



**Australian Government**  
**Department of Health and Ageing**  
**Office of the Gene Technology Regulator**

# **Risk Assessment and Risk Management Plan**

**Application for licence for dealings involving an  
intentional release into the environment**

**DIR 047/2003**

**Title: Field Evaluation of Genetically Modified White  
Clover Resistant to Infection by Alfalfa Mosaic Virus**

**Applicant: Department of Primary Industries (Victoria)**

**July 2004**

## Abbreviations

AMV	Alfalfa mosaic virus
AMV CP	Alfalfa mosaic virus coat protein
AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
CaMV	Cauliflower mosaic virus
CP	coat protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
DPI	Department of Primary Industries
EMBL	European Molecular Biology Laboratory
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
IgE	immunoglobulin E
kD	kilodalton
km	kilometre
m	metre
mRNA	messenger ribonucleic acid
NOS	nopaline synthase
NPTII	neomycin phosphotransferase type II
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PCR	polymerase chain reaction
RARMP	risk assessment and risk management plan
RNA	ribonucleic acid
T-DNA	transfer deoxyribonucleic acid
US EPA	United States Environmental Protection Agency
UTR	untranslated region
US FDA	United States Food and Drug Administration
WHO	World Health Organisation

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## EXECUTIVE SUMMARY

### INTRODUCTION

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety and the environment that can not be managed. As part of the evaluation process, section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. Matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator's decision on whether, or not, to issue a licence.

The Act is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication. The OGTR liaises closely with other regulators to ensure the identification, evaluation and management of risks that may be associated with development and use of gene technology.

The Regulator has made a decision to issue a licence in respect of application DIR 047/2003 from the Department of Primary Industries (DPI) (Victoria).

### THE APPLICATION

The DPI (Victoria) has applied for a licence (application number DIR 047/2003) for the intentional release of genetically modified (GM) virus resistant white clover into the environment, on a limited scale and under controlled conditions. DPI (Victoria) proposes to conduct the field trial of GM white clover on one site in Victoria over four planting seasons between May 2004 and April 2007 consisting of a maximum area of 494 square metres per planting season. The GM white clover contains the Alfalfa mosaic virus coat protein (*AMV CP*) gene that confers resistance to infection by Alfalfa mosaic virus (AMV) and a selectable marker gene (*nptII*) that confers resistance to the antibiotics kanamycin and neomycin.

The coat protein gene, selectable marker gene and associated regulatory sequences were originally introduced into the white clover cultivar 'Irrigation'. Subsequently, GM plants were conventionally bred with the white clover cultivar 'Mink'. It was in turn bred with the white clover cultivar 'Sustain' to produce the GM white clover proposed for release in the current application.

The main aims of the proposed release are the field evaluation of GM white clover resistant to infection by AMV and the production of GM white clover seed for future trials, subject to further approvals. DPI (Victoria) proposes to evaluate agronomic characteristics and resistance to AMV of the GM white clover over two years and then produce seed from a

selection of GM white clover plants showing superior agronomic performance and AMV resistance.

DPI (Victoria) has proposed a number of containment measures to minimise spread and persistence of the GMO and the introduced genetic materials from the trial site. These include surrounding the site by a livestock-proof fence and a rabbit-proof fence, use of a pollen trap, removal of GM flower heads during peak flowering period, isolating the GM plants from non-GM white clover, use of a footbath and washbasin to clean all implements, destroying GM materials not required for subsequent research, and destroying any volunteer GM white clover that may occur in the release area for five years after completion of the trial. To ensure purity of seed produced from specific crosses, the applicant proposes to enclose the GM plot with a bee cage in the second two planting seasons.

None of the white clover plants from the release, or their by-products, will be used for animal feed. Transport of the GM materials will be in accordance with the transport guidelines issued by the Regulator.

GM AMV resistant white clover has not previously been assessed under the current regulatory system. However, under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been four field trials of GM AMV resistant white clover similar to that proposed for release under the current application. Three of the trials were conducted by La Trobe University (PR-64, PR64X and PR 64X2) and the other was by CSIRO (PR-67). The GM white clovers were assessed for plant growth, expression of the introduced gene and resistance to viral infection. Gene flow was also analysed. The size of the releases ranged from two to four hectares and were carried out in the shire of Southern Grampians, Victoria and the shire of Hume, New South Wales. There have been no reports of adverse effects on human health and safety or the environment resulting from the releases.

## **THE EVALUATION PROCESS**

A RARMP has been prepared in relation to licence application DIR 047/2003 from DPI (Victoria) in accordance with the Act, the Regulations, and the Risk Analysis Framework. This framework was developed as part of the establishment of the regulatory arrangements in consultation with the public, State, Territory and Australian Government agencies, key stakeholders and the Gene Technology Technical Advisory Committee, and is available at [www.ogtr.gov.au/pdf/public/rafinal.pdf](http://www.ogtr.gov.au/pdf/public/rafinal.pdf)

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that she must consider in preparing a RARMP, are set out in Appendix 8 of the RARMP. The complete RARMP can be obtained from the OGTR by contacting the Office on 1800 181 030 or from its web site: [www.ogtr.gov.au](http://www.ogtr.gov.au).

The risk assessment considered information relevant to the evaluation of potential impacts on human health and safety and the environment contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, the proposed dealings and containment measures), submissions received during consultation with expert groups and authorities, and current scientific knowledge.

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of the GM white clover were identified. These have

been evaluated to determine whether risks might arise, based on the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised.

The identified potential hazards relate to:

- **toxicity and allergenicity to humans:** could the GM white clover be more toxic or allergenic than non-GM white clover, as a result of the novel gene products or because of unintended effects?
- **toxicity to other organisms:** could the GM white clover be harmful to other organisms as a result of the novel gene products or because of unintended effects?
- **weediness:** could the genetic modifications be harmful to the environment by increasing the potential for the GM white clover to establish as problem weed than non-GM white clover?
- **transfer of introduced genes to other organisms:** could there be adverse consequences from potential transfer of the introduced genes to non-GM white clover, closely related plants, or to other organisms?
- **interactions between introduced viral gene and viruses:** could interactions between the *AMV CP* gene (or its product) and viruses lead to increased disease burden (caused by, for example, an increase in pathogenicity) in white clover or other plants? and
- **anti-viral resistance:** could new AMV variants arise that overcome AMV coat protein-mediated resistance of the GM white clover?

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complementary regulatory role in respect to this application due to its responsibility for agricultural chemical use in Australia. The AMV CP produced by the GM white clover proposed for release falls under the *Agricultural and Veterinary Chemicals Code Act 1994* (Ag Vet Code Act) definition of an agricultural chemical product, and is thus subject to regulation by the APVMA. Further information about the APVMA's assessment and approval processes is contained in Chapter 1 and Appendix 6 of the RARMP.

For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits for experimental work that allow restricted use of an agricultural chemical, for example, for a limited period of time or for a limited area. The APVMA can impose conditions of use on both registrations and permits, and must be satisfied that the proposed use would not present an undue risk to human health and the environment.

DPI (Victoria) has submitted an application to the APVMA for a research permit for the use of the *AMV CP* gene in GM white clover during the proposed trial. The APVMA and the OGTR have worked closely to ensure thorough, coordinated assessments of parallel applications, and, wherever possible, that the decisions by both agencies coincide.

## CONCLUSIONS OF THE RISK ASSESSMENT

It is concluded that the proposed release of the GM virus resistant white clover does not pose significant risks to human health and safety or to the environment as a result of the genetic modification. The Regulator has imposed stringent licence conditions that differ from those proposed by the applicant to minimise potential exposure of humans and other organisms, and to limit the spread and persistence of the GMO and the introduced genes while more data is

gathered on the behaviour and interactions of the GMO in the Australian environment. The risk assessment of each potential hazard identified above is summarised under a separate heading below.

### **Toxicity or allergenicity to humans**

White clover is a well established pasture legume with a long history of safe use. There are no food uses of white clover in Australia. Therefore, humans will not be exposed to material from the GM white clover in food.

Possible exposure of people to the GM white clover will be through working with GM white clover as a part of conducting the proposed field trial, and/or living near the area where the GM white clover is grown. White clover pollen is not transported easily by wind, thus limiting possible exposure to white clover pollen as a potential airborne allergen. Physical contact with non-GM white clover could trigger an allergic response in some people although there are no reports of any major allergic responses. Allergic reactions to white clover tissue tend to be mild and rare.

The GM white clover is unlikely to be more toxic or allergenic to humans via occupational exposure than non-GM white clover. Humans are commonly exposed to the proteins produced by the introduced genes, as the organisms from which they are derived are naturally widespread in the environment. Hence, the risk that GM white clover is toxic or allergenic to humans is very low. The proposed release is very small and conditions have been imposed to limit the exposure to humans and to minimise the spread of the GMO.

### **Toxicity to other organisms**

Non-GM white clover can be toxic to grazing animals if ingested in large quantities or under particular situations, because of the presence of toxic and anti-nutritional factors. These include saponins, which may contribute towards the occurrence of bloat; phytoestrogens, which can interfere with reproduction; and cyanogenic glycosides (linamarin and lotaustralin), which are implicated in nutritional myopathy.

The introduced proteins in the GM white clover are derived from microorganisms that are naturally widespread in the environment and all organisms are commonly exposed to the proteins. The AMV CP and NPTII protein are not known to be toxic to any organisms including mammals, birds, fish, invertebrates and microorganisms.

The proposed release is very small and conditions have been imposed to limit the movement of the GMO and the introduced genes. The GM white clover from the release will not be used as stock feed and the applicant is required to surround the central GM plot by a rabbit-proof fence and the whole release site by a stock-proof fence to minimise access by grazing animals. The Regulator would require information on the toxicity of the GM white clover expressing the AMV CP from livestock feeding studies before a proposal to feed GM white clover to livestock could be considered.

### **Weediness**

Non-GM white clover possesses some characteristics commonly associated with weediness, has a close taxonomic affinity to other weedy species and is known to be a problematic weed in some countries, including Australia. Limitations on the establishment and persistence of white clover populations is likely to be due to complex interactions involving one or more

diseases (including viral diseases), moisture stress, poor soil fertility, grazing pressure and/or competition.

The GM white clover might have the potential to be a more problematic weed than non-GM white clover, either due to expression of the novel gene products or as a result of unintended effects of the genetic modification. This could occur if the GM white clover displayed altered characteristics such as increased fitness or increased fecundity. The introduced *AMV CP* gene in the GM white clover is most likely to affect fitness where AMV is limiting the persistence of white clover. GM white clover may be weedier than non-GM white clover in these circumstances. This could occur in pastoral situations where it is known that AMV does limit white clover growth. However, it is unknown if AMV is limiting the growth and persistence of white clover in areas such as roadsides, home gardens and natural environments. In areas where there is no or a low incidence of AMV, the GM white clover is unlikely to be a more problematic weed than non-GM white clover.

In relation to the proposed trial, it is concluded that the risk of GM virus resistant white clover establishing as a more problematic environmental weed than non-GM white clover is considered to be low because the field trial is small with a maximum of area of 494 square metres in each of the four planting seasons and the area in which the proposed field trial is to take place is not particularly suitable for growing white clover long-term due to hot summers and lack of moisture. Additionally, a number of measures to limit seed dispersal and dormancy (see above for details) and the GM white clover will not be permitted to be used as stockfeed which further reduces the chance of dispersal. Additionally, research on the agronomic characteristics indicative of potential weediness of the GM white clover under Australian field conditions are required.

