in the plant's life cycle the presence of a transgene will have an effect. This will help determine whether the genetic modification is expressed in the growing crop, feed for animals, and/or food for consumers. In addition, the nature of the transgene with regard to time and location of transgene expression is important in determining the consequences of contamination. Use of tissue specific and/or inducible promoters can be used to control the location, intensity and timing of gene expression and can therefore be used to prevent gene expression, for example, in edible plant parts or in pollen.

Crops are grown for a number of purposes but can be divided into four main categories.

1. **Crops grown for further seed multiplication.** Contamination in early generations of the multiplication cycle can lead to greater contamination levels in crop production because the frequency of the transgene can increase rapidly during subsequent generations of multiplication.
2. **Crops for animal and human consumption of seed**, **e.g. cereals, pulses, fruits and oilseed crops.** In these crops pollen contamination must occur at flowering to have any impact.
3. **Crops grown for consumption of vegetative parts prior to flowering, e.g. root crops and leaf vegetables.** Pollen contamination will not affect these crops. Volunteer plants from seed dispersal could cause low level contamination of the crop that is harvested and passed on to consumers.
4. **Crops grown for vegetative consumption by animals after flowering.** This includes perennial grasses and legumes where contamination could accumulate over time. The proportion of new plants recruited each year from pollen or seed contamination is an important factor. Once a transgene is established its impact (and spread) will depend

on whether the introduced gene confers increased vigour or competitiveness in the range of environments in which these species are grown. Another important consideration for crops consumed by animals is whether the transgene or its products are broken down or passed on. The transgenes that have been studied to date are broken down rapidly in the rumen (Biggs and Hancock, 1998).

5.5 Summary

Pollen and seed escape from transgenic plants can be minimised by adoption of the physical and genetic barriers described above. The choice of the appropriate method will need to be considered on a case by case basis dependent on the breeding system of the plant, presence of relatives, end use of the crop, and the type of transgene inserted. The adoption of the seed certification scheme guidelines may in some cases provide sufficient isolation, but in other situations stricter management regimes and use of genetic barriers such as male sterility may be required.

6 Case studies for coexistence in New Zealand

Based on the current status of research in New Zealand and also on the types of crops grown and imported into New Zealand, these case studies focus on potatoes, brassicas, white clover and Bt transgenics. Current environmental research as well as unique factors that need to be considered for each situation are discussed. Further detailed information on each crop already field tested in New Zealand is available from the field trial applications submitted to ERMA for each trial. Additional information on various crops and specific genes is also available from the OECD web site where detailed crop biology documents have been made available for wheat, rice, white spruce, potato and oilseed rape. Information on herbicide resistance genes is also provided at this site.

6.1 GM Potato

Biosafety evaluations of transgenic potatoes have established negligible environmental and food safety risks of potatoes expressing transgenes conferring kanamycin resistance, ß-glucuronidase activity and chlorsulfuron resistance.

Pollen dispersal: Two extensive studies that monitor pollen dispersal from field trials of self-fertile potatoes transgenic for chlorsulfuron resistance have been performed in New Zealand. In a small-scale field trial (22 m x 13.5 m) the frequency of transgenic seedlings from wild-type potato plants growing within the trial was about 1% (n=4,476), whereas only 0.05% of seeds were transgenic (n=40,871) 4.5 m from the trial (Tynan *et al*., 1990). Transgenic seedlings were not recovered from wild-type plants growing 4.5-10 m from the trial (n=16,034). The same transgenic lines were used in a subsequent more comprehensive study (88 m x 24 m). Only 0.05% of the progeny from wild-type plants within the trial were transgenic (n=54,213). From over 250,000 progeny of wild-type plants growing around the trial only 9 (0.004%) transgenic seedlings were recovered, all from rows 3.75m or 6.0 m west of the trial (Conner, 1993).

During five other field trials of transgenic potatoes in New Zealand, minimal transgenic pollen dispersal was detected (Conner, 1993). The frequency of transgenic progeny from wild-type potato plants growing within one trial was 0.04% (n=41,977). No other examples of transgenic pollen being transferred onto wild-type plants either within or surrounding these trials was detected. The dispersal of pollen carrying these transgenes was limited to within 6m of field trials, and then only occurred at exceptionally low frequencies. Consequently, isolation distances between field trials of transgenic potatoes and other potato crops do not have to be great to preclude gene transfer via pollen. Conner and Dale (1996) recommend that isolation distances of 20 m are sufficient to minimise pollen-mediated escape of transgenes from potato.

Crosses to related species: Unsuccessful attempts to hybridise potatoes with other solanaceous plants such as *Nicotiana*, *Petunia*, *Datura*, *Solanum nigrum* and *S. dulcamara* have been reported (Dale, 1993). Despite screening 53,917 seedlings originating from black nightshade (*S. nigrum*) plants growing among transgenic potatoes, interspecific gene transfer to this weedy species was not detected in New Zealand (Conner, 1994).

GM potatoes as weeds: True potato seeds can contribute to a significant weed problem in subsequent crops (Lawson, 1983). Up to 15-250 million true seeds per hectare can be

produced depending on the cultivar, environmental conditions and insect activity. Seeds may remain dormant in soil for up to 2 years and have been reported to retain viability over a seven-year rotation to the next potato crop.

Volunteer potato plants can also arise from tubers remaining in the field following harvest, resulting in the appearance of volunteer plants the next year. After mechanical harvest, up to 367,000 tubers per hectare may remain in the field, although the majority of these fail to survive even the mild winters in temperate climates (Lutman, 1977). The remaining viable volunteer tubers will always sprout the next season. Despite this potential weed problem, potato plants do not have the invasive potential of most weeds, and are rarely seen outside cultivated fields and usually persist for only one to two seasons.

In New Zealand trial sites, although volunteer transgenic potato plants appeared in the seasons following the initial field trials, they were easily managed and eliminated. The density of volunteers ranged from $1.3/m^2$ to $2.9/m^2$ in the year immediately following the field trial, which reduced to $0.02/m^2$ in year two, with no volunteers observed in year 3. The proportion of these volunteers that were transgenic ranged from 3 to 31%.

The single gene transfers resulting from genetic engineering are unlikely to increase the weediness of potato cultivars any more than the introgression of improved resistance to pests, diseases, drought and frosts from wild species via traditional breeding. Transgenic potatoes are therefore no more likely to become weeds outside farming situations than potato cultivars have in the past.

In New Zealand, the most widely grown potato cultivar, Ilam Hardy, is male sterile, though female fertile (Russell Genet, pers. comm.). Use of such a cultivar would remove concerns regarding pollen dispersal, but seed can still form due to pollen transfer from an adjacent field but only on plants within a few metres of each other.

Seed potato certification in New Zealand: In New Zealand, 74% of the total potato crop certified in 99/00 was grown under the strict conditions of the **P**athogen **T**ested (PT) potato scheme operated by Alex McDonalds Merchants Ltd in association with Crop & Food Research. The main goals of the PT scheme are to prevent disease, especially viral, contamination of crops. Most of the rules of the PT scheme are applicable to the situation with coexistence of GM and non-GM crops, e.g. a minimum interval of 5 harvest seasons is required between successive potato crops and adjacent potato crops must be at least 20 m away. However, note that potato seed certification is not concerned with pollen spread as only tubers are harvested. While tubers, not seed, are also the harvested part with GM potatoes, berries could still form. Implementation of a cultivation and/or spray programme after GM potato crops would assist to remove volunteers.

6.2 GM Brassicas and herbicide resistance

Brassicas of importance to New Zealand include oilseed (rape and turnip), vegetable (broccoli, cabbage, cauliflower, Brussels sprouts) and forage (turnip, swede, rape, kale) species. In New Zealand, several field trials of vegetable and forage brassicas have occurred including plants with herbicide resistance and reduced ethylene production (Table 4, Christey *et al.*, 1999; Christey and Braun, 2001). However, as plants were not allowed to flower, pollen dispersal data is not available.

In New Zealand, concerns regarding herbicide tolerant GM crops relate mainly to the release of herbicide tolerant rapeseed and its potential to become an agricultural weed. In addition, there are concerns that genes conferring herbicide tolerance will be transferred to non-GM *Brassica* crops and to wild brassicas. Wild turnip (*B. rapa* var. *slyvestris*) is a potential risk for accidental escape of transgenes in New Zealand because it is one of the few naturalised or native species in this country that is closely related to a crop.

Bourdôt *et al*. (1999) assessed the ecological risks and managerial consequences of growing glyphosate tolerant oilseed rape in New Zealand. They concluded that transgenic glyphosate tolerant oilseed rape is not likely to be any more invasive than already naturalised rape. Field hybridisations between oilseed rape and most other Brassicaceae are highly improbable. However, hybridisation with wild turnip (*B. rap*a) and *B. juncea* is likely but such hybrids will readily succumb to the methods currently employed for controlling volunteer plants of conventional non-GM rape. Hybridisation with conventional non-GM rape is possible but the implementation of the standard isolation distances for rape production (section 5.3.1, Table 8) would essentially prevent this.

In forage rape and other *Brassica* crops, pollen contamination will rarely occur since the agricultural management of these crops usually precludes flowering and seed production (Bourdôt *et al*., 1999). A range of alternative herbicides available with different modes of action could be used for the control of *Brassica* volunteers or weeds that obtain, through crossing, glyphosate resistance. Bourdôt *et al*. (1999) lists 33 different herbicide products with activity against wild turnip. These are spread across nine different mode of action groups.

Jenkins *et al*. (2001) used a chlorsulfuron-resistant mutant (non-GM) rape to model the potential for gene introgression from rape to a New Zealand population of wild turnip. Seed from wild turnip plants was harvested following hand pollination in a greenhouse and natural pollination in field trials. As expected, hand pollinations produced 100% hybrid progeny, which illustrates the high potential for interspecific hybridisation between rape to wild turnip. By contrast, hybrids were very rare under natural field conditions with only 63 hybrids found (n= 14,000, 0.46%). Many wild turnip volunteers appeared at the field trial sites but only one was chlorsulfuron resistant among nearly 11,000 progeny. This plant was triploid as expected and sterile. This reinforces the low frequency of interspecific hybridisation between wild turnip and rape under field conditions.

When considering the case of herbicide resistant GM crops and the concerns raised by the use of these crops, it should be remembered that herbicide tolerant crops developed through traditional breeding are also on the market, e.g. corn resistant to imidazolinone and soybean resistant to sulfonylurea (Shoemaker *et al*., 2001) and brassicas with atrazine resistance.

6.3 GM White clover

About 39% of New Zealand's land mass is covered in well-established grass and clover pastures. A number of open-pollinated grasses (e.g. ryegrass and tall fescue) and legumes (e.g. white clover) are also found in amenity surfaces such as playing fields and garden lawns as well as on roadsides. Therefore the potential for gene transfer to non-GM populations is high. Various transgenes have been introduced into white clover (Table 3) and white clover

has been field-tested (White *et al*., 2000); however, no transgenics have been commercially released yet.