If the applicant applies for future larger scale releases of GM virus resistant white clover, more detailed information would be required to be collected on weediness of the GM white clover under Australian field conditions, including invasiveness, enhanced reproductive capacities, and limitation of white clover persistence by AMV outside of pastoral situations, such as roadsides, home gardens and natural environments.

### **Transfer of introduced genes to other organisms**

White clover must cross with other white clover plants in order to sexually reproduce and therefore gene transfer from GM virus resistant white clover to non-GM white clover is highly likely in the absence of containment measures. Pollen transfer is mediated by insect pollinators, in particular honey bees. White clover pollen is not easily dispersed by wind and, even if there is airborne pollen, it will not result in fertilisation as mechanical damage has been shown to be important in stimulating pollen germination. Transfer of the *AMV CP* gene to non-GM white clover could potentially confer a selective advantage in situations where the virus is limiting the spread and persistence of white clover.

However, it is considered that the risks posed by the proposed trial are low because the field trial is small with a maximum area of 494 square metres in each of the four planting seasons and a number of containment measures have been imposed to limit the dispersal of pollen from trial site.

The applicant had proposed to surround the trial site by a pollen trap consisting of non-GM white clover and other legumes, and to isolate the trial site from all other white clover populations to minimise gene flow and persistence in the first two planting seasons. However,

because there is uncertainty about the potential for enhanced weediness of GM white clover and the ability of AMV to limit white clover growth and persistence in the area surrounding the trial site, the Regulator has taken a cautious approach to manage potential enhanced weediness through gene flow to non-GM white clover plants in the proposed field trial.

Therefore, stringent licence conditions have been imposed to minimise the risk through cross-pollination to non-GM white clover plants outside the release site by enclosing the GM plot in a bee-proof cage during the flowering period of the GM white clover and ensuring that the integrity of the cage is maintained (refer to key licence conditions below). Additionally, any bees used in the pollination of the GM white clover are required to be killed at the end of the period to ensure that no GM pollen is transferred outside the bee cage.

The risk through transfer of the introduced genes in GM white clover to other plant species including other clover species is negligible because of genetic incompatibility which means that viable hybrids will not occur.

Natural transfer of genes from plants to other organisms including humans, other animals and bacteria is extremely rare. Even if such transfer occurred it would be unlikely to pose any hazard to human health and safety and the environment.

### **Interactions between the introduced viral gene and viruses**

Potential hazards may be posed through interactions of the introduced *AMV CP* gene and/or its coat protein product with viruses that are naturally present in the plant or in the environment. These interactions may result in the modification of viral properties, which may in turn lead to increased disease burden in white clover and/or other plants. Increased disease burden can result from increased pathogenicity, a change in host range, increased viral spread, higher viral production in cells or plants, or a new means of transmission of the modified virus.

Modified properties of a virus can be short-term and/or localised due to transient changes. The likelihood of increased disease burden arising and persisting long-term due to transient changes involving interactions between the introduced *AMV CP* gene (or its product) and infecting viruses in the GM white clover plants is possible but likely to be negligible. This is because these interactions do not produce permanent changes, and the proposed field trial is small (an area of 494 square metres at any one time) and only for a short period of time (four planting seasons).

Alternatively, modified properties of a virus may be a permanent phenomenon due to genetic changes resulting from recombination. Increased disease burden arising and persisting long-term due to a genetic change between the introduced *AMV CP* gene (or its product) and infecting viruses is possible but likely to be very low.

Interactions between the introduced *AMV CP* gene and/or its coat protein product with viruses are considered in detail in Appendix 5 of the RARMP.

### **Anti-viral resistance**

New AMV variants could arise as a result of the trial that overcome AMV coat protein (CP)-mediated resistance (anti-viral action) of the GM white clover and could potentially cause increased disease burden in white clover plants and other plant species. However, given the

limited scope of the release in both scale and time, the likelihood of this risk resulting from the release is negligible. This issue is discussed in Appendix 6 of the RARMP.

The APVMA will also assess the hazard of AMV variants arising that overcome the anti-viral action of GM white clover plants in considering the research permit application from DPI (Victoria) to use the *AMV CP* gene.

### **THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)**

As part of the evaluation process for this licence application, a risk management plan has been developed to address the risks identified (refer to conclusions of the risk assessment, above). This plan has been given effect by the licence conditions imposed. The key licence conditions are outlined below.

#### **Toxicity or allergenicity to humans**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- prevent GM materials from entering the human food chain;
- destroy all GM materials not required for testing or future trials;
- securely transport and store retained GM materials; and
- report any adverse impacts on human health and safety.

#### **Toxicity to other organisms**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- surround the GM plot with a rabbit-proof fence and stock-proof fence;
- prevent GM materials from being used as stockfeed;
- destroy all GM materials not required for testing or future trials; and
- securely transport and store retained GM materials.

#### **Weediness**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- surround the GM plot with a rabbit- and stock-proof fence;
- prevent GM materials from being used as stockfeed;
- destroy all GM materials not required for testing or future trials;
- securely transport and store retain GM materials;
- clean equipment used at the release site;
- encourage germination of any GM white clover seed bank during post harvest monitoring period; and

- monitor release area during and after trial and destroy volunteers before flowering.

### **Transfer of introduced genes**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- enclose the GM plot by a bee-proof cage and ensure integrity of the cage is maintained during the flowering period of the GM white clover;
- kill any bees within the bee-proof cage once pollination of the GM white clover is complete;
- prevent GM materials from being used as stockfeed;
- destroy all GM materials not required for testing or future trials;
- securely transport and store retained GM materials;
- clean equipment used at the release site; and
- monitor release area during and after trial and destroy volunteers before flowering.

### **Interactions between the introduced viral gene and viruses**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release.

### **Anti-viral resistance**

No conditions have been imposed in relation to anti-viral resistance management, as this risk is considered negligible to human health and safety and the environment. The applicant's obligation to comply with any conditions imposed by the APVMA is noted in the licence.

### **General conditions**

Any licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example:

- identification of the persons or classes of person covered by the licence;
- a requirement that the applicant allows access to the release site by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing; and
- a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

Chapter 2 of the risk assessment and risk management plan provides a tabulated summary of assessment conclusions and corresponding management conditions. Full details of the licence conditions are provided in Appendix 7.

### **Research requirements**

The licence conditions include the requirement that the applicant collect and provide to the Regulator further information regarding:

- expression levels of the proteins of the introduced *AMV CP* and *nptII* genes in different parts of the plants under Australian field conditions;
- agronomic characteristics indicative of potential weediness of the GM white clover under Australian field conditions; and
- examination of the disease status of the GM white clover plants during the trial to determine whether novel viruses emerge.

### **Additional data**

The proposed limited and controlled release is a small scale, single site trial over four planting seasons. If the applicant makes any future application for larger scale releases of GM virus resistant white clover, more detailed information would be required to be collected on:

- molecular characterisation of the introduced genetic materials;
- the levels of the natural toxicants e.g. cyanogenic glucosides, phytoestrogens or saponins in the GM white clover;
- toxicity of the GM white clover expressing the AMV CP, via livestock feeding studies;
- weediness of the GM white clover under Australian field conditions, including invasiveness, enhanced reproductive capacities, and limitation of white clover persistence by AMV outside of pastoral situations, such as roadsides, home gardens and natural environments; and
- gene transfer to non-GM white clover.

### **Monitoring and enforcement of compliance by the OGTR**

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling (which takes into account biological, seasonal, geographical and ecological risk factors), to determine whether licence holders are complying with the licence conditions, or whether there are any unintended effects.

## CHAPTER 1 BACKGROUND

1. This chapter provides background information about the application and previous releases of relevant genetically modified organisms (GMOs) into the environment.

### SECTION 1 THE APPLICATION

2. The OGTR has received an application (licence application number DIR 047/2003) from the Department of Primary Industries (DPI) (Victoria) for the intentional release of genetically modified (GM) virus resistant white clover into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

<b>Project Title:</b>	<b>Field Evaluation of Genetically Modified White Clover Resistant to Infection by Alfalfa Mosaic Virus</b>
<b>Applicant:</b>	Department of Primary Industries (Victoria) PO Box 500 East Melbourne VIC 3002
<b>Common name of the parent organism:</b>	White clover
<b>Scientific name of the parent organism:</b>	<i>Trifolium repens</i> L.
<b>Modified trait(s):</b>	Viral disease resistance, Antibiotic resistance
<b>Identity of the gene(s) responsible for the modified trait(s):</b>	<ul style="list-style-type: none"> <li>• <i>AMV CP</i> gene from Alfalfa mosaic virus (viral disease resistance)</li> <li>• <i>nptII</i> gene from the bacterial Tn5 transposon (antibiotic resistance)</li> </ul>
<b>Proposed Release Location:</b>	Shire of Southern Grampians, Victoria
<b>Proposed Release Size:</b>	Maximum area of 494 square metres at one site per planting season
<b>Proposed Release Date:</b>	May 2004 - April 2007 (four planting seasons)

#### Section 1.1 The proposed dealings

3. Department of Primary Industries (DPI) (Victoria) seeks approval for the limited and controlled release of a genetically modified (GM) virus resistant white clover into the environment.

4. DPI (Victoria) proposes to conduct the field trial on one site covering a maximum of 2 hectares (consisting of a 26 x 19 metre GM white clover plot surrounded by a 32 metre wide (minimum) pollen trap of non-GM white clover and other legumes) in the Shire of Southern Grampians, Victoria. The release is planned for May 2004 to April 2007 and encompasses four planting seasons.

5. The main aims of the proposed release are the field evaluation of a GM white clover resistant to infection by Alfalfa mosaic virus (AMV) and the production of GM white clover seed for future trials, subject to further approvals. DPI (Victoria) proposes to evaluate agronomic characteristics and resistance to AMV of the GM white clover over two years and

then produce seed from a selection of GM white clover plants showing superior agronomic performance and AMV resistance.

6. Six hundred GM white clover plants, grown in containment facilities, will initially be planted at the trial site. Molecular analysis of a selection of superior performing GM white clover plants will be undertaken in the laboratory. Because white clover is self-incompatible, the superior GM white clover plants will be caged and crosses using bees will be undertaken to produce seed. Seed collected from the second generation of GM white clover produced by crossing will be stored in secure facilities for future releases, if approved. The applicant proposes that any GM white clover plants not required for further analysis at the completion of the trial will be destroyed by herbicide application.

7. To minimise dissemination of the GM white clover, DPI (Victoria) proposes a number of containment measures including:

- specific containment, transport and storage conditions in accordance with OGTR guidelines;
- surrounding the release site by both a livestock-proof and a rabbit-proof fence to prevent seed dispersal by grazing animals;
- surrounding the GM white clover plot by a pollen trap, consisting of an inner one metre wide band of non-GM white clover, concentric 10 metre wide (minimum) bands of red clover, lucerne and Persian clover, side strips of red clover and an outer one metre wide band of non-GM white clover;
- removal of flowers from the GM white clover plants in the event of the pollen trap plants not flowering simultaneously;
- removal of mature flower heads during peak flowering time to reduce the build-up of a seed bank;
- monitoring for, and destroying any, white clover plants that may occur within 500 metres of the release site;
- destroying GM materials not required for subsequent research;
- placing a footbath and washbasin at gate of release site to clean all implements; and
- monitoring for, and destroying any, volunteer GM white clover that may occur in the release area for five years after completion of the trial.

8. None of the white clover plants from the release, or their by-products, will be used for animal feed or human food.

## **Section 1.2 Parent organism**

9. The parent organism is white clover (*Trifolium repens* L.), which is exotic to Australia and is grown as a component of improved pastures in south-eastern Australia and in the wetter parts of Western Australia. It is the dominant pasture clover for the Australian dairy industry. More detailed information on white clover can be found in a review document 'The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia' (OGTR 2004), that was produced in order to inform the risk assessment process for this licence application involving GM white clover. This document is available at [www.ogtr.gov.au](http://www.ogtr.gov.au).

### Section 1.3 Genetic modification and its effect

10. The GM white clover proposed for release contains two genes, the coat protein gene from Alfalfa mosaic virus (*AMV CP*) and the neomycin phosphotransferase type II (*nptII*) selectable marker gene from *Escherichia coli*.

11. The *AMV CP* gene confers resistance to infection by AMV (see Section 3 of Appendix 1 for more details). AMV is a single stranded RNA virus and belongs to the genus *Alfavirus*, family *Bromoviridae* (ICTV website). It is widespread in the environment, being reported to infect at least 599 plant species in 245 genera in 68 families (Edwardson & Christie 1986). The virus is transmitted by at least 14 species of aphids in the Aphididae family, in particular *Myzus persicae*, and can also be transmitted by mechanical inoculation and by grafting. In AMV infected plants, virions are found in all parts of the plant (Büchen-Osmond 2002).

12. The *nptII* gene confers resistance to aminoglycoside antibiotics related to kanamycin and neomycin. It was used as a selectable marker in the early laboratory stages of development of the GM plants, to enable selection of plant cells containing the desired genetic modification. It will also be used to confirm the presence of the genetic modification both in the laboratory and in the field, using the polymerase chain reaction (PCR) technique. The use of this marker for identification is important because primers targeted to the *AMV CP* gene cannot discriminate between the introduced *AMV CP* gene and any indigenous *AMV CP* sequences present in the plant through infection of the virus.

13. Short regulatory sequences (promoters and terminators) that control the expression of the introduced genes are also present in the GM white clover. The *AMV CP* gene is under the control of the enhanced Cauliflower mosaic virus 35S promoter (CaMV 35S) and the pea ribulose-1,5-bisphosphate carboxylase small subunit gene (*rbcS-E9*) terminator. The *nptII* gene is controlled by the nopaline synthase (*nos*) gene promoter and the terminator from the common soil bacterium *Agrobacterium tumefaciens*.

14. Further details on the introduced genes and their encoded proteins are provided in Appendix 1, Section 3.

### Section 1.4 Method of gene transfer

15. The *AMV CP*, *nptII* and associated regulatory sequences were originally introduced into the white clover cultivar 'Irrigation' by *Agrobacterium* (*A. tumefaciens*)-mediated DNA transformation (Zambryski 1992). The vector carried by the *A. tumefaciens* has been disabled, which means that it does not contain the genes which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). The GM white clover (cultivar 'Irrigation') was then conventionally bred with the white clover cultivar 'Mink'. It was in turn bred with the white clover cultivar 'Sustain' to produce the GM white clover proposed for release in the current application.

## SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

### Section 2.1 Previous Australian releases of similar GM white clovers

16. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been four field trials of GM AMV resistant white clover

which involved different white clover cultivars to that proposed for release under the current application. Three of the trials were conducted by La Trobe University (PR-64, PR64X and PR 64X(2)) and the other was by CSIRO (PR-67). The GM white clovers were assessed for plant growth, expression of the introduced gene and resistance to viral infection. Gene flow was also analysed. The size of the releases ranged from two to four hectares and were carried out in the Shire of Southern Grampians, Victoria and the Shire of Hume, New South Wales and were subject to similar containment conditions to those proposed by the applicant. There have been no reports of adverse effects on human health and safety or the environment resulting from the releases.

17. However, there has been no such trials under the current regulatory system established by the *Gene Technology Act 2000*.

## **Section 2.2 Approvals by other Australian government agencies**

18. The OGTR is responsible for assessing the risks to human health and safety and the environment associated with development and use of gene technology. Other government regulatory requirements may be relevant in respect of the release of GMOs, and the use of products of the GMOs, including the responsibilities of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standards Australia New Zealand (FSANZ).

### **2.2.1 Australian Pesticides and Veterinary Medicines Authority**

19. The APVMA has a complementary regulatory role in respect to this application due to its responsibility for agricultural chemical use in Australia. The viral coat protein produced by the GM white clover proposed for release falls under the *Agricultural and Veterinary Chemicals Code Act 1994* (Ag Vet Code Act) definition of an agricultural chemical product, and is thus subject to regulation by the APVMA. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an agricultural chemical product, for example for a limited period of time or for a limited area.

20. DPI (Victoria) submitted an application to the APVMA for a research permit for the use of the *AMV CP* gene in GM white clover during the proposed trial. The APVMA and the OGTR work closely to ensure thorough, coordinated assessments of parallel applications, and, wherever possible, that the decisions by both agencies coincide.

21. In considering applications for registrations or research permits, the APVMA considers and, if necessary, imposes conditions in relation to a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of agricultural chemical products and resistance development.

22. Further information is available from the APVMA:

Australian Pesticides and Veterinary Medicines Authority

PO Box E240

KINGSTON ACT 2604

Phone: (02) 6272 5158

Fax: (02) 6272 4753

Email: [contact@apvma.gov.au](mailto:contact@apvma.gov.au)

<http://www.apvma.gov.au>

### 2.2.2 Food Standards Australia New Zealand

23. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. As white clover is not used in human food, the applicant is not required to apply to FSANZ for food use of the GM white clover from the proposed release.

### Section 2.3 International approvals

24. GM white clover expressing the *AMV CP* gene has not been released in other countries. However, other GM plants containing the *AMV CP* gene have been trialed in USA, including pea (*Pisum sativum*) (Timmerman-Vaughan et al. 2001), alfalfa (*Medicago sativa*) (USDA-APHIS 1994), and tobacco (*Nicotiana tabacum*) (Xu et al. 1998). Environmental assessments carried out by the Animal and Plant Health Inspection Service (APHIS) of the USA concluded that the GM alfalfa and tobacco have no significant impact on the environment (USDA-APHIS 1994; USDA-APHIS 1988).

25. GM plants with introduced viral coat protein genes have been field trialed overseas. For example, in the USA, tomato plants with either tobacco mosaic virus CP gene (Nielsen et al. 1998; Sanders et al. 1992) or tomato mosaic virus CP gene (Sanders et al. 1992), and squash plants with cucumber mosaic cucumovirus (CMV) CP gene, zucchini yellow mosaic virus CP gene or watermelon virus 2 potyvirus CP gene (Fuchs et al. 1998) have been field trialed. Other countries have also trialed GM plants with a viral CP gene in the field, including Italy for tomato plants with CMV CP gene (Tomassoli et al. 1999) and Canada for potato plants with potato leaf roll luteovirus CP gene (Kawchuk et al. 1997).

26. No adverse effects from the field trials of GM plants containing viral CPs have been reported.

## **CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN**

27. The Act and the Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 8).

### **SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN**

28. Comments received in response to the consultation with expert groups and authorities on the preparation of the risk assessment and risk management plan (RARMP) under Section 50 of the Act and with the same stakeholders and the public on the RARMP, under Section 52 of the Act (see Appendix 8), were very important in finalising the plan, which formed the basis of the Regulator's final decision on the application.

29. Written submissions in relation to DIR 047/2003 received from the agencies and authorities suggested the following issues relating to human health and safety or the environment, which have been addressed in the RARMP:

- the potential toxicity of the GM white clover (Appendix 2 refers);
- the potential for increased competitiveness/fitness of the GM white clover which could result in enhanced weediness (Appendix 3 refers);
- the potential for, and management of, gene transfer to non-GM white clover (Appendices 4 and 7 refer);
- the potential for ecological impacts arising from gene transfer to other organisms including plants and microbes (Appendix 4 refers);
- the potential for recombination or other viral interactions between the introduced viral gene and infecting viruses resulting in novel viral properties (Appendices 5 and 6 refer);
- the persistence of the GM white clover, or its introduced proteins, at the release site (Appendices 2 and 3 refer);
- the potential for dissemination of the GM white clover beyond the release site (Appendices 3 and 4 refer);
- the potential for unintended effects due to insertion of the introduced gene in white clover (Appendix 1 refers); and
- the prevention of GM white clover entering the food chain (Appendix 7 and Chapter 2 refer).

30. These submissions also raised the necessity for additional data requirements for the future development of the GMO including characterisation of the site of insertion, competitive advantage of the GM white clover, role of AMV limiting the spread of white clover populations, recombination events leading to the production of novel viruses, and viral interactions leading to long-term adverse effects (Chapter 2 and Appendices 3, 5 and 6 refer).

31. The agencies and authorities also raised some issues, including segregation, that are outside the scope of evaluation conducted under the Act and therefore have not been considered as part of the assessment process.

32. The Regulator received three submissions from members of the public on this application. A summary of these written submissions is provided in Appendix 9. The key issues raised by the public that related to human health and safety or the environment were:

- potential for long-term adverse impacts on human and animal health (Appendix 2 refers);
- potential for adverse impacts on the environment (Appendices 3, 4, 5 and 6 refers);
- stability of the genetic modification (Appendix 1 refers); and
- the potential for gene transfer (Appendices 4 refers).

33. The public submissions also raised issues relating to the effect of GMOs on export markets and ethical concerns, which are outside the scope of evaluation conducted under the Act and therefore have not been considered as part of the assessment process.

34. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to risk to human health and safety and the environment in finalising the RARMP. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

## **SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN**

35. The Regulator has conducted a risk assessment in relation to the proposed dealings and prepared a risk management plan in accordance with the Act and the Regulations. The risk assessment process used a Risk Analysis Framework developed in consultation with the public and key stakeholders (available from the OGTR website [www.ogtr.gov.au](http://www.ogtr.gov.au)). A number of hazards were identified that may be posed by the proposed dealings. The risks posed by these hazards were assessed as being either 'negligible', 'very low', 'low', 'moderate', 'high' or 'very high' by considering:

- the likelihood of the hazard occurring; and
- the likely consequences (impact) of the hazard, were it to be realised.

36. The following table (Table 1) lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed *Summary of Risk Assessment*.

37. Where it is considered, on the basis of a combination of possible adverse impacts and likelihood of occurrence, that risk management may be required to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the methods selected to limit the potential for risk exposure and the reasons they were chosen. The risk management plan for the proposed dealing are given effect by specific conditions

within the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 7.

### **SECTION 3 RESEARCH REQUIREMENTS**

38. The licence conditions include the requirement that the applicant collect and provide to the Regulator further information regarding:

- expression levels of the proteins of the introduced *AMV CP* and *nptII* genes in different parts of the plants under Australian field conditions;
- agronomic characteristics indicative of potential weediness of the GM white clover under Australian field conditions; and
- examine the disease status of the GM white clover plants during the trial to determine whether novel viruses emerge.

### **SECTION 4 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES**

39. The proposed limited and controlled release is a small scale, single site trial over four planting seasons. Additional information would be required to assess future applications for larger scale releases of GM AMV resistant white clover, and before reduced containment conditions or an approval for commercial release could be contemplated. Data would need to be provided on:

- molecular characterisation of the introduced genetic material;
- the levels of the natural toxicants e.g. cyanogenic glucosides, phytoestrogens or saponins in the GM white clover;
- toxicity of the GM white clover expressing the AMV CP, via livestock feeding studies;
- weediness of the GM white clover under Australian field conditions, including invasiveness, enhanced reproductive capacities, and limitation of white clover persistence by AMV outside of pastoral situations, such as roadsides, home gardens and natural environments; and
- gene transfer to non-GM white clover.