Crosses to related species: Transfer of genes from GM white clover to related species is unlikely since there are no wild relatives present in New Zealand with which viable seed can be produced. Of the species that have been successfully hybridised with white clover only caucasian clover (*Trifolium ambiguum*) is currently widely used in New Zealand, and in this case embryo rescue (under sterile conditions in the laboratory) has been required. However, wild and cultivated populations of white clover are common and offer ample opportunity for gene transfer. White clover is naturalised throughout New Zealand and over 7,500 hectares of white clover are grown annually for commercial seed production.

Pollen dispersal: White clover plants flower profusely throughout spring and summer, providing prolonged opportunity for pollen dispersal. Honeybees and bumblebees are the principal pollinators, with honeybees being the more numerous and effective agents of pollen transfer. Experiments to determine gene flow with white clover pollen found that 99% of the pollen spread by bees was deposited within 24 m of the pollen source. However, a very low level (<1%) were transferred to greater distance which is consistent with expectations based on honeybee foraging. However, adoption of suitable isolation distances would reduce this source of contamination. In addition, the impact of transgene "escape" will ultimately depend on whether the introduced gene confers increased vigour or competitiveness in the range of environments in which white clover is grown.

Seed dispersal: Seed dispersal is unavoidable in both commercial seed production and in grazed pastures and must be considered before releasing transgenic forage plants. In New Zealand commercial seed production situations yields of over 500 kg/ha are common for white clover. Seed losses prior to and during harvest contribute between 40 to 210 kg/ha of hard seed to the buried seed pool, and these seeds can remain dormant for many years (Clifford *et al*., 1985; Harris, 1987). Whenever soil disturbance (e.g. cultivation) occurs, a proportion of the buried seed germinates and contributes to the resulting plant population (Clifford *et al*., 1985). However, in competitive swards the number of plants that regenerate from buried seed and become fully established is very low (Chapman, 1987; Archer and Robinson, 1989). The most effective means of containing transgenic material in an open-pollinated species such as white clover may be to prevent or retard flower production. This can be achieved to a limited extent by intensive grazing management during the flowering season, but molecular techniques that modify floral initiation or induce male sterility offer a longer-term solution.

6.4 Bt transgenics

In the USA commercial cultivars of Bt maize, cotton, canola, soybean and potato are grown over large areas. Bt-transgenic plants can greatly reduce the use of broader spectrum insecticides, but there are concerns that insect resistance may develop through the increased exposure to Bt gene products. The development of insect resistance may hinder this technology and also affect the efficacy of foliar Bt sprays, which are widely used by the organic industry. Insect resistance management plans are being implemented to ensure the prolonged effectiveness of these products. Present resistance management strategies rely on a "refuge" composed of non-Bt plants. In the USA, the Bt corn industry developed an insect

resistance management which was accepted by the EPA for the 2000 planting season. This plan features a 20% refuge requirement of non-Bt in the mid-west corn growing region and a 50% refuge requirement in areas of overlapping corn and cotton production. In addition, growers must locate the non-Bt refuge within a specified distance of Bt fields. If not treated with insecticides, the non-Bt refuge must be within 0.5 mile of Bt corn. If treated, the non-Bt refuge must be within 0.25 mile and must not be treated with Bt sprays (http://www.monsanto.com).

In Canada, a growers handbook is available which outlines an Insect Resistance Management Plan to be used with Bt corn. A "high dose/refuge strategy" has been adopted which involves high expression levels of Bt and a 20% refuge.

Shelton *et al*. (2000) have used Bt-transgenic broccoli plants to examine resistance management strategies. They concluded that each insect/Bt crop system may have unique management requirements. In addition, other strategies for managing overall resistance to Bt need to be developed. Having Bt expressed in plants so that the insect population is subjected to selection pressure for particular periods of time (e.g. inducible promoter) or in particular plant parts (tissue specific promoter) may provide larger refuges. Theoretical models suggest that stacking two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance more effectively than single toxin plants released spatially or temporally (Rousch, 1997; Tabashnik *et al*., 1997) and may allow smaller refuges.

Summary: In the case of GM plants containing Bt genes, coexistence needs to consider concerns regarding the development of insect resistance. While numerous strategies have been proposed to prevent Bt resistance, experimental evaluation of the effectiveness of such resistance management tactics is vital to help provide guidelines for the deployment of transgenic insecticidal crops. The large amount of research being conducted overseas and the implementation of Bt management strategies already in place, indicate that such schemes could be adapted for use in New Zealand. As Bt crops have been released overseas for several years now and carefully monitored during that time any resistance development will be detected and an appropriately modified management plan developed prior to release in New Zealand. Development of any such management plan requires adequate grower education to ensure it is implemented effectively.

7 Segregating GM and non-GM products

Coexistence of GM and non-GM crops requires segregation in the field to prevent cross contamination (section 5.3), but it also requires segregation of produce from the field to the end user. In some cases segregation systems are already in place to ensure that certain commodity crops are kept separate to avoid contamination during harvesting, loading and unloading, storing and transporting. Such a handling process has been used for some time for speciality grains, such as high oil corn (Shoemaker *et al*., 2001) and is widely used in the organics industry. These processes could be applied to GM crops to ensure segregation from non-GM crops.

7.1 Imported seed and GM detection

New Zealand imports a large amount of seed and this includes seed from countries where GM crops are grown. Examples of such seed crops include maize, sweet corn, tomatoes, squash, canola and soybean. Therefore the potential exists for the imported seed to be from a GM source or contaminated with GM material. Since no GM organisms have been approved for release in New Zealand, verification and/or detection systems at the border are required.

Seed imported into New Zealand for sowing needs to follow the import specification and entry conditions outlined in MAF Regulatory Authority (MAFRA) Standard 155.02.05 "Importation of Seed for Sowing". These standards are designed to ensure that seed imported into New Zealand is pure, weed-, soil- and pest-free. They do not cover GM seed that needs a separate application to ERMA for importation and for release. This MAFRA protocol is concerned with phytosanitary issues, and does not include a question on GM status of the imported seed.

MAFRA prepares an import health standard for each crop that includes all pests and diseases of concern. It is feasible that this could also indicate whether GM status was of concern because of the crop type, region from which it came or origin of seed. Where there are concerns over whether a seed lot contains GM material, then documentation or testing would be required to determine GM status. Certification could be provided as part of this scheme, either by appropriate certified documentation from the country of origin or at the border. Random sampling and testing may be required to ensure required standards are met.

As molecular detection for pathogens is already conducted with some imported seeds, the addition of an extra PCR (section 7.4), is not a huge requirement. This could be circumvented in cases where appropriate documentation is provided by an accredited company. For example, importation of new potato varieties into New Zealand generally involves the entry of tissue culture material/tubers that are then grown in vitro. As part of the disease testing scheme required by MAF before imported material can be grown in the field, plant material is checked for a range of diseases using protein and DNA based assays. Twelve viruses are checked for using Enzyme Linked Immunosorbant Assay (ELISA) and a herbaceous host assay, 5 phytoplasmas are checked using nested PCR, and RT PCR is used to check for the presence of 1 viroid - 4 rely on area freedom at the source of the material (John Fletcher, pers. comm.).

A protocol has already been drafted by ERMA, MFAT and MAF covering the importation of sweet corn seed to prevent GM contamination (Appendix 2). While this protocol only relates

to sweet corn seed, it could be adopted for other imported seed where concerns exist about its GM content. This protocol covers in detail all aspects needed to ensure detection of GM content with high certainty.

7.2 Harvest/storage/transport of produce

Identity preservation (IP) is the stringent process of differentiating commodities and requires strict separation to be maintained at all times. This is already used successfully in the several industries to preserve unique end use characteristics. For example, virtually all high oil corn varieties are marketed under the OPTIMUM brand developed by Dupont. Seed corn and waxy corn growers have been segregating and preserving the identity of their corn crops for many years. In addition, the organics industry has standards and guidelines in place to ensure that organic and non-organic produce is not mixed (section 7.3).

In Tasmania, crop segregation and identity preservation are already an important part of many industries. Closed supply chains (production systems involving a single product managed by a single operator) are used in the pyrethrum, poppy and seed crop industries.

So Good® (Sanitarium Health food company) products now carry a label to indicate that they are made from non-GM soy. An IP process was implemented to track the soy used at every stage, from seed through to the final manufacturing step. This also involved audited certification at each stage to maintain segregation and to minimise the possibility of mixing GM and non-GM produce.

Segregation of produce such as fruit and vegetables should be easier than seed or products used for processing, as fresh fruit and vegetable are not bulked and stored but instead are often transported to the market in small lots. Therefore, on-farm labelling is probably feasible. In New Zealand systems are already in place in the apple, kiwifruit and meat industries that enable detailed tracking of produce. These systems could be further developed for use for GM produce.

As greenhouse crops such as tomatoes and flowers are grown indoors it is possible to contain plant material more easily and reduce the chances of contamination. GM material could be segregated and labelled at harvest to keep track of the plant material.

Such segregated marketing requires rapid, accurate and economical tests. Several tests are available for detecting GM content in a range of plant material including grains and seeds and their processed products (section 7.4).

For seed certification, strict rules are in place covering the identification of seed after harvest, seed cleaning, labelling and sealing. Field-dressed seed is tagged by the grower using appropriately marked labels issued by AgriQuality. Before such seed is accepted for processing at the cleaning plant, the grower must return a certificate of identification to AgriQuality's Seed Certification Bureau. Cleaning of seed must be carried out in approved seed stores/seed cleaning plants. After cleaning, all certified seed must be sampled according to approved seed sampling procedures, by a licensed sampling officer. Seed is not finally certified until an analysis certificate issued by the National Seed Laboratory, Palmerston North, has been completed indicating that the standards for the appropriate class have been met. A DNA-based test for GM contamination could be added as part of this seed certification process using the seed samples sent to the National Seed Laboratory.

7.3 Organic production

Organic production systems that enable the coexistence of organic and non-organic produce at all stages of production have been in place worldwide for many years. Guidelines for the production, processing, labelling and marketing of organically produced foods have been established in several countries including New Zealand, Australia, Japan, USA, and the EU. The three countries with the greatest areas planted in transgenic crops are the USA, Argentina and Canada. All three have commercially successful organic production sectors. In addition, The Codex Alimentarius Commission has produced Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods. These are intended to be a step towards standardising international requirements for organic products in terms of production and marketing standards, inspection arrangements and labelling.

Organic standards set out principles for organic production at farm, preparation, storage, transport, labelling and marketing stages. They provide lists of permitted substances for the production of organic foods. The guidelines also outline rules for inspection and certification systems, including minimum inspection requirements. In all cases, organic standards preclude the use of GM products. However, the setting of limits for accidental contamination from GM material are being discussed in several countries. For example, the USA National Organic Program "Principles of Organic production and Handling" (May 7 2001 draft) states that "Although organic standards prohibit the use of certain materials such as synthetic fertilisers, pesticides and genetically engineered organisms, they cannot ensure that organic products are completely free of residues due to background levels in the environment". The International Federation of Organic Agricultural Movements is also aware that GM pollution is becoming a problem.