40. It should be noted that provision of the above data during the proposed release are not required to ensure the management of risks to human health and safety and the environment from the proposed release. The risk management measures summarised in Table 1 and given effect by the licence conditions, will achieve this purpose.

### **SECTION 5 DECISION ON THE APPLICATION**

41. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 8. It is important to note that the legislation requires the Regulator to base the licence decision on whether risk posed by the dealings are able to be managed so as to protect human health and safety and the environment.

42. It is concluded that the proposed release of the GM virus resistant white clover does not pose significant risks to public health and safety or to the environment as a result of the

genetic modification. The Regulator has imposed licence conditions to minimise potential exposure of humans and other organisms, and to limit the spread and persistence of the GMO and the introduced genes while more data is gathered on the behaviour and interactions of the GMO in the Australian environment. Detailed risk analyses based on the available scientific information are provided in Appendices 2-6 in support of this conclusion.

43. Therefore, the Regulator has issued licence DIR 047/2003 in respect of this application.

**Table 1 Summary of the risk assessment and the risk management plan (including licence conditions)**

GM white clover: the genetically modified white clover proposed for release.  
 AMV: Alfalfa mosaic virus.  
 AMV CP: Alfalfa mosaic virus coat protein.  
*nptII* gene: an antibiotic resistance marker gene from the bacterial Tn5 transposon, encoding NPTII protein  
 NPTII protein: neomycin phosphotransferase type II, encoded by an antibiotic resistance gene (*nptII*) which enables selection of GM plant cells when grown in the presence of antibiotics (kanamycin or neomycin) in the laboratory.  
 N/A: Not Applicable

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Food	Very Low	See Appendix 2 <ul style="list-style-type: none"> <li>the field trial is small consisting of a maximum area of 494 square metres at any one time;</li> <li>white clover is not used in human food; and</li> <li>none of the GM material from the release will be used in human food.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases likelihood of exposure.</li> <li><b>Prevent GM material from entering human food supply:</b> prevents exposure through food.</li> <li><b>Destroy all GM material not required for testing for further trials:</b> prevents unintended exposure.</li> <li><b>Ensure secure transport and storage of retained GM material:</b> prevents unintended exposure.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Prohibit entry into human food supply:</b> no GM white clover material to enter human food supply.</li> <li><b>Destroy GM material:</b> destroy all GM material not required for future trials.</li> <li><b>Secure transport and storage:</b> the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM white clover is stored within.</li> </ul>
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	Very Low	See Appendix 2 <ul style="list-style-type: none"> <li>the field trial is small consisting of a maximum area of 494 square metres at any one time;</li> <li>pleiotropic effects of the genetic modification could potentially alter aspects of plant metabolism;</li> <li>white clover pollen is not easily dispersed by wind and therefore unlikely to be an airborne allergen;</li> <li>exposure to the introduced proteins through working with white clover plants is very low;</li> <li>humans are commonly exposed to the AMV CP and NPTII proteins, as these proteins are naturally widespread in the environment;</li> <li>evidence indicates that the introduced proteins are not allergenic, nor do they have properties of known allergenic proteins; and</li> <li>there have been no reported toxic or allergic effects from similar GM white clovers expressing the same proteins that have been previously field trialed in Australia.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases likelihood of exposure.</li> <li><b>Destroy all GM material not required for testing for further trials:</b> prevents unintended exposure.</li> <li><b>Ensure secure transport and storage of retained GM material:</b> prevents unintended exposure.</li> <li><b>Report any adverse impacts on human health and safety:</b> ensures identification of unexpected adverse impacts.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Destroy GM material:</b> destroy all GM material not required for future trials.</li> <li><b>Secure transport and storage:</b> the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM white clover is stored within.</li> <li><b>Report adverse impacts:</b> any adverse impacts on human health and safety must be reported to the Regulator.</li> </ul>

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
<p><b>TOXICITY FOR OTHER ORGANISMS:</b> livestock, wildlife, invertebrates, microorganisms</p>	<p><b>Very Low</b></p>	<p><b>See Appendix 2</b></p> <ul style="list-style-type: none"> <li>the applicant proposes to surround the central GM plot with a rabbit proof fence and the whole release site with a stock proof fence which minimises exposure to animals;</li> <li>none of the GM white clover from the release will be used in animal feed;</li> <li>animals and micro-organisms are commonly exposed to the AMV CP and the NPTII protein, as these proteins are naturally widespread in the environment;</li> <li>the AMV CP and NPTII protein are not known to be toxic to any organism;</li> <li>however, expression levels of the introduced proteins in the GM white clover under Australian field conditions is not known.</li> </ul>	<p>Yes</p>	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases likelihood of exposure.</li> <li><b>Surround the GM plot with a rabbit- and stock-proof fence:</b> prevents dispersal by grazing animals.</li> <li><b>Prevent GM material from being used as stockfeed:</b> prevents exposure of animals.</li> <li><b>Destroy all GM material not required for testing for further trials:</b> prevents unintended exposure.</li> <li><b>Ensure secure transport and storage of retained GM material:</b> prevents unintended exposure.</li> <li><b>Further research:</b> provides information on the expression of the introduced proteins and validates the stringency of containment measures</li> </ul>	<p>Yes</p>	<ul style="list-style-type: none"> <li><u>Limit scale:</u> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><u>Surround the GM plot with a rabbit- and stock-proof fence:</u> inner rabbit-proof fence and outer stock-proof fence around GM plot.</li> <li><u>Prohibit seed from being used as stockfeed:</u> no GM material to be used as stockfeed.</li> <li><u>Destroy GM material:</u> destroy all GM material not required for future trials.</li> <li><u>Secure transport and storage:</u> the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM white clover is stored within.</li> <li><u>Require further research:</u> on expression of introduced proteins under Australian field conditions.</li> </ul>

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
WEEDINESS	Low	<p>See Appendix 3</p> <ul style="list-style-type: none"> <li>non-GM white clover possesses some characteristics commonly associated with weediness, has a close taxonomic affinity to other weedy species and is known to be a problematic weed in some countries, including Australia. It is a naturalised pasture legume in some parts of Australia.</li> <li>limitations on the establishment and persistence of white clover populations is likely to be due to complex interactions involving one or more diseases (including viral diseases), moisture stress, soil fertility, grazing pressure and/or competition.</li> <li>the introduced <i>AMV CP</i> gene in the GM white clover is likely to affect its fitness where AMV is limiting the persistence and therefore GM white clover may be more weedy than non-GM white clover in these circumstances.</li> <li>Therefore, in the <b>absence</b> of isolation/containment measures, the likelihood of the GM white clover establishing and persisting as a weed in various parts of Australia at higher levels than that of non-GM white clover can be divided into three parts: <ul style="list-style-type: none"> <li>the likelihood of the GM white clover having enhanced growth and persistence in pastoral situations where AMV is limiting the growth and persistence of white clover is high (but in this situation it would not be considered to be a more problematic weed);</li> <li>the likelihood of the GM white clover becoming a more problematic environmental weed than non-GM white clover in areas such as roadsides, home gardens and natural environments is uncertain, because it is unknown whether AMV is limiting the growth and persistence of white clover in these areas; and</li> <li>the likelihood of the GM white clover becoming a more problematic weed than non-GM white clover in areas with no or low incidence of AMV is very low.</li> </ul> </li> <li>However, in relation to the proposed trial, it is concluded that the risk of GM virus resistant white clover establishing as a problematic environmental weed is low because: <ul style="list-style-type: none"> <li>the field trial is small with a maximum area of 494 square metres in each of the four planting seasons;</li> <li>the area in which the proposed field trial is to take place is not particularly suitable for growing white clover long-term due to hot summers and lack of moisture;</li> <li>the GM white clover will not be used as stockfeed which minimises the chances of dispersal; and</li> <li>extensive measures to limit seed and pollen dispersal are proposed by the applicant.</li> </ul> </li> <li>more information is required on agronomic characteristics of the GM white clover under Australian field conditions, including stolon growth and flower numbers.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases likelihood of escape.</li> <li><b>Prevent GM material from being used as stockfeed:</b> prevents dispersal by grazing animals.</li> <li><b>Surround the GM plot with a rabbit- and stock-proof fence:</b> prevents dispersal by grazing animals.</li> <li><b>Destroy all GM material not required for testing for further trials:</b> prevents unintended dispersal.</li> <li><b>Ensure secure transport and storage of retained GM material:</b> prevents escape of GM plant material outside the release site.</li> <li><b>Clean equipment used at the release site:</b> prevents escape of GM plant material into the environment outside the release site.</li> <li><b>Encourage germination of seed bank and destroy any volunteers</b> prevents persistence.</li> <li><b>Further research:</b> informs the ongoing review of the data on spread/persistence of the GMO in the environment and validates the efficacy of containment measures.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Prohibit GM material from being used as stockfeed:</b> no GM material to be used as stockfeed.</li> <li><b>Surround the GM plot with a rabbit- and stock-proof fence:</b> inner rabbit-proof fence and outer stock-proof fence around GM plot.</li> <li><b>Destroy GM material:</b> destroy all GM material not required for future trials.</li> <li><b>Secure transport and storage:</b> the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM white clover is stored within.</li> <li><b>Clean equipment used at the release site:</b> equipment must be cleaned before it is used for any other purpose.</li> <li><b>Encourage germination of any GM white clover seed bank:</b> encourage germination of any GM white clover seed bank during post harvest monitoring period.</li> <li><b>Destroy volunteers:</b> the release site must be monitored at least once every month during the trial and after harvest for at least 5 years and any white clover volunteers destroyed before flowering.</li> <li><b>Research conditions:</b> collect data on agronomic characteristics indicative of the potential enhanced weediness of the GM white clover compared to non-GM white clover under Australian field conditions.</li> </ul>

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
GENE TRANSFER: Plants Other white clover plants	Low	<p><b>See Appendix 4</b></p> <ul style="list-style-type: none"> <li>transfer of the <i>AMV CP</i> gene to non-GM white clover could potentially confer a selective advantage in situations where the virus is limiting the spread and persistence of white clover;</li> <li>gene transfer from GM virus resistant white clover to cultivated or naturalised white clover is highly likely in the absence of isolation/containment measures.</li> <li>It is considered that the risks posed by the proposed trial are low because: <ul style="list-style-type: none"> <li>the field trial is small with a maximum area of 494 square metres in each of the four planting seasons; and</li> <li>extensive containment measures are proposed by the applicant.</li> </ul> </li> <li>The applicant proposed to surround the trial site by a pollen trap composed of non-GM white clover and other legumes, and to isolate the trial from all other white clover populations to minimise gene flow and persistence.</li> <li>However, because there is uncertainty about the potential for enhanced weediness of GM white clover and the ability of AMV to limit white clover growth and persistence in the area surrounding the trial site, the Regulator considers that a cautious approach using a bee-proof cage, should be used to manage potential enhanced weediness through gene flow to non-GM white clover plants in the proposed field trial.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases potential transfer.</li> <li><b>Surround the GM white clover with a bee-proof cage:</b> minimises spread of the introduced genes beyond the release site via pollen flow.</li> <li><b>Kill bees used in pollination of the GM white clover:</b> minimises the spread of genes beyond the release site via pollen flow</li> <li><b>Prevent GM material from being used as stockfeed:</b> prevents dispersal by grazing animals.</li> <li><b>Destroy all GM material not required for testing for further trials:</b> prevents unintended dispersal.</li> <li><b>Ensure secure transport and storage of retained GM material:</b> prevents escape of GM plant material outside the release site.</li> <li><b>Clean equipment used at the release site:</b> prevents escape of GM plant material into the environment outside the release site.</li> <li><b>Destroy any volunteers</b> prevents persistence.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Surround the GM white clover with a bee-proof cage:</b> the GM white clover plot must be surrounded by a bee-proof cage and the integrity of the cage maintained.</li> <li><b>Kill bees used in the pollination of GM white clover:</b> any bees involved in the pollination of the GM white clover within the bee cage must be killed before leaving the bee cage.</li> <li><b>Prevent GM material from being used as stockfeed:</b> no GM material to be used as stockfeed.</li> <li><b>Destroy GM material:</b> destroy all GM material not required for future trials.</li> <li><b>Secure transport and storage:</b> the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM white clover is stored within.</li> <li><b>Clean equipment used at the release site:</b> equipment must be cleaned before it is used for any other purpose.</li> <li><b>Destroy volunteers:</b> the release site must be monitored at least once every month during the trial and after harvest for at least 5 years and any white clover volunteers destroyed before flowering.</li> </ul>
GENE TRANSFER: Plants Other species and genera	Negligible	<p><b>See Appendix 4</b></p> <ul style="list-style-type: none"> <li>well established genetic incompatibility prevents successful cross pollination with other plant species.</li> </ul>	No	N/A	N/A	None Required