Standards require that production, preparation and storage of organic products be clearly separated from produce not prepared in accordance with the standards/guidelines. All practical measures are to be taken at the level of the unit to ensure compliance. Samples of the final product may be tested by the certification body if it is suspected that prohibited products (e.g. pesticides) have been used. The obligation is on the producer/handler to ensure the conditions set out in the standards are met. This is then verified by the certification body.

In New Zealand, BIO-GRO New Zealand certifies over 700 producers across a wide range of primary industry sectors, products, processes and services for organic production. BIO-GRO has a series of Producer Guides covering organic dairy farming, livestock farming, vegetable, crops, orchards and tree crops. The BIO-GRO Standards set the production rules and certification process which BIO-GRO certified producers must comply with to use the BIO-GRO trademark and logo. These standards are available on their web site (www.bio-gro.co.nz) and cover in detail the certification process. Module 4.2 "Crop Production Standard" includes detailed guidelines for harvesting, packing, storing and transporting organic produce including recommendations for situations where parallel production occurs. In addition, BIO-GRO organic production standards have international accreditation. AgriQuality also certifies organic produce for the export market through Certenz which has ISO/IEC Guide 65 accreditation awarded by International Accreditation New Zealand (IANZ).

Organic growers already accept that contamination from neighbouring properties, for example from spray drift, is inevitable and therefore limits are allowed. Genetic contamination of

various kinds is inevitable in field grown crops from both GM and non-GM crops. This is an issue already faced by farmers growing seed production crops and regulations already exist to ensure contamination is below an agreed level. Contamination from either pollen or seed from GM sources cannot be entirely eliminated. However, if organic growers accept levels of contamination similar to those for certified seed, then the same growing practices can be adopted.

7.4 GM detection

Analytical tests are needed that can rapidly and accurately check for GM content due to consumer requests about the GM status of specific products and the need to be able to segregate GM and non-GM produce. In addition, GM produce poses many challenges to international trade. Certain GM crops have not been approved for human food use in Europe and Japan, yet they have been approved and commercialised in countries such as the USA. Other products have been approved for animal feed in the USA but not for human consumption.

GM food can be identified by either detecting the inserted DNA using polymerase chain reaction (PCR) or the new protein produced from the insertion using enzyme-linked immunosorbent assay (ELISA). PCR is the most sensitive of the two methods and can detect very few copies of the target sequence. The use of the TaqMan® PCR system is more sensitive than conventional PCR and enables quantification of how much target sequence occurs in a sample. This ability to determine the percentage of an ingredient or produce that is GM is important to food manufacturers since the labelling regulations developed by ANZFA (section 8.1) that come into force from December 2001 include threshold levels which permit a low level of contamination.

GM detection services are available both in New Zealand and overseas. At Crop & Food Research, tests have been developed to detect the two sequences most commonly found in commercially grown GM crops, 35S and Nos. Additional tests can also be used that are specific for the actual gene inserted such as Bt genes or herbicide resistant genes. Such construct-specific PCR tests could be used to distinguish between GM maize food products that have been approved for human consumption and those that have not been approved for human consumption, like StarLink maize, since these contain different Bt genes. The tests developed at Crop & Food Research are very sensitive and easily detect the GM content in foods and have a sensitivity better than 0.1% for foods or ingredients containing DNA (Timmerman-Vaughan, 2001; pers. comm.).

7.4.1 Australia

GeneScan Australia Pty Ltd provides an analytical GM detection service for the 35S and Nos regions which are present in 17 of 19 GM crops undergoing assessment by ANZFA. In addition, GM specific primer systems are available for the transgenes in all 19 GM crops assessed by ANZFA. GeneScan Australia has kits that can identify Roundup Ready® (RR) soy, Liberty Link® corn, BT176 corn, Maximizer® Bt maize, Bt11 corn, Yield Gard® corn and Starlink corn. No field kits are available as all kits require access to PCR (Stephen Wilcox, GeneScan pers. comm.). In addition, chip technology is now available for GM testing, enabling a single test to determine the plant species and type of GM DNA present.

There is no GM accreditation system in Australia at present. GeneScan labs have been set up according to their European counterparts which have been accredited. At the moment Stephen Wilcox from GeneScan is a member of a National Association of Testing Authorities, Australia (NATA) committee determining whether it is feasible to accredit laboratories for GM testing (both PCR and ELISA) (Stephen Wilcox, GeneScan pers. comm.).

Australian Government Analytical Laboratories (AGAL) also has the capacity to analyse samples for the presence of GM material. AGAL has facilities throughout Australia and uses a comprehensive Chain of Custody system when transporting samples between labs which generates evidence of receipt and provides rapid follow up.

7.4.2 USA

The Grain Inspection, Packers and Stockyards Administration (GIPSA) of the USDA established a Biotechnology Laboratory in January 2001 at the Technical Center in Kansas City, MO. This laboratory will be used to evaluate and verify the validity of analytical procedures applied to the detection and quantification of bioengineered traits in grains and oilseeds. It will also be used to establish sampling procedures for use in testing biotechnology-derived grains and oilseeds. GIPSA also intends to develop guidelines for accreditation of laboratories providing DNA-based testing. This DNA-Based Laboratory Accreditation is likely to be implemented this summer (Don Kendall, GIPSA, pers. comm.).

GIPSA has conducted test kit evaluations on kits that are commercially available in the USA to test for the presence or absence of GM content (http://www.usda.gov/gipsa/biotech/biotech/evalaccredit.htm). GIPSA has performance verified 8 rapid test kits for analysis of StarLink/Cry9C in grains. The sensitivity of these kits ranges from 1 in 10,000 (0.01%) kernels for a Microtiter Well Plate Assay to 1 in 800 (0.1%) kernels for a Lateral Flow Strip. Lateral Flow Strips are ELISA based and can be conducted in the field or at processing plants to rapidly detect biotech content.

7.4.3 UK

In the UK, Genetic ID was the first lab to receive accreditation for quantitative and varietal testing for GMOs from the United Kingdom Accreditation Service (UKAS). UKAS accreditation ensures that Genetic ID's laboratory complies with ISO/IEC Guide 25 and EN 45001 standards. Genetic ID, Inc., has developed unique and proprietary Varietal IDSM tests that are designed to detect and quantify GM varieties that are not approved in a particular country or region. Genetic ID is developing a program to combine its testing with a certification program called “CertID” for producers who want to sell segregated non-biotech crops.

7.4.4 Summary and recommendations

GM detection services are widely available. However, the disadvantages of current testing systems are the relatively long time taken and the lack of rapid tests that can be conducted in the field. Samples need to be moved from the site to a testing lab, increasing the chances of further contamination. More ideal would be rapid, accurate, on-site testing at minimal cost. New Zealand needs accredited testing facilities not only to satisfy New Zealand consumers

and labelling requirements but also to provide some of our trade partners with assurances about the GM status of our produce.

8 Regulation and regulatory agencies

8.1 ANZFA

The Australia New Zealand Food Authority (ANZFA) develops food standards and other regulatory measures for Australia and New Zealand. GM foods in Australia and New Zealand are regulated by Food Standard A18: Food Produced Using Gene Technology, which is currently under revision. The standard has two provisions:

1. mandatory pre-market safety assessment requirement, and
2. mandatory labelling requirement.

Labelling of GM food is required where novel DNA and/or protein is present in the final food; and where the food has altered characteristics. The following are exempt from these requirements:

- highly refined food where the effect of the refining process is to remove novel DNA and/or protein,
- processing aids and food additives except those where novel DNA and/or protein is present in the final food,
- flavours which are present in a concentration less than or equal to 0.1% in the final food, and
- food prepared at the point of sale.

Ministers also resolved to exempt ingredients from GM labelling where they contain less than 1% of GM material, but only where its presence is unintended. To assist food businesses to comply with the new labelling requirements for GM food an Intergovernmental Task Force has developed a Draft Compliance Guide to Standard A18 entitled: "Labelling Genetically Modified Food".

Producers of GM foods must also apply to ANZFA to seek approval for the food to enter the food supply in the two countries. As of August 2000 there were 19 GM crops from 6 different crop types undergoing ANZFA assessment (Table 9). Of these 10 have been approved.

Table 9: Crops undergoing ANZFA assessment

Crop	Completed	Currently undergoing	Traits
Canola (3)	1	2	Herbicide tolerance
Corn (7)	2	5	Herbicide tolerance, Bt, Herbicide tolerance + Bt
Cotton (3)	2	1	Herbicide tolerance, Bt
Soybean (2)	2	0	Herbicide tolerance, high oleic
Sugar beet (1)	0	1	Herbicide tolerance
Potato (3)	3	0	Bt, Bt + virus resistant

Source: GeneScan and ANZFA websites. As of August 2000.

8.2 HSNO Act

HSNO Act Part V Section 38 (2) states: "Any approval to import an organism for release, or to release an organism from containment, shall be granted without controls".

As already presented to the Royal Commission (Timmerman-Vaughan, Royal Commission witness brief), we recommend a modification to the HSNO Act and ERMA regulatory processes to provide a third category of field release which is intermediate between field trials

under containment and general release. This level of containment might be called "Release with Controls". This intermediate level of release would be valuable in a number of situations, for example to conduct environmental risk assessment on an appropriate scale, or as the final release level for plants genetically modified to produce bioactive compounds such as pharmaceuticals. There are several cases where the release of GM plants with controls is appropriate to ensure coexistence of GM and non-GM crops can occur. For example, current research indicates that the growing of Bt crops should not be conducted without the use of a non-Bt refuge (section 5.5.4). Controls may also be needed where GM plants are being grown to produce high value products, such as hybrid seed for overseas markets, but where isolation from other crops is required given the pollination characteristics of the species or near relatives. Maintaining controls might be prudent for many reasons, however, including that the GM plants or animals may not be suitable for food.

A total of 68 crop-gene combinations have been deregulated overseas and therefore those recommendations should be studied in detail as a starting point to determine if the same crop-gene combination could also be deregulated in New Zealand. Where a crop-gene combination has already been deregulated overseas then a streamlined process for deregulation in New Zealand should be considered. Factors unique to New Zealand must be included in a streamlined procedure. For example, relatives of the crop present in New Zealand that were not present in the country where deregulation occurred, and secondly, any increased likelihood of the crop-gene combination having greater propensity for invasiveness or weediness in New Zealand. This process will be on a case by case basis as different regulations will be needed depending on the breeding system of the crop, the nature of the transgene, and the end use of crop, e.g. seed vs. vegetable. In some cases, the specific gene will also need to be considered, e.g. a Bt refuge strategy may have to be adopted for Bt plants. Field trial applications submitted to ERMA cover in great detail all the potential concerns associated with field testing a particular plant. Any monitoring results from these existing and ongoing trials will provide vital information on the conditions to be placed on general release.

8.3 Resource Management Act 1991

The purpose of this Act is to promote the sustainable management of natural and physical resources. All growers of primary produce are obliged to meet the requirement of this Act. While changes to the HSNO Act are the appropriate area for regulations concerning the growing of GM crops, local councils may want to impose further guidelines on the growing of such crops in their jurisdiction due to the concerns of local people and communities. However, regulations for growing GM crops should be addressed as national policy due to the practical difficulties that are envisaged when dealing with such complex issues at a local level.

8.4 Liability

While the above recommendations and guidelines may facilitate the enable the co-existence of GM and non-GM crops, they cannot guarantee 100% purity. This is true of any segregation system and is why limits for contamination are set for organic production. GM detection systems are extremely sensitive and thus capable of detecting very low levels of contamination. Such levels of contamination by non-organic sources could not be detected previously in organic crops. The organics industry is likely to have to adopt acceptable limits

for accidental GM contamination. The area of liability for the consequences of any GM contamination problems is a complex legal issue that will need to be addressed prior to deregulation of any GM crop in New Zealand. Co-operation between GM and non-GM producers will be required to ensure that co-existence can occur.

9 Acknowledgements

We are grateful for the assistance of Tony Conner in providing research data and detailed comments on the report. We thank Robert Braun, Nick Roberts, Jo Smith, Tracy Webster, Tracy Williams and Nadene Winchester for their assistance in compiling this report; as well as colleagues and staff of MAF, PT scheme and BIO-GRO for useful discussions and providing information. We thank Denise McDonald and Anne Rose from ERMA for the GM field trial summary information. We thank Amy Pope from Monsanto for useful discussions and providing the Roundup Ready® cotton manual.

10 References cited

10.1 Scientific papers

AgriQuality 2000-2001. Seed Certification Field and laboratory standards. 103 p.

AgriQuality 2001. Seed Certification Statistics 2000-2001. 61 p.

Archer, KA, Robinson, GG 1989. The role of stolons and seedlings in the persistence and production of white clover (*Trifolium repens* L. cv. Huia) in temperate pastures in the northern Tablelands, New South Wales. *Australian Journal of Agricultural Research* 40: 605-616.

Biggs, DR, Hancock, KR 1998. In vitro digestion of bacterial and plant fructans and effects on ammonia accumulation in cow and sheep rumen fluids. *Journal of General and Applied Microbiology* 44:167-171.

Bourdôt, G, Conner, T, Jenkins, T 1999. *Ecological risks and managerial consequences of Roundup Ready oilseed rape in New Zealand*. New Zealand Institute for Crop & Food Research Ltd, Christchurch, New Zealand. 67pp.

Chapman, DF 1987. Natural reseeding and *Trifolium repens* demography in grazed hill pastures. II. Seedling appearance and survival. *Journal of Applied Ecology* 24: 1037-1043.

Christey, MC**,** Braun, RH 2001. Transgenic Vegetable and Forage *Brassica* Species: Rape, Kale, Turnip and Rutabaga (Swede). Biotechnology in Agriculture and Forestry, Vol. 47 Transgenic Crops II. YPS Bajaj (ed). Pp87-101.

Christey, MC**,** Braun, RH, Reader, JK 1999. Field performance of transgenic vegetable brassicas (*Brassica oleracea* and *B. rapa*) transformed with *Agrobacterium rhizogenes*. *SABRAO Journal of Breeding and Genetics* 31(2): 93-108.

Clifford, PTP, Rolston, MP, Williams, WM 1985. Possible solutions to contamination of white clover seed crops by buried seeds. *In:* Hare, M.D. and J.L. Brock (eds) *Producing herbage seeds*. Grassland Research and Practice Series No. 2: 25-31.

Clifford, PTP, Sparks, GA, Woodfield, DR 1996. The intensifying requirements for white clover cultivar change. *Grassland Research and Practice Series No. 6*. Pp 19-24.

Conner, AJ 1993. Monitoring "escapes" from field trials of transgenic potatoes: A basis for assessing environmental risks. In: *Seminar on Scientific Approaches for the Assessment of Research Trials with Genetically Modified Plants, Jouy-en-Josas, France, April 1992*. Organisation for Economic Co-operation and Development, Paris. Pp. 34-40.

Conner, AJ 1994. Biosafety assessment of transgenic potatoes: environmental monitoring and food safety evaluation. *In:* DD Jones (ed) Proceedings of the 3rd international symposium on The biosafety results of field tests of genetically modified plants and microorganisms. Pp 245-262.

Conner, AJ, Dale, PJ 1996. Reconsideration of pollen dispersal data from field trials of transgenic potatoes. *Theoretical and Applied Genetics* 92: 505-508.

Crawley, MJ, Brown, SL, Hails, RS, Kohn, DD, Rees, M 2001. Transgenic crops in natural environments. *Nature* 409: 682-683.

Dale, PJ 1993. Potato - general presentation. *In*: Seminar on Scientific Approaches for the Assessment of Research Trials with Genetically Modified Plants, Jouy-en-Josas, France, April 1992. Organisation for Economic Co-operation and Development, Paris. Pp. 12-20.

Daniell, H, Datta, R, Varma, S, Gray, S, Lee, SB 1998. Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnology* 16: 345-348.

Dumas, C, Mogensen, HL 1993. Gametes and fertilization: Maize as a model system for experimental embryogenesis in flowering plants. *The Plant Cell* 5: 1337-1348.

Grant, JE, Dommisse, EM, Christey, MC, Conner, AJ 1991. Gene transfer to plants using Agrobacterium. *In*: Advanced methods in plant breeding and biotechnology. Murray, DR (ed). CAB International. Pp 50-73.

Harris, W 1987. Population dynamics and competition. *In*: Baker MJ, Williams WM (eds.). White clover. CAB International, UK. Pp 203-298.

Hill, RR Jr, Shenk, JS, Barnes, RF 1988. Breeding for yield and quality. *In*: Hanson AA, Barnes DK, Hill RR Jr. (eds). Alfalfa and alfalfa improvement. *Agronomy Monograph* 29: 809-825.

Hokanson, SC, Hancock, JF, Grumet, R 1997. Direct comparison of pollen-mediated movement of native and engineered genes. *Euphytica* 96: 397-403.

James, C. 2000. Global review of commerialized transgenic plants. ISAAA Briefs No. 21: Available at http://www.isaaa.org/publications/briefs/Brief_21.htm

Jenkins, TE, Conner, AJ, Frampton, CM 2001. Investigating gene introgression from rape to wild turnip. *New Zealand Plant Protection* 54: in press.

Lawson, HM 1983. True potato seed as arable weeds. *Potato Research* 26: 237-246.

Lutman, PJW 1977. Investigations into some aspects of the biology of potatoes as weeds. *Weed Research* 17:123-132.

Molina-Fraener, F, Jain, SK 1992. Inheritance of male-sterility in *Trifolium hirtum* All. *Genetica* 85: 153-161.

Moyes, CL, Dale, PJ 1999. Organic farming and gene transfer from genetically modified crops. www.gmissues.org/orgreport.htm.

Negri, V, Rosellini, D 1996. Inheritance of male-sterility in some genotypes of *Lotus corniculatus* L. *Journal of Genetics and Breeding* 50: 23-28.

Richards, AJ 1986. Plant breeding systems. George Allen & Unwin Ltd, London.

Roush, RT 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pesticide Sci*ence 51: 328-334.

Shelton, AM, Tang, JD, Roush, RT, Metz, TD, Earle, ED 2000. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnology* 18: 339-342.

Shoemaker, R, Harwood, J, Day-Rubenstein, K, Dunahay, T, Heisey, P, Hoffman, L, Klotz-Ingham, C, Lin, W, Mitchell, L, McBride, W, Fernandez-Cornejo, J 2001. Economic issues in agricultural biotechnology. U.S. Department of Agriculture. Agriculture Information Bulletin No. 762 (also www.ers.usda.gov/publications/aib762).

Tabashnik, BE *et al*. 1997. Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis*. *Proceedings of the National Academy of Sciences USA* 94: 12780-12785.

Thill, DC, Mallory-Smith, CA 1997. The nature and consequence of weed spread in cropping systems. *Weed Science* 45: 337-342.

Tiedje, JM, Colwell, RK, Grossman, RL, Hodson, RE, Lenski, RE, Mack, RN, Regal, PJ 1989. The planned introduction of genetically engineered organisms - ecological considerations and recommendations. *Ecology* 70: 298-315.

Timmerman-Vaughan, G 2001. The reality of PCR and ELISA. *Food Technology in New Zealand.* April: 39.

Tynan, JL, Williams, MK, Conner, AJ 1990. Low frequency of pollen dispersal from a field trial of transgenic potatoes. *Journal of Genetics and Breeding* 44: 303-305.

White, DWR, Woodfield, DR, Dudas, B, Forster, RLS, Beck, DL 2000. White clover molecular genetics. *Plant Breeding Reviews* 17: 191-223.

Young, WC, Youngberg, M 1996. Cropping systems for perennial ryegrass seed production 2. Minimum tillage systems for changing cultivars in certified seed production. *Agronomy Journal* 88: 78-82.

10.2 Web sites

Web sites consulted during the preparation of this contract.

Agriculture & Biotechnology Strategies (Canada) Inc.: http://www.agbios.com/default.asp

AgriQuality: http://www.agriquality.co.nz/ Go to "hot tips" for information on Certenz.

ANZFA: http://www.anzfa.govt.nz/

APHIS: home page: http://www.aphis.usda.gov./

Current status of Petitions http://www.aphis.usda.gov/biotech/petday.html

Petitions of Nonregulated Status Granted by APHIS as of 10-12-2000. http://www.aphis.usda.gov/biotech/not_reg.html

Guide for Preparing and Submitting a Petition for Genetically Engineered Plants http://www.aphis.usda.gov/biotech/petguide.html

Petitions for Determination of Nonregulated Status of various crop-trait combinations:

http://www.aphis.usda.gov/biotech/dec_docs/9425701p_ea.HTM

http://www.aphis.usda.gov/biotech/dec_docs/9917301p__ea.HTM

http://www.aphis.usda.gov/biotech/dec_docs/9827801p_det.HTM

http://www.aphis.usda.gov/biotech/dec_docs/9821601p_ea.HTM

Australian Government Analytical Laboratories (AGAL): www.agal.gov.au/index.html

Aventis: http://www.aventis.com

The Bio Dynamic Farming and Gardening Association in New Zealand Inc: http://www.biodynamic.org.nz/ Information on Biodynamics and Demeter certification.

BioGro: www.bio-gro.co.nz

Biotechnology Australia: http://www.biotechnology.gov.au/

Canadian Biotechnology Advisory Committee: http://cbac.gc.ca/

DEFRA: UK Department for Environment, Transport and Rural Affairs http://www.defra.gov.uk/environment/fse/index.htm interim reports on FSEs

http://www.environment.detr.gov.uk/acre/index.htm ACRE home page

http://www.environment.detr.gov.uk/acre/background/index.htm ACRE background papers on the release of GMs in the EU.

DETR: http://www.dtlr.gov.uk/ home page for the former UK Department of the Environment, Transport and the Regions. In the process of being amalgamated into DEFRA.