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
GENE TRANSFER: Microorganisms (bacteria)	Negligible	<p>See Appendix 4</p> <ul style="list-style-type: none"> <li>the introduced genes in GM AMV resistant white clover are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and</li> <li>gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.</li> </ul>	No	N/A	N/A	None Required
GENE TRANSFER: Humans and other animals	Negligible	<p>See Appendix 4</p> <ul style="list-style-type: none"> <li>the introduced genes in the GM white clover are already widespread in the environment;</li> <li>the GM AMV resistant white clover will neither be fed to livestock nor enter in the human food supply;</li> <li>the probability of interaction, uptake and integration of intact plant DNA by other organisms occurring is extremely low, especially if it involves unrelated sequences (non-homologous recombination); and</li> <li>natural events of horizontal gene flow from plants to distantly related organisms are extremely rare.</li> </ul>	No	N/A	N/A	None Required

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed?	Licence conditions (see Appendix 7 for detailed licence conditions)
<b>VIRAL INTERACTIONS</b> Non-persistent (e.g. complementation, transcapsidation, synergism)	Negligible	See Appendix 5 <ul style="list-style-type: none"> <li>the likelihood that long term adverse impacts will result from transient interactions (such as complementation, transcapsidation, synergism, resurgence of non-target virus) between the introduced <i>AMV CP</i> gene (or its product) and viruses is negligible.</li> </ul>	No	N/A	N/A	None required.
<b>VIRAL INTERACTIONS</b> homologous recombination	Very Low	See Appendix 5 <ul style="list-style-type: none"> <li>the proposed field trial is small (an area of 494 square metres at any one time) and for only for a short period of time (four planting seasons);</li> <li>the <i>AMV CP</i> gene (and its product) is naturally widespread in the environment; and</li> <li>the likelihood of homologous recombination between the introduced <i>AMV CP</i> gene and infecting AMV is uncertain; but even if homologous recombination does occur, the likelihood that it will have an adverse impact is very low.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases potential interactions.</li> <li><b>Further Research:</b> determine whether novel viruses emerge.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Research conditions:</b> examine the disease status of the GM white clover plants during the trial to determine whether novel viruses emerge.</li> </ul>
<b>VIRAL INTERACTIONS</b> heterologous recombination	Very Low	See Appendix 5 <ul style="list-style-type: none"> <li>the proposed field trial is small (an area of 494 square metres at any one time) and for only for a short period of time (four planting seasons);</li> <li>the <i>AMV CP</i> gene (and its product) is naturally widespread in the environment;</li> <li>natural heterologous recombination rarely produces adverse effects; and</li> <li>the likelihood of heterologous recombination between the introduced <i>AMV CP</i> gene and infecting viruses in the GM white clover is uncertain; but if heterologous recombination does occur, the likelihood that it will have an adverse impact is very low.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale of release:</b> decreases potential interactions.</li> <li><b>Further Research:</b> determine whether novel viruses emerge.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><b>Limit scale:</b> restrict area of GM plot to 494 square metres at any one time over four planting seasons at one individual site.</li> <li><b>Research conditions:</b> examine the disease status of the GM white clover plants during the trial to determine whether novel viruses emerge.</li> </ul>
<b>ANTI-VIRAL RESISTANCE</b>	Negligible	See Appendix 6 <ul style="list-style-type: none"> <li>the risk of AMV variants arising that overcome the CP-mediated resistance (anti-viral action) of GM white clover and cause increased disease burden in white clover or other plant species is negligible;</li> <li>the proposed field trial is small (an area of 494 square metres at any one time) and for only for a short period of time (four planting seasons); and</li> <li>this hazard is currently being assessed further by the APVMA</li> </ul>	Assessed by the APVMA	APVMA would impose appropriate conditions.	N/A	Licence notes the requirement to adhere to the APVMA conditions, including any anti-viral resistance management strategies.

## APPENDIX 1 INFORMATION ABOUT THE GMO

44. In preparing the risk assessment and risk management plan, the Regulator is required under Section 49 (2) of the Act to consider the properties of the parent organism and the effects of the genetic modification.

45. This Appendix addresses these matters and provides detailed information about the GMO proposed for release, the parent organism, the genetic modification process, the genes that have been introduced and the new proteins that are expressed in the GM white clover.

### SECTION 1 SUMMARY INFORMATION ABOUT THE GMO

46. In application DIR 047/2003, the Department of Primary Industries (DPI) (Victoria) proposes to release GM white clover plants conferring resistance to infection by Alfalfa mosaic virus (AMV).

47. The white clover was genetically modified by the introduction of two genes, the coat protein gene from AMV (*AMV CP*) and the neomycin phosphotransferase type II (*nptII*) antibiotic resistance selectable marker gene from *Escherichia coli*.

48. The *AMV CP* gene confers resistance to the AMV. See Section 3 of this Appendix for details on the coat protein mediated mechanism of resistance. AMV is a single stranded RNA virus and belongs to the genus *Alfavirus*, family *Bromoviridae* (Büchen-Osmond 2002). AMV is widespread in the environment, being reported to infect at least 599 plant species in 245 genera in 68 families (Edwardson & Christie 1986). The virus is transmitted in a non-persistent manner by at least 14 species of aphids in the *Aphididae* family, in particular *Myzus persicae*, and can also be transmitted by mechanical inoculation and by grafting. In AMV infected plants, virions are found in all parts of the plant (Büchen-Osmond 2002).

49. The *nptII* gene in the GM white clover confers resistance to the antibiotics kanamycin and neomycin. This gene was used as a selectable marker in the early laboratory stages of development of the GM plants, to enable selection of plant cells containing the desired genetic modification. It will also be used to confirm the presence of the genetic modification both in the laboratory and in the field, using the polymerase chain reaction (PCR) technique. The use of this marker for identification is important because primers targeted to the *AMV CP* gene cannot discriminate between the introduced *AMV CP* gene and any endogenous *AMV CP* sequences present in the plant through infection with the virus.

50. Short regulatory sequences (promoters and terminators) that control the expression of the introduced genes are also present in the GM white clover. The *AMV CP* gene is under the control of the enhanced Cauliflower mosaic virus 35S promoter (CaMV 35S) and the pea ribulose-1,5-bisphosphate carboxylase small subunit gene (*rbcS-E9*) terminator. The *nptII* gene is under the control of the nopaline synthase (*nos*) gene promoter and terminator from the common soil bacterium *Agrobacterium tumefaciens*.

51. The *AMV CP* gene, *nptII* gene and associated regulatory sequences were originally introduced into the white clover cultivar ‘Irrigation’ by *Agrobacterium*-mediated transformation (Zambryski 1992). See Section 4 of this Appendix for more detail. The GM plants produced were then conventionally bred with the white clover cultivar ‘Mink’, and the GM progeny further bred with the white clover cultivar ‘Sustain’. GM plants containing the

*AMV CP* gene in a homozygous state in cultivar ‘Sustain’ are proposed for release in this field trial.

52. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been four field trials of GM white clover containing the same genetic modification (transformation) event as in the current application but involving different white clover cultivars. Three of these trials were conducted by La Trobe University (PR-64, PR-64X and PR-64X(2)) and one was by CSIRO (PR-67). The field trials were carried out between 1996 and 2003. These trials involved plants from the initial transformation event in the cultivar ‘Irrigation’ and plants generated from breeding with the cultivar ‘Mink’, whereas the current application involves the progeny of the ‘Mink’ GM white clover conventionally bred with the cultivar ‘Sustain’.

53. Further details on the introduced genes, their products and mechanism of action are provided in Section 3 of this Appendix.

## **SECTION 2 THE PARENT ORGANISM**

54. A comprehensive review of the parent organism, *Trifolium repens* L. (white clover), is provided in the document, 'The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia' (OGTR 2004), that was produced in order to inform the risk assessment process for this licence application involving GM white clover. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au).

55. The white clover cultivar used for the original transformation (i.e. genetic modification) was ‘Irrigation’, due to its positive response to the tissue culture system used in producing GM plants. The GM plants produced were then conventionally bred with the white clover cultivar ‘Mink’, and the GM progeny of which were further bred with the white clover cultivar ‘Sustain’.

56. Characteristics of white clover cultivar ‘Sustain’ include medium-large leaf size, high stolon growing point density and heavy seed set potential (Caradus et al. 1997). Both ‘Mink’ and ‘Sustain’ are agronomically superior to ‘Irrigation’.

## **SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS**

### **Section 3.1 The *AMV CP* gene and encoded protein**

57. The AMV genome is tripartite and consists of RNAs1, 2 and 3, with a fourth subgenomic coat protein mRNA (sgRNA4). The sequence of the sgRNA4 is present in the RNA3 genome sequence and is derived during the replication of RNA3, hence the term subgenomic.

58. The *AMV CP* gene, which confers resistance to AMV, corresponds to the non-replicating sgRNA4 of the AMV, including part of the 5’ untranslated region (UTR) and all of the 3’ UTR. The complementary DNA (cDNA) was generated using the polymerase chain reaction (PCR) technique from the sgRNA4 of the Victorian AMV white clover isolate WC31. Thus the *AMV CP* gene in the GM white clover contains 35 base pairs (bps) of the 5’ UTR, 663 bps of the complete coding region of the coat protein and 182 bps of the 3’ UTR. The *AMV CP* gene in the GM white clover is not able to self-replicate nor is it infectious.

59. The *AMV CP* gene encodes the AMV coat protein (AMV CP), one of four proteins encoded by the AMV genome. The coat protein makes up the outer shell of the virus particle. The AMV CP expressed in the GM white clover is identical to that produced in plants infected with AMV. The AMV CP is a structural protein but is also involved in multiple steps of the replication cycle of AMV and cell-to-cell movement of AMV. Although the AMV CP is not infectious by itself, the protein, or its encoding RNA (sgRNA4), is required for infectivity of AMV. This early function of the CP has been termed ‘genome activation’ (Bol 1999; Smit et al. 1981). This is a specific feature of AMV and ilarviruses as other related tripartite plant viruses do not require the presence of the CP or its encoding RNA for infectivity (Neeleman & Bol 1999).

60. AMV particles consist of the three genomic RNAs encapsidated by 132- 240 AMV CP molecules in a bacilliform structure. In the absence of RNA, the CPs form empty icosahedral particles (Bol 1999). The AMV CP is not naturally present in uninfected plants but upon infection by AMV, the virus is rapidly spread systemically through the plant.