Dupont home page http://www.dupont.com/

ERMA: http://www.ermanz.govt.nz/ Source of information on New Zealand GM research. Also has links to the HSNO and RMA Acts.

Farm Scale Evaluations of GM Crop Trials-UK: http://www.gm-info.gov.uk/1999/gmcroptrials.htm

FDA: http://vm.cfsan.fda.gov/~lrd/biocon.html List of Completed Consultations on Bioengineered Foods.

Florigene: http://www.florigene.com.au/web/florigenecomau/florigenecomauhp.nsf/web/index.html

Food and Agriculture organization of the United Nations: http://www.fao.org/

GeneScan: http://www.genescan.com.au/

Genetic ID; http://www.genetic-id.com/href/gmotesting.asp

Genetic Manipulation Advisory Committee (GMAC), Australia:

http://www.health.gov.au/tga/gmac/gmachome.htm

Gene watch: http://www.genewatch.org/Home.htm

GIPSA: http://www.usda.gov/gipsa/biotech/biotech.htm Test Kit Performance Evaluation: http://www.usda.gov/gipsa/biotech/biotech.htm

Her Majesty's Stationery Office: http://www.hmso.gov.uk/ Source of full text of UK legislation.

International Service for the Acquisition of Agri-biotech Applications: http://www.isaaa.org/

International Organization for Standardization: (ISO): http://www.iso.ch/iso/en/ISOOnline.frontpage

John Innes Centre, Norwich, UK: http://www.gmissues.org/ Source of Moyes and Dale, 1999 reference.

Life Sciences Network: http://www.lifesciencenz.com/

Monsanto: http://www.monsanto.com/

http://www.monsanto.com/monsanto/biotechnology/default.htm for Bt Corn Insect Resistance Management Survey — 2000 Growing Season.

Monsanto Australia: http://www.monsanto.com.au/

The National Organic Program, USA: http://www.ams.usda.gov/nop/

New Zealand Royal Commission on Genetic Modification: http://www.gmcommission.govt.nz/Source of witness briefs

OECD: http://www.oecd.org/

Summary of Data from OECD's Database of Field Trials:

http://www.oecd.org/ehs/summary.htm

(http://www.oecd.org/ehs/public.htm). "Harmonization in Biotechnology" Web Site. Source of Consensus Documents on biology of various plants and general information on some genes and their enzymes.

Office of the Gene Technology Regulator, Australia. http://www.ogtr.gov.au/ or:.http://www.health.gov.au/ogtr/general/index.htm

Ontario Corn Producers' Association: http://www.ontariocorn.org/. Source of Bt corn growers guide for Canada.

Sanitarium: http://www.sanitarium.co.nz/ or http://www.sanitarium.com.au/

Statistics New Zealand: http://www.stats.govt.nz

Tasmanian joint select committee report on gene technology (http://www.parliament.tas.gov.au/CTEE/REPORTS/Gene2001.pdf)

Wales-legislation : http://www.wales-legislation.hmso.gov.uk/

Wales buffer zone:

http://www.lifesciencenz.com/repository/external_news_material/0516_buffer.pdf

11 Appendices

Appendix 1: Roundup Ready® Cotton Technical Manual

This appendix includes the Table of Contents and Appendix 2 from the Roundup Ready® Cotton Technical Manual, published by Monsanto Australia Limited, April 2001. Reproduced by permission of Amy Pope, Monsanto Australia. The full report is available from Mary Christey at Crop & Food Research on request.

TABLE OF CONTENTS

1. Introduction 4

2. Benefits of Using Roundup Ready® Cotton 5

3. Resistance Management strategy for Roundup Ready® Cotton 6

4. How Does Roundup Ready® Technology work 9

5. Developing a Roundup Ready® cotton variety 10

6. Weed Management Strategies in Cotton 12

   6.1 Ipomoea spp

   6.2 Cyperus spp

   6.3 Hibiscus spp

   6.4 Echinochloa spp

   6.5 Understanding Weeds and their Implications for Management

   6.6 Life Cycle of Weeds

7. How Does Roundup Ready® Herbicide work 19

   7.1 Roundup Ready® Herbicide Performance Factors

   7.2 Entry into the Plant

   7.3 Movement in the Plant

8. The Performance of Roundup Ready® Cotton Varieties and Technology 22

   8.1 Quality Assurance Program

   8.2 Fruit Retention and its Relationship to Yield and Earliness

   8.3 Effect on Lint Quality – US data

9. Tolerance of Roundup Ready® Cotton to Roundup Ready® Herbicide 31

10. Weed Management Options in a Roundup Ready® System 34

    10.1 Pre - plant Residual Herbicides

    10.2 Ratoon and Volunteer Cotton

11. Ultra Narrow Row Production 41

11.1 "Gundaline" Site
12. Application 44
12.1 Application timing
12.2 Application rates / Number of applications per crop
12.3 Tank Mixes
12.4 Spray Equipment
12.5 Off target drift
12.6 Aerial application
12.7 Over the top applications
12.8 Directed or shielded spray applications
12.9 Decontamination and cleaning of spray equipment
12.10 Pre harvest use of Roundup Ready® Herbicide
13. Non Crop Situations 53
14. Roundup Ready® Herbicide Formulation 54
14.1 Mixing
14.2 Handling, Storage and Disposal
15. For Best Results 56
16. References 58
Appendix 1 - Roundup Ready Herbicide label 60
Appendix 2 - Crop Management Plan 67
Appendix 3 - Herbicide Group Classifications 71
Appendix 4 - Regulatory Processes 73

Appendix 2: Monsanto Crop Management Plan (CMP)

The purpose of the Roundup Ready Cotton Weed Management Strategy (WMS) is the prevention of the evolution of herbicide resistant weeds. Prevention is best achieved by following integrated weed management guidelines:

- Use as many different weed control options (chemical and non-chemical) as possible in both crop and fallow phases.
- Enter a cropping phase with low weed numbers.
- Make every herbicide application count - use the registered rate that kills.
- Rotate herbicides with different modes of action.

In Roundup Ready Cotton an alternative method of weed control must be used to stop the seed set of weeds that have been exposed to Roundup Ready® herbicide.

1. Training and Accreditation

The following stakeholders must complete and pass the Roundup Ready Cotton Accreditation Programme before they can purchase seed:

i) The Technology Service Provider (TSP), at least one employee from a TSP outlet must have passed the accreditation course.

ii) The grower or person responsible for making the weed management decision in Roundup Ready Cotton on farm.

The accreditation course includes training days and a Technical Manual which covers all aspects of managing Roundup Ready Cotton. A copy of the Technical Manual is available on request.

All accredited persons will receive updates and amendments to the Technical Manual as they become available. Accredited persons will be required to take a refresher course after a number of years.

It should be noted that agronomists have a duty of care to ensure that all recommendations made are in accordance with the CMP, Technical Manual, Roundup Ready herbicide label and seed label.

2. Communication

The CMP will form part of the Technology User Agreement (TUA). Details of the CMP will be included as part of the accreditation course.

3. Compliance, Auditing and Enforcement

3.1 Technology Service Providers

Only accredited TSPs will be able to sell Roundup Ready Cotton.

3.2 Growers

Monsanto will reward growers who comply with the CMP by offering a rebate on the cost of the Roundup Ready Cotton system. Growers will be rewarded when they comply with all of the following:

1. Adhere to all requirements of the CMP
2. Adhere to all requirements of the TUA
3. Use only glyphosate that is registered for use in Roundup Ready Cotton.
4. Provide Monsanto with required information and documentation from the second audit.

3.3 Auditing

Two audits are conducted during the cotton season.

3.4 Audit 1

Completed by the TSP by the date as set down in the TUA. Information required includes:

- Number of hectares sown
- Location of Roundup Ready Cotton
- Date of sowing
- Planned Weed Management Strategy
- Details about the first Roundup Ready Herbicide in crop application.

3.5 Audit 2

Completed by the grower and provided to Monsanto by the date as set down in the TUA in order to claim the rebate. Information required includes:

- Assessment of labelled weed incidence on 3 x 100 m rows for each 40 ha of Roundup Ready Cotton prior to seed set of escape weeds
- The effective remedial action taken to stop seed set
- Details of the weed management programme during the season (herbicides, rates of application, number of applications)
- Comments about the level of weed control achieved in Roundup Ready Cotton
- Adverse event reporting

4. Adverse event reporting

Growers are required to report any adverse events, such as suspected weed resistance, to Monsanto as soon as it is identified.

Monsanto will investigate the incident and produce a report if weed resistance is confirmed.

5. Non compliance with CMP

Growers who do not comply with the requirements of this CMP may put at jeopardy the benefits of the technology. Consequently, Monsanto may deny these growers access to the technology in the future and they may refer these growers details to the regulatory agencies.

6. Who to contact for assistance

6.1 Monsanto Australia Ltd

12/600 St Kilda Road
Melbourne Vic 3004
Tel: 03 9522 7122
Fax: 03 9525 2253

6.2 Technology Service Provider

Accredited distributor who sold Roundup Ready Cotton. Details to be kept by grower on purchase of the seed.

6.3 Cotton Planting Seed company

Company who provided the seed. Details to be kept by grower on purchase of the seed. Crop tolerance and gene purity data to be provided to Monsanto before any variety can be released commercially. (Monsanto Quality Assurance Programme).

Appendix 2: Protocol for preventing GM contamination in sweet corn seed imports

Drafted by ERMA New Zealand, MFAT and MAF

Used with permission from Dave Nendick, MAF

Introduction and Summary

1. This protocol aims to prevent unapproved genetically modified (GM) seeds from being grown in New Zealand through contamination of non-GM sweet corn seed imported for planting.
2. GM organisms, including viable seeds, are new organisms under the Hazardous Substances and New Organisms (HSNO) Act 1996. The purpose of this act is to protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of hazardous substances and new organisms. The act prohibits the importation, field-testing or release of any new organism without an approval from the Environmental Risk Management Authority (ERMA). The HSNO Act is enforced at the border through Section 28 of the Biosecurity Act 1993 (as amended by Schedule 4 of the HSNO Act):
   "An inspector shall not give biosecurity clearance for goods that are or contain … a new organism…"
3. To date (April 2001), no GM organisms have been approved for release in New Zealand.
4. This protocol applies only to sweet corn seeds, although it may be applied to other seeds if there is good reason to suspect that GM material is present in a consignment (the government is considering general measures for other seeds). It does not apply to seeds imported for use in food, but only to seeds imported for planting. Importers may voluntarily agree to apply these measures to other types of seeds imported for planting.
5. Importers will have the option of providing written assurances that imported seeds are non-GM and have been produced and handled in a way that prevents contamination with GM seeds. MAF will require some consignments to be tested for audit, with the frequency depending on MAF's confidence in the seed production (quality assurance) system. These tests can be performed when the consignment arrives at the border, or by sending a sample of seeds to MAF before shipment, or at an overseas laboratory that has been accredited by MAF. If tests show that GM material is present, then the consignment will not be given biosecurity clearance and every subsequent consignment must be tested until MAF is satisfied that consignments can return to an assurance and audit based regime.
6. Alternatively, if sufficient assurances are not provided, every consignment of imported seeds must be tested before receiving biosecurity clearance. These tests can be performed when the consignment arrives at the border, or by sending a sample of seeds to MAF before shipment, or at an overseas laboratory that has been accredited by MAF. If tests show that GM material is present, then the consignment will not be

given biosecurity clearance. The importer has the option of applying to ERMA for approval to release the GM seeds[1]

7. For small quantities of seeds for experimental purposes, importers will be required to provide written assurances that the seeds are non-GM and have been produced and handled in a way that prevents contamination with GM seeds. The seeds will be directed to a transitional facility isolated from other corn crops. The importer will have the option of either testing a sample of the imported seeds, or testing the plants during growth.
8. The test outlined in this protocol has a 99% chance of detecting one GM seed in 200 "non-GM" seeds. Lower levels of contamination may be detected, but the chances of detection are less than 99%.
9. All costs associated with sampling and testing will be borne by the importer and all associated MAF activities will be charged on a user pays basis.