61. Expression of the *AMV CP* gene in white clover confers resistance to AMV. The mechanism of resistance is believed to be protein based (information supplied by the applicant). High levels of the AMV CP in the GM white clover plants are thought to block the uncoating (i.e. removal of the AMV CP) of any incoming AMV particles and inhibit translation of its RNA, preventing the virus from reproducing. This limits damage to the GM plants and also limits further transmission of the virus to other plants.

62. One copy of the *AMV CP* gene is present in the GM white clover, under the control of the enhanced CaMV 35S promoter (Kay et al. 1987). A promoter is a region of DNA linked to a gene that determines the levels of gene expression, including timing and plant tissue specificity. The 35S promoter directs the *AMV CP* gene to be expressed in most plant tissues and throughout the plant lifecycle. This kind of expression is called constitutive expression.

63. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The 3’ termination region influences mRNA production, stability of the mRNA and protein expression from the mRNA. The mRNA termination region for the *AMV CP* gene in the GM white clover is derived from the pea *rbcS-E9* terminator (Coruzzi et al. 1984; Morelli et al. 1985).

64. Potential hazards relating to the toxicity and allergenicity of the CP are discussed in Appendix 2, and those relating to gene transfer in Appendix 4.

### **Section 3.2 The *nptII* antibiotic resistance marker gene and encoded protein**

65. The GM white clover contains the *nptII* antibiotic resistance marker gene from *E. coli*. This gene was used in the initial laboratory stages of development of GM white clover plants, to enable selection of cells containing the desired genetic modification. The *nptII* gene is in common use as a selectable marker in the production of GM plants.

66. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. The NPTII enzyme uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII producing cell. The *nptII* gene functioned as a selectable marker in the initial laboratory stages of development of the GM white clover, allowing genetically

modified cells to grow in the presence of kanamycin, which inhibited the growth of non-modified cells. It will also be used to confirm the presence of the genetic modification both in the glasshouse and in the field.

67. The NPTII enzyme is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992).

68. Expression of the *nptII* gene in GM white clover plants is controlled by the nopaline synthase (*nos*) gene promoter and the *nos* gene mRNA termination region from *A. tumefaciens*.

69. Potential hazards relating to the toxicity and allergenicity of the NPTII protein are discussed in Appendix 2, and those relating to antibiotic resistance gene transfer in Appendix 4.

#### SECTION 4 METHOD OF GENETIC MODIFICATION

70. The *AMV CP* and *nptII* genes were introduced into the genome of white clover by *Agrobacterium*-mediated DNA transformation (Zambryski 1992).

71. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically transformed (modified) by the transfer of DNA (T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens*, through the mediation of genes from the *vir* (virulence) region of Ti plasmids.

72. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989).

73. The plasmids used contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing biosafety problems.

74. In this instance, a single disarmed plasmid vector was used to introduce the two linked genes, *AMV CP* and *nptII*, into the white clover cultivar 'Irrigation'. Genetically modified plants were selected in the laboratory by resistance to kanamycin.

75. The GM white clover was then conventionally bred with the white clover cultivar 'Mink'. These plants were in turn bred with the white clover cultivar 'Sustain' to produce the GM white clover proposed for release in the current application. The cultivars 'Mink' and 'Sustain' are less amenable to transformation and regeneration in tissue culture than 'Irrigation', hence the choice of cultivar 'Irrigation' for the initial transformation event.

## **SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIALS AND STABILITY OF THE GENETIC MODIFICATION**

76. Preliminary data, obtained using Southern blotting, indicate that all of the introduced genetic materials, including coding regions, non-coding regions and regulatory elements, are present as a single copy insertion, stably integrated in the genome of the GM white clover. No plasmid vector sequences, other than the intended T-DNA region, are present (information supplied by the applicant). The GM white clover plants proposed for release are homozygous for the inserted genes.

77. The virus-resistant phenotype has been found, in glasshouse conditions and in four previous field trials under GMAC, to be stable in both the hemizygous and homozygous states and in different genetic backgrounds. The inserted elements are inherited over several generations in a normal Mendelian manner for single, dominant genes (information supplied by the applicant).

78. More detailed information on the characterisation of the introduced genetic material would be required for consideration in any future application for a larger scale or commercial release (subject to separate application and assessment process) of the GM AMV resistant white clover.

## **SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS**

79. It is expected that the constitutive CaMV 35S promoter will lead to expression of the *AMV CP* gene in most parts of the GM white clover plants (Kay et al. 1987). Expression of the *AMV CP* gene has been confirmed by detection of the encoded mRNA in leaf tissue, using the Northern blot technique. Levels of *AMV CP* RNA in GM white clover are several-fold lower than that found in conventional white clover plants infected by AMV. Protein expression has not been quantified.

80. Expression of the NPTII enzyme in the GM white clover has been confirmed, through the ability of the plants to grow in the presence of kanamycin, in the laboratory during development of the GM plants. Expression of mRNA has been confirmed in leaf tissue, using the Northern blot technique. Protein expression has not been quantified.

81. The applicant is required to gather data on the levels of expression of the introduced AMV CP and NPTII proteins in different parts of the GM white clover plant under Australian field conditions as part of the proposed field trial. This information would be essential for consideration in any future application for a larger scale or commercial release of the GM AMV resistant white clover (which requires a separate application and assessment process).

## **SECTION 7 OTHER EFFECTS OF THE GENETIC MODIFICATION**

82. A genetic modification may have unintended effects, either due to physical disruption of an existing gene at the site of insertion of a new gene, or due to pleiotropic effects (i.e. the effects of a single gene on other apparently unrelated genes or traits).

83. No unintended characteristics have been observed in the GM white clover plants during glasshouse or four field trials conducted under GMAC oversight. The GM white clover plants proposed for field trial under the current application have shown no unintended or secondary effects in the glasshouse.

84. The applicant proposes to conduct evaluation of agronomic and phenotypic traits of the GM white clover as part of the proposed release. These traits include diameter, height, stolon density at plant centre, stolon density at plant edge and flower numbers. The assessment of these traits in the field will provide further evidence for any unintended effects of the genetic modification.

## APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS AND OTHER ORGANISMS

85. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed to human health and safety as a result of any toxicity or allergenicity of the GMO or its novel proteins, and to other organisms as a result of any toxicity of the GMO or its novel proteins.

### SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

86. A toxic response to a chemical is shown by the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000).

87. Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies known as IgE, or sensitisation of specific tissue bound lymphocytes (Taylor 2000; FAO & WHO 2000; Taylor & Lehrer 1996). Allergic responses can have severe consequences for an individual. For example, anaphylaxis is a shock syndrome caused by a massive release of histamine and other allergic mediators from even minute exposures to an antigen. Food proteins are common causes of anaphylaxis, especially peanut and shell fish (Frick 1995). Allergic responses have a well-defined etiology (i.e. biochemical cause) that is quite different from toxicity.

88. Predicting allergenicity is difficult and has been based on sequence, structural and biochemical comparisons with known allergens. Protein allergens usually share a number of characteristics (ANZFA 2001a; Flavell et al. 1992; Fuchs et al. 1993c; Fuchs et al. 1993b; Fuchs & Astwood 1996; Metcalfe et al. 1996; Taylor 1995; Fuchs et al. 1993a; Davies 1986). They typically have molecular weight ranges between 15-70 kD, are glycosylated and are present as the major protein component in the specific food. Common food allergens also tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein is sufficiently stable to reach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion (Kimber et al. 1999).

89. The GM virus resistant white clover differs from conventional white clover in the expression of two additional proteins. These are the Alfalfa mosaic virus (AMV) coat protein (CP) and the neomycin phosphotransferase II (NPTII) protein. The AMV CP confers resistant to infection by AMV in the GM white clover plants. The NPTII protein confers resistance to the antibiotic kanamycin. The potential for this GM white clover to be toxic or allergenic to humans or other organisms due to either expression of the novel gene products or because of unintended effects of the genetic modification is considered in this Appendix.

90. GM white clover containing the AMV coat protein (*AMV CP*) gene and the *nptII* gene have been trialed in Australia under the previous voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) (see PR 64, PR64X, PR64X2 and PR 67). No adverse effects relating to the toxicity or allergenicity have been reported from these trials.

## SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

91. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM virus resistant clover to humans, or toxicity to other organisms, a number of factors were considered including:

- the inherent toxicity and allergenicity of non-GM white clover (OGTR 2004);
- the potential for exposure to the GM white clover;
- exposure to the introduced proteins (AMV CP and NPTII) from other sources;
- the potential toxicity and allergenicity of the introduced proteins expressed in the GM white clover; and
- the potential toxicity and allergenicity of the GM virus resistant white clover.

### Section 2.1 Toxicity and allergenicity of non-GM white clover

92. White clover is a well established pasture legume with a long history of safe use. A comprehensive review of conventional white clover, including information on its toxicity and allergenicity, is provided in the document ‘The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia’ (OGTR 2004) that was produced in order to inform the risk assessment process for this licence application involving GM white clover. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au). Information on non-GM white clover is included here to establish a baseline for comparison with the GM white clover being considered in this risk assessment.

93. White clover can potentially be toxic to grazing animals if ingested in large quantities or under particular situations, for example deficiencies in body iodine reserves, because of the presence of toxic and anti-nutritional factors. These include saponins, which may contribute towards the occurrence of bloat; phytoestrogens, which can interfere with reproduction; and cyanogenic glycosides (linamarin and lotaustralin), which are implicated in nutritional myopathy (OGTR 2004).

94. White clover pollen is not transported easily by wind (OGTR 2004), and therefore its potential to act as an airborne allergen is extremely low. However, physical contact with white clover could potentially trigger an allergic response in some people although there are no reports of any major allergic responses.

### Section 2.2 Potential for exposure to the GM white clover

#### 2.2.1 Exposure of people to the GM white clover

95. The applicant proposes to destroy the GM clover plants and any samples taken for laboratory analyses. White clover has no food uses in Australia. Therefore, humans will not be exposed to materials from the GM clover in food. White clover may be used in complementary medicines in Australia, but since all GM white clover plants used in the trial will be destroyed, there will be no opportunity for human exposure via medicines. Therefore, exposure of people to the GM clover will be through:

- working with GM white clover as a part of conducting the proposed field trials; or

- living near the area where the GM white clover is grown (general environmental exposure, e.g. people breathing white clover pollen).

96. Humans working with GM white clover plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the hard seed coat and to pollen. Any proteins present in the cuticle or seed coat are not accessible. Dermal exposure of workers to white clover pollen is possible, but the amounts to which workers may be exposed is expected to be very low, particularly as the trial consists of only an area of 494 square metres at any one time. Exposure to proteins (including the new proteins expressed in the GM white clover) or to other cellular components of the white clover plants will only occur if plant cells are ruptured.

97. Exposure to GM white clover by persons living close to the trial site could occur through direct contact with the plants or by inhalation of white clover pollen. Direct contact with the GM white clover plants is unlikely to occur because the field trial is small, containment measures are imposed to limit the spread of GM white clover and only authorised people will have access to the site. Exposure to pollen would also be unlikely because the field trial is small and white clover pollen is not transported easily by wind (OGTR 2004), limiting possible exposure to the pollen as a potential airborne allergen.

98. Expression levels of the introduced proteins have not been determined. However, these proteins are not expected to be a major component of any tissue of the GM white clover, based on the knowledge of the promoters controlling expression of the introduced genes (Kay et al. 1987). The applicant is required to gather data on the levels of expression of the introduced AMVCP and NPTII proteins in different parts of the GM white clover plant under Australian field conditions as part of the proposed field trial.

99. Even if exposure to the introduced proteins in the GM white clover occurs, these proteins are not toxic or allergenic to humans (see Section 2.4 for details).

### **2.2.2 Exposure of livestock and wildlife to the GM white clover**

100. None of the GM white clover plants from the release, or their by-products, will be used as stockfeed. The applicant proposes to limit access to the site by surrounding the trial site by a stock-proof fence. The applicant also proposes to surround the 26 x 19 metre GM white clover plot by a rabbit-proof fence, thereby limiting access by relatively small animals. Refer to Chapter 1 for the containment measures proposed by the applicant.

101. The bee proof cage imposed as a licence condition (See Appendix 4, Section 1.3.1) would prevent animals smaller than rabbits as well as birds accessing the GM plot.

102. The field trial is very small consisting of a maximum area of 494 square metres at any one time, which reduces the chance of exposure to the GM white clover.

103. White clover seed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation of the site or rainfall will produce minimal water run-off as the proposed field trial is on a level site (information supplied by the applicant). Additionally, other plants/crops grown within the outer stock-proof fence will greatly restrict any seed movement from the GM white clover site if any excess water run-off does occur.

104. The licence includes a requirement to separate the white clover trial site from natural waterways by at least 50 metres.

105. Even if exposure to the introduced proteins in the GM white clover occurs, these proteins are not toxic to stock and wildlife (See Section 2.4 for details).

### **2.2.3 Exposure of invertebrates to GM white clover**

106. Invertebrates may be exposed to the GM white clover and to the introduced proteins directly through feeding on the plants, including pollen. White clover is affected by many types of invertebrate pests (OGTR 2004) and relative exposure will be greatest for herbivorous species feeding on the white clover plants. Indirect exposure to the introduced proteins may potentially occur in species feeding on invertebrates that have been exposed to the proteins or via the soil, from root exudates or if white clover tissues break down and are incorporated into the soil.

107. Levels of the introduced proteins have not been determined. However, even if exposure to the introduced proteins in the GM white clover occurs, these proteins are unlikely to be toxic to invertebrates (See Section 2.4). The applicant is required to gather data on the levels of expression of the introduced AMV CP and NPTII proteins in different parts of the GM white clover plant under Australian field conditions as part of the proposed field trial. Furthermore, the Regulator has imposed a bee-proof cage as a licence condition to limit gene flow (See Appendix 4, Section 1.3.1) which will reduce the access of some invertebrates to the GM white clover plants. Although the applicant proposes to release bees within the cage to encourage pollination, these will be killed after pollination.

### **2.2.4 Exposure of microorganisms to GM white clover**

108. Microorganisms may be exposed to the GM white clover plants during growth or during decomposition of plant material. Herbicide treatment and manual removal of the GM white clover plants will result in minimal decomposition of the GM white clover in the soil, so that soil microorganisms are likely to be exposed to only low levels of the introduced proteins. Exposure of organisms in soil to the introduced proteins may also occur as a result of root exudations or root breakage.

109. Even if exposure to the introduced proteins in the GM white clover occurs, these proteins unlikely to be toxic to microorganisms (See Section 2.4).

## **Section 2.3 Other sources of the AMV CP and NPTII in the environment**

110. The native AMV CP is naturally produced by the Alfalfa mosaic virus, which belongs to the genus *Alfavirus*, family *Bromoviridae* (Büchen-Osmond 2002). AMV is widespread in the environment, being reported to infect at least 599 plant species in 245 genera in 68 families (Edwardson & Christie 1986). AMV infects many plants consumed by humans such as tomatoes, capsicums, potatoes, beans and other pulses, grapes and melons (Edwardson & Christie 1986) and therefore humans are constantly exposed to high levels of the AMV CP. AMV also infects many types of clover and other pasture crops and therefore livestock and many other organisms such as invertebrates and microorganisms present in pastures are commonly exposed to AMV CP.

111. AMV is transmitted by at least 14 species of aphids in the Aphididae family, in particular *Myzus persicae* (Büchen-Osmond 2002). Therefore exposure of aphids to the AMV CP is extremely high in nature.

112. The NPTII protein is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992). For humans, food, especially raw salad, is the major source of NPTII: at a conservative estimate, each human ingests  $1.2 \times 10^6$  kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998), estimated at  $10^{12}$  per person (Flavell et al. 1992).

## **Section 2.4 The potential toxicity and allergenicity of the introduced proteins in the GM white clover**

113. There are two novel proteins expressed in the GM white clover, AMV CP and NPTII.

### **2.4.1 AMV CP**

114. The *AMV CP* gene was derived from the AMV isolate WC31, which has at least 92% sequence identity to all other known AMV isolates. The protein produced is identical to that produced by the virus in nature and therefore the introduced protein has the same physiological function as the native CP (see Appendix 1, Section 3.1 for details on the roles of AMV CP). Expression of the AMV CP in the GM white clover plants may result in the CP being expressed in cells not normally infected by AMV as well as increased numbers of CP subunits containing no viral RNA (instead of the normal capsids consisting of CP subunits and viral RNA).

115. Expression of the *AMV CP* gene in the white clover confers resistant to infection by AMV and the mechanism of resistance is believed to be protein based. Viral coat proteins expressed in plants act in a very specific fashion, adversely affecting only viruses by blocking or limiting their ability to infect, replicate, and/or translocate within the plant. This specificity minimises the potential for viral coat proteins produced in plants to adversely affect nonviral organisms (Beachy 1997).

116. There have been no studies to determine acute oral toxicity of viral coat proteins (including AMV CP). This is because virus-infected plants currently are, and have always been, a part of both the human and domestic animal food supply and hence the consumption of viral CPs is very common with no adverse effects having ever been reported. Levels of AMV CP RNA are reported to be several-fold lower in the GM white clover compared to infected non-GM plants (information supplied by the applicant). Expression of the introduced proteins has not been quantified in the GM white clover plants. Additionally, there is no significant amino acid sequence similarity between the AMV CP and known toxins.

117. The licence holder is required to determine the expression levels of the introduced proteins in various tissues of the GM white clover under Australian field conditions during the proposed release.

118. The Australian Pesticides and Veterinary Medicines Authority (APVMA) is currently assessing a research permit application by the applicant for the use of AMV CP as an agricultural chemical product in white clover plants. As part of their assessment of chemical

use, the APVMA considers any potential human health effects, for example hazards arising through occupational exposure or residues in the environment.

119. The United States Environment Protection Agency (US EPA) is currently considering the addition of plant-incorporated protectants (PIPs) based on viral coat proteins to its PIPs exemptions. Substances which plants produce for protection against pests, and the genetic material necessary to produce them, are pesticides under the US Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), if humans intend to use these substances to ‘prevent, repel or mitigate any pest’. These substances are also ‘pesticide chemical residues’ under the US Federal Food, Drug, and Cosmetic Act (FFDCA) and EPA is also considering the exemption of PIPs based on viral coat proteins from the requirement of a tolerance under FFDCA. The final decision on whether viral coat proteins will be exempt is expected by November 2004 (EPA 2003).

120. AMV CP as well as other viral coat proteins are now being used as carrier molecules for the delivery of vaccines. Antigens from rabies virus, HIV and human syncytial virus have all been cloned into the open reading frame of AMV CP, and the subsequent transcripts expressed in plants. Purified virus particles consisting of the recombinant CP or whole plant tissue is being trialed in mice and humans for immunisations against the above diseases (Belanger et al. 2000; Yusibov et al. 1997; Yusibov et al. 2002). These reports further indicate the lack of toxic or allergenic properties of the AMV CP.

121. To date, no allergenic reaction has been attributed to a plant virus or plant viral gene product, although humans are often exposed to high levels of viral gene products (in particular coat protein) in the diets through viral infections/contamination of fruits and vegetables.

122. The AMV CP shows no significant sequence homology to known allergens assembled from the Genpept, Pir and SwissProt protein databases (Metcalfe et al. 1996). Furthermore, there have been no reports of allergic reactions to AMV CP or other viral CPs.

123. Neither the GM white clover nor any of its products from this trial will be used in human food or animal feed. However, even if exposure to the AMV CP does occur, the protein appears not to be toxic or allergenic to humans and other organisms including livestock, wildlife, invertebrates and microorganisms.

124. The Regulator would require information on the toxicity of the GM white clover expressing the AMV CP from livestock feeding studies before a proposal to feed GM white clover to livestock could be considered (separate application and assessment process).

#### **2.4.2 NPT II protein**

125. NPTII is a phosphorylating enzyme, which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). The function of this enzyme is the phosphorylation (inactivation) of the antibiotic kanamycin (and the related neomycin). In the environment, this enzyme is not likely to be active outside of living cells, as it requires specific chemical conditions for activity, including the availability of specific co-factors.

126. An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993, Monsanto Unpublished). The US

FDA has concluded that NPTII does not possess any properties that would distinguish it toxicologically from other phosphorylating enzymes in the food supply, and which are present in all plants and animals. NPTII is approved as an additive in food for human consumption in the US (FDA 1994). The US EPA has also established an exemption for NPTII from the requirement for a residue tolerance limit when used as a plant pesticide inert ingredient (EPA 1994).

127. As noted above, none of the white clover plants, or their by-products, would be used as stockfeed. However, it is worth noting that the introduced NPTII protein is present in many internationally released GM plants, including some approved for commercial release in Australia and used as stockfeed (e.g. DIRs 012/2002, 022/2002 and 023/2002). Studies using the purified forms of the NPTII protein have been conducted and detailed descriptions of the results of these studies are available in the risk assessment and risk management plans for DIR 012/2002, DIR 022/2002 and DIR 023/2002.

128. Although antibiotic production by non-pathogenic bacteria has been implicated in suppression of some plant diseases (Brimecombe et al. 2001), no evidence for the involvement of neomycin or kanamycin has been found in a search of the scientific literature. Neither are these antibiotics used in agriculture for controlling soil borne disease. Thus the presence of NPTII in soil is not expected to impact on microbial populations or plant disease susceptibility. Furthermore, expression of NPTII in a variety of crop plants (for example, canola, corn, cotton, and tomato), over several years of agronomic performance testing and commercial cultivation, has not been linked to any increased occurrence of disease.

129. The NPTII protein does not display characteristics common to known food allergen proteins (ANZFA 2000; ANZFA 2001b; FDA 1994; FDA 1998; Fuchs et al. 1993c). The NPTII protein is heat labile and degrades rapidly in simulated human gastric fluid. Fuchs et al. (1993) reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot and enzymatic activity. NPTII shows no significant DNA or protein sequence homology to known allergens in the EMBL, Genbank, Pir and SwissProt protein databases (Fuchs & Astwood 1996).

## **Section 2.5 Potential toxicity and allergenicity of the GM white clover**

130. An important consideration in assessing the GM white clover plants is whether the toxicity or allergenicity of the plant has been enhanced. Pleiotropic effects of the genetic modification could potentially alter aspects of plant metabolism. However, because the GM white clover proposed for release represents early stage research, no data have been obtained on the level of toxic compounds in the GM white clover. Information on the levels of the natural toxicants e.g. cyanogenic glucosides, phytoestrogens or saponins in the GM white clover would be required for consideration of any future larger scale or commercial release of the GM white clover (which would require a separate application and assessment process).