Import permits requirements

10. Individual sweet corn shipments may only enter with single issue import permits (primarily for tracking purposes). The import health standard (IHS) for Zea mays, Commodity class: Seed for Sowing will specify both the phytosanitary requirements for entry and the need for a permit to cover each individual consignment imported into New Zealand.

Options for sweet corn seed imports

11. Importers of sweet corn seeds will have the following three options:

Option 1: Assurances and audit testing

- Importer must provide written assurances (based on comprehensive testing) that imported seeds are non-GM and meet international standards (none have been developed at this stage) or MAF accredited standards for segregation (i.e. production and handling that prevents contamination with GM seeds).
- MAF will require some consignments to be audited for GM content, with the frequency depending on MAF's confidence in the quality assurance system.
- Testing will be conducted across random selections from each consignment. If tests confirm GM contamination is found then the particular consignment will not be given biosecurity clearance. Every subsequent consignment from that particular exporter/producer (where contamination was found) must be tested for GM content until MAF is satisfied that consignments can return to an assurance and audit based regime.

Option 2: Insufficient assurances

- A sample from each consignment will be tested. Tests can be undertaken by either New Zealand laboratories on arrival of the consignment, or a sample sent pre-shipment or pre-shipment in the supply country by a MAF accredited laboratory.

Option 3: Experimental seeds

- The consignment of seeds must weigh less than [to be announced].

[1] Currently there is a voluntary moratorium on applications to release GM organisms in New Zealand. The moratorium is scheduled to expire on 31 August 2001.

- The importer must provide written assurances that the imported seeds are non-GM and have met the international standards or the New Zealand MAF standards for segregation (i.e. production and handling that prevents contamination with GM seeds).
- Seeds will be directed to a transitional facility (level 1 quarantine facility).
- The seeds will be grown in plots isolated (by at least 500m) from other Zea mays crops.
- Importers shall choose one of the following testing options:
  - a sample of seeds tested from many randomly selected small packets; or
  - a random selection of whole packets of seeds; or
  - during growth, leaf disc samples tested by MAF.
  - · When the seed trial is complete and testing confirms seeds/plants are non-GM then:
  - the seed lines will receive biosecurity clearance and importers are free to dispose of the plants or seeds as they see fit;
- If leaf disc testing confirms that the seeds/plants are contaminated with GM material then:
  - all plots must be mowed and ploughed under; and
  - any eventual emerging volunteer plants must be sprayed with herbicide and ploughed under.
  - The requirements and testing for experimental seeds will be monitored by MAF or a MAF accredited service provider.

Sampling

12. The sampling procedure is designed to collect a sample that is representative of the consignment as a whole. Several assumptions have been made including:
    - Individual seeds are either GM or not GM.
    - Any GM contamination will be randomly distributed throughout the consignment.
    - The sample will be ground up and analysed as a whole, seeds will not be analysed individually.

    Sampling can be undertaken either on arrival of the consignment at the New Zealand border or pre-shipment in the supply country.

13. A sample size of 1377 seeds provides a 99% chance that contamination of 1 seed in 200 will be detected (see attached spreadsheet). A sample (weight basis) drawn from a consignment for testing must contain at least 1377 seeds. The weight of the sample size can be calculated by multiplying the standard 20 seed weight by 70 (= 1400 seeds) and rounding up to the nearest 20 grams. The weight of any seed dressing must be included if the standard 20 seed weight does not include the seed dressing.
14. The sample will be collected using standard International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology.
    - Consignments up to 100kg:

Number of bags or containers per consignment	1-4	5-8	9-15	16-30	31-59	more
Number of sub-samples	3 from each bag or container	2 from each bag or container	1 from each bag or container	15 total each bag or container	20 total each bag or container	30 total each bag or container

- Consignments exceeding100kg:

Weight of line	100-500 kg	501-3,000 kg	3001-20,000 kg	20,001 kg & above
Number of sub-samples	5	1 per 300kg but not less than 5	1 per 500kg but not less than 10	1 per 700kg but not less than 40

Combine the sub-samples evenly to form one uniform collection then reduce it to get a sample of approximately but not less than 1377 seeds.

15. The sample will be collected under controlled conditions by MAF staff in a MAF accredited transitional facility (at the border or industry premises). The sample will be held under MAF supervision until it can be sent to an accredited testing facility. The rest of the consignment will be held in a MAF accredited transitional facility until testing is completed.
16. Records of sampling will be kept by MAF staff and forwarded to the National Adviser, Plant Imports (Seeds and Nursery stock) for reference. Copies of the records will also be provided to ERMA for information.

Testing (subject to further details and amendments)

17. The test procedure must be able to provide a greater than 99% probability of detecting one GM seed from a sample of 200 seeds.
18. Polymerase Chain Reaction (PCR): The tests will be based on the polymerase chain reaction (PCR). PCR procedures are specific for the combination of equipment and reagents that are being used. PCR reaction mixtures should be adjusted for about 10 – 20 nanograms of DNA. Conditions for thermocycling equipment should be based upon manufacturers recommendations and optimised for a strong signal on a 0.5% or 1% standard for each target sequence. In general this will involve up to 40 cycles for each reaction. Analysis of the PCR can be either by gel electrophoresis of the fragments produced by the reaction, or by real time measurement techniques, such as the Taqman system (Perkin Elmer Corporation).
19. Selectivity of method: Genetic sequences to be tested should be determined in advance. Target sequences largely fall into two categories; generic type sequences that are present in most GM crop plants, and construct-specific sequences.
20. First level testing (i.e., screening for presence or absence of genetic modification) will involve one or more generic type sequences, such as the cauliflower mosaic virus 35S promoter, and the nos 3' terminator from the soil bacterium *Agrobacterium tumefaciens*. These sequences are used in most of the GM crops currently grown commercially. The actual selection will be based upon knowledge of the likely range of genetically modified contaminants. The nos sequence is derived from the soil bacterium *Agrobacterium tumefaciens*. The test may give a false positive result if the bacterium is present as a contaminant in the sample. Similarly, the 35S sequence is obtained from the cauliflower mosaic virus that may be present in cruciferous plants (while cruciferous seeds are unlikely to be present in sweet corn samples, care must be taken to actively exclude them otherwise there is another risk of false positive results).
21. Second level testing for specific constructs would only occur if the precise identification of a GMO contamination or if confirmation of the presence of GM material were required. This could include testing for genes that express Bacillus thuringiensis ("Bt") proteins (cry gene sequences), or confer tolerance to the herbicides glyphosate and glufosinate, or the barnase sequences for male sterility. Testing for the protein products of these genes could also be performed using ELISA (enzyme-linked

immunosorbent assay). However, from the biosecurity perspective the nature of the GM crop is irrelevant since to date no GM organisms have been approved for release in New Zealand.

22. Since the nos sequence comes from a common soil bacterium, a test may give a false positive result if this organism (or close relatives) is present in the sample. Soil or extraneous plant tissue should, therefore, not be included in a sample. Similarly, the 35S sequence is obtained from the cauliflower mosaic virus, which may be present in cruciferous plants (e.g., brassicas). While these plants are unlikely to be present in sweet corn samples, care must be taken to actively exclude them otherwise there is a risk of false positive results.
23. Sample extraction and purification: There are several methods for extracting and purifying DNA from a sample. All methods should have been optimised in the laboratory, and evidence provided that the extracted DNA is of PCR quality. Laboratory manuals should contain the detailed steps for extracting, purifying and checking.
24. Sensitivity: Test results should include evidence of the sensitivity and selectivity of the analysis method. Results should also include the confidence of detecting low levels of GM contamination, determined in the laboratory by analysing a series of replicate standard samples, and tested over time to provide a time variation. The range of GM contamination in these samples should vary between 1 seed in 200 (0.5%) and 1 seed in 100 (1%), with a variety of sources on contaminants.
25. Quality assurance procedures: These procedures should include the following quality assurance samples:
    - reagent blanks,
    - sample replicates,
    - sample preparation (pre- and post- grinding) controls,
    - positive PCR controls for sample DNA extraction quality
    - positive controls of DNA from certified standards of known contamination level,
    - negative controls (DNA from Zea mays known to be non-modified), and for positive results verification of the expected size of the target sequences by gel electrophoresis procedures
26. Reporting: Test results should clearly indicate how the testing was performed. The analyst should record either the weight of the sample or the number of seeds analysed. Details of any seed cleaning procedures to remove seed dressings that may interfere with the PCR method and any soil particles need to be recorded.

Annex A

Justification for Sampling Schemes

p1	**0.99**	probability of finding a contaminated sample unit (confidence)
p2	**0.991**	probability that the lab gives a positive result for a contaminated sample at the targ
p3	**0.005**	maximum allowable proportion of units that can be contaminated (tolerance)
n	**1377**	**number of units to have analysed**

Equation: $p1 = p2 [1 - (1-p3)^n]$ to solve for n: $n = \log(1-p1/p2) / \log(1-p3$

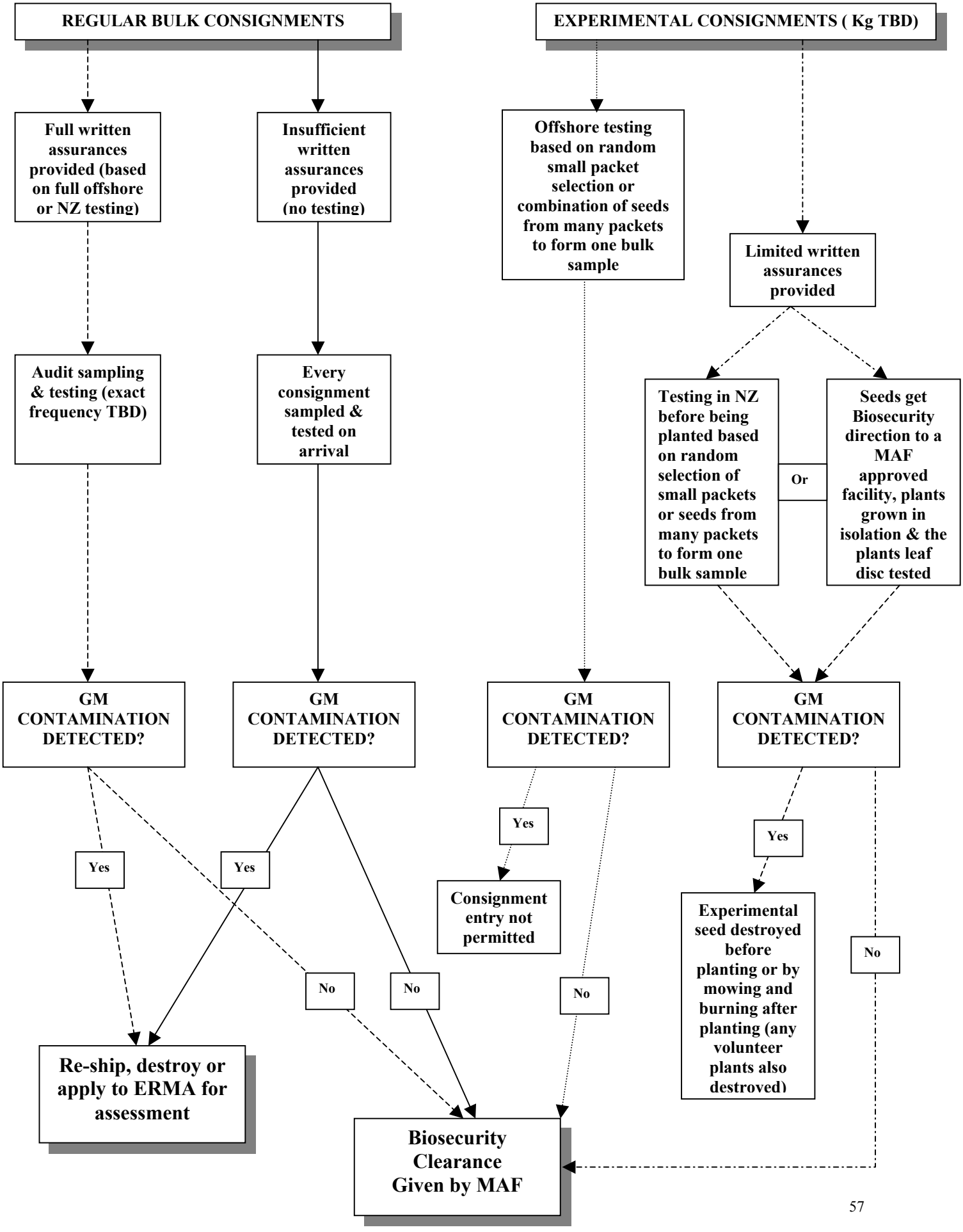
These equations should be used in cases where all the sampled units will be tested in a single test
The confidence in the result (p1) must be less than the confidence in the lab test (p2)

If all the sample units are tested individually the following equations should be used instead:

Equation: $p1 = [1 - (1-p2p3)^n]$ to solve for n: $n = \log(1-p1)/\log(1-p2p3)$

In this case, you can have a greater degree of confidence overall than the confidence in the individual test, as each unit is teste
Crop Surveys, for example.

SEED CONSIGNMENTS WITH THE POSSIBILITY OF GM CONTAMINATION



Appendix 3: EPA assessment of glyphosate tolerant canola

Response to Monsanto Petition 98-216-01p for Determination of Nonregulated Status for Glyphosate-Tolerant Canola Line RT73

Environmental Assessment and Finding of No Significant Impact

January 1999

Finding of No Significant Impact

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment prior to issuing a determination in response to a petition (APHIS Number 98-216-01p) received from Monsanto Company regarding the status of glyphosate-tolerant canola line RT73 under APHIS regulations at 7 CFR Part 340. Canola line RT73 has been engineered to express a CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene and a modified glyphosate oxidoreductase (goxv247) gene. The CP4 EPSPS gene encodes a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme and the goxv247 gene encodes a glyphosate oxidoreductase (GOXv247) protein.

These two proteins confer tolerance to the herbicide glyphosate in transgenic canola. Based upon the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that glyphosate-tolerant canola line RT73 and its progeny shall no longer be regulated articles.

S))Q

Rebecca A. Bech, Assistant Director

Scientific Services

Biotechnology and Biological Analysis

Plant Protection and Quarantine

Animal and Plant Health Inspection Service

U.S. Department of Agriculture

Date:

TABLE OF CONTENTS

I. SUMMARY 1

II. INTRODUCTION 2

III. PURPOSE AND NEED 3

IV. ALTERNATIVES 4

V. POTENTIAL ENVIRONMENTAL IMPACTS 4

VI. CONCLUSIONS 8

VII. LITERATURE CITED 9

VIII. PREPARERS AND REVIEWERS 11

IX. AGENCY CONTACT 11

APPENDICES

Appendix A: Determination of Nonregulated Status for Glyphosate-tolerant Canola

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 98-216-01p) from Monsanto Company (Monsanto) regarding glyphosate-tolerant canola line RT73 (canola line RT73). Monsanto seeks a determination that canola line RT73 does not present a plant pest risk and should therefore no longer be a regulated article under regulations at 7 CFR

Part 340.

Canola line RT73 has been engineered to express a CP4 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4 and a modified glyphosate oxidoreductase (goxv247) gene from Ochrobactrum anthropi LBAA. The gene EPSPS encodes a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme and goxv247 produces a glyphosate oxidoreductase (GOXv247) protein. The genes were introduced into canola via a *Agrobacterium*- mediated transformation protocol. The presence of these proteins in canola line RT73 confers tolerance to the herbicide glyphosate.

Field trials of Line RT73 have been conducted under permits and notification acknowledged by APHIS according to regulations at 7 CFR Part 340. Performance standards and conditions for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), an Environmental Assessment (EA) was prepared prior to granting permits for field trials of glyphosate-tolerant canola. The EA for the previous introductions of glyphosate-tolerant canola addressed plant pest risk issues relative to the conduct of field trials under physical and reproductive confinement. This EA specifically addresses the potential for impacts to the human environment through use in agriculture of glyphosate-tolerant canola. Similarly, notifications were acknowledged based on the scientific review and the applicant's certification. The consultation process with the Food and Drug Administration (FDA) was completed in September, 1994.

Monsanto submitted a package to EPA in April 1998 for registration of glyphosate for over-the-top application on transgenic canola.

APHIS has considered the information provided by Monsanto in its petition as well as other scientific data relating to the potential plant pest risk of glyphosate-tolerant canola. A thorough evaluation of the potential for significant impact to the human environment through the unconfined, agricultural use of glyphosate-tolerant canola has brought APHIS to a Finding of No Significant Impact (FONSI). This conclusion is based upon:

1. Neither the genes that result in accumulation of CP4 EPSPS and GOXv247, nor the CP4 EPSPS and GOXv247 proteins, nor their associated regulatory sequences, confer on glyphosate-tolerant canola or its progeny any plant pest characteristic.

2. In nature, the gene that results in accumulation of CP4 EPSPS and GOXv247 proteins will not provide glyphosate-tolerant canola or its progeny with any measurable selective advantage over nontransformed canola plants in their ability to disseminate or to become established in the environment. There is no reason to believe that glyphosate- tolerant canola exhibits any increased weediness relative to that of traditional varieties.
3. The use of glyphosate-tolerant canola or its progeny in agriculture will not lead to an increase in weediness in any plant with which it can successfully interbreed.
4. The use of glyphosate-tolerant canola or its progeny in agriculture will not cause damage to raw or processed agricultural commodities.
5. The use of glyphosate-tolerant canola or its progeny in agriculture will not have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

In conjunction with the FONSI, APHIS has made the determination that canola line RT73 and its progeny have no potential to pose a plant pest risk, and are, therefore, no longer regulated articles under regulations at 7CFR part 340.

II. INTRODUCTION

This EA examines potential environmental impacts from the unrestricted introduction of glyphosate-tolerant canola.

Glyphosate-tolerant canola has been extensively field tested in Canada, Europe, and the United States. Monsanto has submitted field data reports for the U.S. release permits and notifications granted by APHIS. Monsanto has also submitted data from the Canadian trials. These reports give information on the biological and agronomic characteristics of the plant and the toxicant and compositional analysis of seeds and seed oil. All these traits fall well within the range of commercial varieties of canola. The only significant consistent difference between glyphosate- tolerant canola and the parental nontransformed variety is the increase in the CP4 EPSPS enzyme and GOXv247 protein that confer tolerance to glyphosate.

Testing in the U. S. has been conducted under USDA permits and notifications since 1995 (APHIS authorization numbers: 95-279-01r, 96-045-01r, 96-061-02r, 96-211-01r, 96-274-01r, 97-022-01r, 97-024-01r, 97-254-02n, 97-254-04n, 97-324-06n, and 97- 309-03n). Field trial reports from these tests demonstrate no deleterious effects on plants, nontarget organisms, or the environment. Field trials in the United States were performed under conditions of physical and reproductive confinement. Further discussions of the biology of canola as well as of the genetic components of glyphosate- tolerant canola are found in the APHIS Determination of Nonregulated Status (Appendix A.).

Prior to issuing a permit or notification for a field release, APHIS analyzes the potential impacts associated with the proposed introduction in accordance with regulations and procedures implementing the National Environmental Policy Act (NEPA), as amended (42 U.S.C. 4321 et seq.); 40 CFR Parts 1500-1508; 7 CFR Part 1b; 7 CFR Part 372. APHIS also evaluates the potential for significant impact to the human environment from its determination of non-regulated status.

A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of

the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. The transgenic canola plants described in the Monsanto petition have been considered regulated articles because they contain DNA sequences derived from the plant pathogens figwort mosaic virus and *Agrobacterium* sp. CP4 and because the plant pathogen *Agrobacterium tumefaciens* was used as a vector agent.

III. PURPOSE AND NEED

The purpose of this EA is to ascertain whether the approval of a petition submitted to USDA/APHIS for the determination of non-regulated status of glyphosate-tolerant canola, which will allow the unconfined introduction of the article, will have a significant impact on the environment. A petition was submitted to APHIS pursuant to regulations codified in 7 CFR Part 340 entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question. Permits and notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of that article or its progeny. Normal agronomic practices with it, e.g., cultivation, propagation, movement, and cross-breeding could then be conducted without APHIS approval.

The FDA has authority to ensure the safety and wholesomeness of all food(s). The FDA policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from glyphosate-tolerant canola lines is under the jurisdiction of the FDA. FDA has granted a finding of 'No Concern' for canola line RT73 in September, 1994, (please see the FDA Home Page listed as below):

(http://www.cfsan.fda.gov/~lrd/biocon.html).

The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended, (7 U.S.C. 136 et seq.). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 et seq.), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA. A tolerance exemption for CP4 EPSPS was received on August 2, 1996 and for GOX on October 8, 1997 from the EPA (please see the EPA Federal Register notices):

(http://www.epa.gov/docs/fedrgstr/EPA-PEST/1996/August/Day-02/pr-840DIR/pr-840.html),

(http://www.epa.gov/fedrgstr/EPA-PEST/1997/October/Day-08/p26190.htm) for respective proteins.

Monsanto submitted a package to EPA in April 1998 for registration for use of glyphosate for the over-the-top application on transgenic canola.

IV. ALTERNATIVES

In the course of preparing the environmental assessment for this petition, APHIS considered the following two alternatives: (1) deny the petition, so that glyphosate- tolerant canola would continue to be regulated under 7 CFR Part 340; and (2) approve the petition, so that permits would no longer be required from APHIS under 7 CFR Part 340 for glyphosate-tolerant canola when grown in the United States and its territories. Based on the biology of canola, the nature of the genetic change, data and information presented by Monsanto, and scientific literature, APHIS could not find any basis for denying the petition (Alternative 1).

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of glyphosate-tolerant canola in the absence of confinement.

Potential impacts based on increased weediness of glyphosate-tolerant canola relative to traditionally bred canola

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje *et al*. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

Despite its ability to volunteer, escape from cultivated fields, and form temporary occasional populations, the parent plant in this petition, Brassica napus, is not a weed under conditions found in the United States. B. napus is listed as a weed in Weed Science Society of America (1992). The comprehensive world list of Holm *et al*. (1991) does not list it as a serious or principal weed anywhere in the world; they do, however, give two listings as a common weed: one in Finland and one in Kenya. B. napus is mentioned as an "occasional weed" by Munz (1968), and "sometimes escaped" by Bailey (1949). Monsanto has submitted substantial evidence to indicate the lack of weedy nature of transformed canolas under agricultural conditions. They have submitted data or information on germination, seed production, pest and disease resistance, response to abiotic factors (such as drought, heat, and frost), on salinity, seed dormancy, and sensitivity to herbicides other than glyphosate, and other fitness characteristics. None of these characteristics indicate an increase in weediness potential for canola line RT73.

The relevant introduced trait, glyphosate tolerance, is unlikely to increase weediness of this canola unless glyphosate is the only alternative for control of the plant. Such an alteration, because it does not confer any pest resistance or alter reproductive biology or change any physiology related to survival, does not confer a competitive advantage favoring the canola plants over unmodified varieties. To increase weediness of the canola plant there would have to be selection pressure on glyphosate-tolerant canola (Tiedje *et al*., 1989; Office of Technology Assessment, 1988). Monsanto data from field trials show no obvious increase in volunteers from seed, increase in seed dormancy, or other variation indicative of increased weediness. Moreover, Monsanto presents evidence that glyphosate-tolerant canola is as readily controlled with non-glyphosate herbicides as the nontransformed canola.

Potential impacts from outcrossing of glyphosate-tolerant canola to wild relatives Whereas intra-specific crosses between B. napus cultivars occur readily, inter-specific crosses between B. napus and related species occur with varying degrees of success and are influenced greatly by the direction of the cross. Even where there is a possibility of hybridization between B. napus and a related species growing in the vicinity of a release, poor vigor and high sterility in the hybrids will generally mean that hybrids and their progeny will not survive in either an agricultural or natural habitat (Scheffler and Dale, 1994).

The potential of a gene movement, at very low level, from B. napus to other Brassica spp. such as B. juncea or B. rapa, will be subject to the availability of the target organism and the reduced fertility of the hybrids. B. napus can cross with B. rapa (under co-cultivation 1.3% hybrid seed was formed) and produce hybrids of much reduced fertility; B. napus can also cross at low frequency with B. juncea (under field co-cultivation 4.7% hybrid seed formed) and these hybrids can produce a small amount of seed and fertile progeny (Bing, 1991). The gene that codes for glyphosate tolerance should not confer a competitive advantage in these species unless glyphosate is used for control.

Gene movement is also possible to other members of the Brassicaceae, e.g. Herschfeldia incana (Brassica adpressa), and Raphanus raphanistrum. Gene movement is at extremely low levels, and as with members of the genus Brassica, it is unlikely that the gene that codes for glyphosate tolerance would confer a competitive advantage in these species unless glyphosate is used for control.

Potential impact on nontarget organisms, including beneficial organisms such as bees and earthworms, and endangered or threatened species

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms and endangered or threatened species, would result from the cultivation of glyphosate-tolerant canola. The CP4 EPSPS enzyme and GOXv247 protein encoded by EPSPS and goxv247 genes respectively confer tolerance to the herbicide glyphosate in canola line RT73. Both proteins and the genes are not known to have any toxic properties.

Consideration of potential environmental impacts associated with the cultivation of glyphosate-tolerant canola outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with the potential approval of this glyphosate-tolerant canola in the United States.

Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of these canola lines or their progeny.

Any international traffic in the canolas subject to this determination would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (105 countries as of October, 1996). The treaty, now administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into force on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS has played a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within the RPPO for our region, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection.

APHIS participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. In addition, APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner, including those in regions where canola originated or is cultivated in significant quantities (e.g., China, Japan, Korea, Association of South East Asian Nations member States, India, Pakistan, African States, and more). We have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology, and sponsored several workshops on safeguards for planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues.

In the course of these wide-ranging studies and interactions, APHIS has not identified any significant impacts on the environment that might be relevant to glyphosate-tolerant canola or follow from the unconfined cultivation of canola line RT73 in the United States and its territories, or abroad which could not be mitigated by reasonable agricultural practices. In addition to the assurance provided by the analysis leading APHIS to a finding of no significant impact for the introduction of this canola, it should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new canola cultivars internationally apply equally to those covered by this determination.

Potential impacts on biodiversity

Our analysis determined that genetically engineered glyphosate-tolerant canola line RT73 is no more likely to become weed than any line developed by traditional breeding techniques, is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which this line can interbreed, and will not harm threatened and endangered species and non-target organisms. Based on this analysis, APHIS concludes that there is no potential impact of this line on biodiversity.

Potential impacts on agricultural and cultivation practices.

Based on the APHIS analysis, there is unlikely to be any significant adverse impact on agricultural practices associated with the use of these lines. However, it is of concern that there is a likelihood of canola volunteers possessing a combination of two different herbicides resistance genes and how such volunteers would be managed by growers. It is known that glyphosate is not employed to any significant degree for the control of canola volunteers. This glyphosate-tolerant line has been in commercial production in Canada since 1996 and the Canadian Government has suggested the need for sound crop management practices for volunteer management control and potential outcrossing concerns in its Document DD95-02 (March 1995). Monsanto has provided information regarding the use of alternative herbicides which could be used to control Brassica volunteers or weed should they obtain, through crossing, resistance to glyphosate and/or other herbicides with different modes of action.

Potential damage to processed agricultural commodities.

An analysis of the components and processing characteristics of these lines reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

VI. CONCLUSIONS

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of a proposed action, i.e, reaching the determination that glyphosate-tolerant canola has no potential to pose a plant pest risk and should no longer be considered a regulated article under the regulations at 7 CFR Part 340. After careful analysis of the available information, APHIS concludes that its proposed action will not have a significant impact on the environment, and that the proper alternative is to approve the petition. This conclusion is based on factors discussed herein or in the determination included as Appendix A, as well as the following conclusions:

1. Neither the genes that result in accumulation of CP4 EPSPS and GOXv247, nor the CP4 EPSPS and GOXv247 proteins, nor their associated regulatory sequences, confer on glyphosate-tolerant canola or its progeny any plant pest characteristic.
2. In nature, the gene that results in accumulation of CP4 EPSPS and GOXv247 proteins will not provide glyphosate-tolerant canola or its progeny with any measurable selective advantage over nontransformed canola plants in their ability to disseminate or to become established in the environment. There is no reason to believe that glyphosate- tolerant canola exhibits any increased weediness relative to that of traditional varieties.
3. The use of glyphosate-tolerant canola or its progeny in agriculture will not lead to an increase in weediness in any plant with which it can successfully interbreed.

4. The use of glyphosate-tolerant canola or its progeny in agriculture will not cause damage to raw or processed agricultural commodities.
5. The use of glyphosate-tolerant canola or its progeny in agriculture will not have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

VII. LITERATURE CITED

Agriculture and Agri-Food Canada. 1995, Determination of environmental safety of Monsanto Canada Inc.'s Roundup Herbicide-Tolerant Brassica napus canola line GT73. DD95-02. Plant Products Division. March 24, 1995.

Baker, H. G. 1965. Characteristics and Modes of Origin of Weeds. In: The Genetics of Colonizing Species. pp. 147-172.

Baker, H. G., Stebbins, G. L. (eds.). Academic Press, New York and London.

Bailey, L. H. 1949. Manual of Cultivated Plants. Macmillan Publishing Company, New York. 1116 pp.

Bing, D. J. 1991. Potential of Gene Transfer Among Oilseed Brassica and their Weedy Relatives. Master of Science Thesis,University of Saskatchewan. 155 pp.

de Wet, J. M. J., Harlan, J. R. 1975. Weeds and Domesticates: Evolution in the Man- Made Habitat. Economic Botany. 29:99-107.

Holm, L., Pancho, J. V., Herbarger, J. P., Plucknett, D. L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Keeler, K. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, Ithaca and London. 586 pp.

Munz, P. A. 1968. A California Flora. University of California Press, Berkeley and Los Angeles. 1681 pp.

Office of Technology Assessment, United States Congress. 1988. New Developments in Biotechnology-- 3. Field-Testing Engineered Organisms: Genetic and Ecological Issues. U.S. Government Printing Office, Washington, DC. 150 pp.

Scheffler, J. A. and Dale P. J. 1994. Opportunities for gene transfer from transgenic oilseed rape (Brassica napus) to related species. Transgenic Research 3:263-278.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., Regal, P. J. 1989. The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations. Ecology 70:298-315.

Weed Science Society of America. 1992. Crop losses due to weeds in the United States. Weed Science Society of America, Champaign, Illinois.

VIII. PREPARERS AND REVIEWERS

Scientific Services

Rebecca A. Bech, Assistant Director

Arnold Foudin, Ph.D., Assistant Director

Biotechnology and Biological Analysis

Subhash Gupta, Ph.D., Biotechnologist (Preparer)

David S. Heron, Ph.D., Biotechnologist

Karen E. Hokanson, Ph.D., Biotechnologist

Susan M. Koehler, Ph.D., Biotechnologist (Reviewer)

James Lackey, Ph.D., Biological Safety Officer

Craig R. Roseland, Ph.D., Biotechnologist

Sivramiah Shantharam, Ph.D., Senior Operations Manager

John Turner, Ph.D., Biotechnologist

James L. White, Ph.D., Senior Operations Manager

Safeguarding and Pest Management

Michael Lidsky, J.D., Assistant Director

Shirley P. Ingebritsen, M.A., Regulatory Analyst (Reviewer)

IX. AGENCY CONTACT

Ms. Kay Peterson, Regulatory Analyst

Scientific Services

USDA, APHIS, PPQ

4700 River Road, Unit 147

Riverdale, MD 20737-1237

kay.peterson@usda.gov

Phone: (301) 734-4885

Fax: (301) 734-8669