131. GM plants with introduced viral coat protein genes have been field trialed overseas. For example, in the USA, tomato plants with either tobacco mosaic virus CP gene (Nielsen et al. 1998; Sanders et al. 1992) or tomato mosaic virus CP gene (Sanders et al. 1992), and squash plants with cucumber mosaic cucumovirus (CMV) CP gene, zucchini yellow mosaic virus CP gene or watermelon virus 2 potyvirus CP gene (Fuchs et al. 1998) have been field trialed. Other countries have also trialed GM plants with a viral CP gene in the field, including Italy for tomato plants with CMV CP gene (Tomassoli et al. 1999) and Canada for potato plants

with potato leaf roll luteovirus CP gene (Kawchuk et al. 1997). No adverse effects from the field trials of GM plants expressing viral CPs have been reported.

132. It is unlikely that the GM white clover would be more toxic or allergenic than non-GM white clover. As none of the GM white clover plants from this release or their by-products will be used for human food or animal feed and the size of the field trial is small, the potential for adverse effects due to toxicity or allergenicity of the GM white clover is very low.

### SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

133. It is considered that the risk of GM white clover being toxic or allergenic for humans or other organisms is *very low* because:

- the field trial is very small consisting of a maximum area of 494 square metres at any one time;
- white clover pollen is not easily dispersed by wind and therefore unlikely to be an airborne allergen;
- none of the GM white clover from the release will be used in human food or animal feed;
- exposure to the introduced proteins through working with white clover plants is very low;
- the applicant proposes to surround the central GM plot with a rabbit proof fence and the whole release site with a stock proof fence which minimises exposure to animals;
- humans, animals and micro-organisms are commonly exposed to the AMV CP and the NPTII protein, as these proteins are naturally widespread in the environment, including human and animal food;
- feeding studies indicate that the NPTII protein is not toxic;
- evidence indicates that the introduced proteins are not allergenic, nor do they have properties of known allergenic proteins;
- pleiotropic effects of the genetic modification could potentially alter aspects of plant metabolism, so more information would be required for consideration if the applicant proposes larger scale releases; and
- there have been no reported toxic or allergic effects from similar GM white clovers expressing the same proteins that have been previously field trialed in Australia.

134. Licence conditions (Appendix 7) have been imposed to limit the spread and persistence of the GMO and ensure that none of the GM white clover plants from the release or their by-products will enter the human food supply or animal feed.

135. During the proposed release, the licence holder is required to report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the GM white clover) or to the environment.

### SECTION 4 RESEARCH REQUIREMENTS

136. During the proposed release, the licence holder is required to determine:

- expression levels of the introduced proteins in various tissues of the GM white clover under Australian field conditions.

137. Before any proposal to feed GM white clover to livestock could be considered, more detailed information would be required on:

- the levels of the natural toxicants such as cyanogenic glucosides, phytoestrogens and saponins in the GM white clover; and
- toxicity of the GM white clover expressing the AMV CP, via livestock feeding studies.

## APPENDIX 3 WEEDINESS

138. Under Section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. In this Appendix, risks posed by the proposed dealings to the environment are considered in relation to the potential for the GMO to become a problematic weed.

### SECTION 1 NATURE OF THE WEEDINESS HAZARD

139. There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weeds may also represent a source of food to various organisms, hence the introduction of weeds to an environment may also bring about ecological change by altering the structure of food webs.

140. Weeds are thought to share a number of life history characters that enable them to rapidly colonise and persist in ecosystems, particularly those that are regularly disturbed (Baker 1965; Roy 1990; Bazzaz 1986; Noble 1989; Williamson & Fitter 1996). These characteristics include:

- ability to germinate, survive, and reproduce under a wide range of environmental conditions;
- long-lived seed with extended dormancy periods;
- rapid seedling growth;
- rapid growth to reproductive stage;
- long continuous seed production;
- ability to self-pollinate but not exclusively autogamous;
- use of unspecialised pollinators or wind when outcrossing;
- high seed output under favourable conditions;
- special adaptations for long distance and short distance dispersal; and
- being good competitors.

141. However, because environmental conditions have a big influence on these attributes, and other factors such as plant community composition and availability of key resources (e.g. space, water, light and nutrients) influence the potential of a plant species to invade, weedy characters alone are not enough to determine if a plant will become a problematic weed. Therefore, the most successful predictors of weediness remain taxonomic affinity to other weedy species and the history of a given species weediness elsewhere in the world (Panetta 1993; Pheloung et al. 1999).

142. The GM virus resistant white clover differs from non-GM white clover in the expression of two additional proteins. These are the Alfalfa mosaic virus (AMV) coat protein (CP) and the neomycin phosphotransferase type II (NPTII) protein, which confer resistance to infection by AMV and the antibiotic kanamycin, respectively (see Appendix 1 for details).

143. The possibility was considered that the GM white clover might have the potential to be harmful to the environment, because of increased potential for weediness, either due to

expression of the novel gene products or as a result of unintended effects of the genetic modification.

144. This could occur if the GM white clover displayed altered characteristics such as increased fitness or increased fecundity as compared to non-GM white clover. If the GM white clover were to spread in the environment as a more problematic weed, this could result in impacts such as loss of native biodiversity or other adverse environmental effects.

## **SECTION 2 LIKELIHOOD OF THE ENHANCED WEEDINESS HAZARD OCCURRING**

145. In assessing the likelihood of adverse impacts due to the enhanced weediness of GM white clover, a number of factors were considered, including the:

- weediness of non-GM white clover;
- potential selective advantage conferred by the introduced AMV CP and NPTII protein;
- potential enhanced weediness of GM white clover;
- potential for spread of GM white clover into the environment; and
- potential for enhanced persistence of GM white clover at the site of release.

### **Section 2.1 Weediness of non-GM white clover**

146. Attributes of non-GM white clover associated with potential weediness are discussed in the document 'The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia' (OGTR 2004) that was produced in order to inform the risk assessment process for this licence application involving GM white clover. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au). In summary, the document concludes that non-GM white clover is a problematic weed (both in natural and agricultural ecosystems) in many States of Australia, although factors such as soil moisture and nutrient levels restrict the establishment and/or persistence of white clover seedlings to particular areas of Australia. It is considered a naturalised pasture legume in the coastal regions and tablelands of eastern Australia (Hill & Donald 1998). Information on the weediness of non-GM white clover is included here to establish a baseline for comparison with the GM white clover being considered.

147. Depending on genotype and environmental conditions, white clover has some of the weedy characteristics mentioned in Section 1 such as seed dormancy (under some environmental conditions, resulting in increased persistence of seed in the soil), long continuous seed production, high seed output and seeds that can be dispersed over long distances by animals (OGTR 2004). Other characteristics of white clover that increase the likelihood of its persistence in the environment include its ability to regenerate by either seedling recruitment or by vegetative perennation through the stolon system, and its highly heterozygous nature, due to outcrossing, which allows rapid adaptation.

148. For agricultural seed production, when white clover cultivars are changed, a break from white clover cultivation is required in order to maintain seed purity, due to the presence of hard dormant white clover seeds in soil seed banks. In New Zealand conditions, a five year break coupled with either annual cultivation or herbicide treatment has been found to reduce volunteer plants to less than 1 per 10 m<sup>2</sup> (Clifford et al. 1990; Clifford et al. 1996).

149. As mentioned above, the most successful predictors of weediness are taxonomic affinity to other weedy species and the history of a given species weediness elsewhere in the world (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). There are more than 300 species of *Trifolium*, and seventy of these, including white clover, are listed as weeds (including naturalised and/or environmental weeds) in a comprehensive compilation of the world's weed flora (Randall 2002). Many of these listed *Trifolium* species are classified as weeds in a number of different countries, including Australia, highlighting the successful spread and establishment of the genus *Trifolium* in many countries.

## **Section 2.2 Potential selective advantage conferred by the introduced proteins**

### **2.2.1 AMV CP**

150. The AMV CP could only confer a selective advantage in the presence of AMV and if AMV is limiting the establishment, growth and/or persistence of white clover.

151. Infection of white clover with AMV decreases growth, flowering and seed number (Barnett & Gibson 1977; Gibson et al. 1981; Gibson et al. 1982). For example, glasshouse studies with white clover experimentally infected with AMV have shown dry mass losses of up to 60% due to reduction in leaf area and leaf number (Garrett 1991) and in field studies, a loss in dry mass yield of 24% has been reported (Gibson et al. 1982). AMV, in combination with clover yellow vein virus and white clover mosaic virus, may reduce white clover pasture production by up to 30% through reduced foliage yield and quality, reduced nitrogen capacity and reduced vegetative persistence (Kalla et al. 2001).

152. AMV has a very wide host range, infecting 599 plant species in 245 genera in 68 families (Edwardson & Christie 1986). AMV is transmitted in a non-persistent manner by aphids (ICTV database). Non-persistent viruses can survive for a few hours in aphids and can be carried in the stylet or foregut of the aphid (Garrett 1973). Many non-persistent viruses such as Cauliflower mosaic virus in cauliflower, lettuce necrotic yellows virus in lettuce and subterranean clover stunt virus in French beans tend to spread over short distances of 10 metres or less (Garrett & McLean 1983). There is little information on the transfer of AMV in white clover. AMV spreads slowly over a number of years in white clover pastures (Mckirdy & Jones 1995). Information supplied by the applicant reports that white clover plants proximal (0.5 metres) to an AMV source plant have 6-8 fold higher AMV infection rates than those plants more distal (1 metre) over a nine month period. AMV is seed-borne in lucerne and possibly other legumes but there have been no reports that AMV is seed-borne for white clover (Garrett 1991).

153. AMV incidence in white clover is likely to be strongly linked to both aphid distribution and white clover density (or the density of another plant host of AMV).

154. There is very little information on population of aphids in different habitats in Australia. Extremely low numbers of aphids are found in native grasslands (Garrett & Chu 1997). In pastoral situations where host plants are present, aphid numbers can be extremely high. Aphid populations decline in hot, dry summers and cool wet winters of southern Australia, but aphid populations are not totally eliminated if suitable host plants are available (Garrett & McLean 1983).

155. Levels of AMV infection of white clover in pastoral populations are substantial in many sites across Australia. At many pasture sites, over 40% of white clover plants are infected

with AMV, and in some cases the level of infection exceeds 90% (Garrett 1991; Mckirdy & Jones 1995; Norton & Johnstone 1998). Hence, in an agricultural setting due to the prevalence of AMV, the GM white clover is likely to have increased fitness.

156. There is little information published on the incidence of AMV outside of a pastoral situation in more marginal areas such as roadsides, native environments and home gardens. In subalpine regions of New South Wales and the Australian Capital Territory, the incidence of AMV in native grasslands is very low with only one of the 31 white clover populations surveyed being infected with AMV. Additionally, seven alpine sites were surveyed in Victoria and there was no AMV detected native grasslands in the conserved parts of the national parks, although AMV infection was common in local tourist parks (Godfree et al. 2004). Information on AMV distribution is not available for other natural environments, roadsides or home gardens in Australia where white clover may have a significant presence.

157. Even if white clover populations, aphid populations and AMV are all present in a particular location, the likelihood of GM white clover becoming a more problematic weed than non-GM white clover at that location cannot be determined until it is established whether AMV is limiting the spread and persistence of non-GM white clover.

158. More information on the presence of AMV and its impact on the persistence of white clover populations in the Australian environment, including roadsides, home gardens, natural areas close to pastures, and isolated natural environments would be required for consideration in any larger scale or commercial release applications involving GM AMV resistant white clover (which would require a separate application and assessment process).

### **2.2.2 NPTII**

159. The NPTII protein could confer a selective advantage to GM white clover plants in the presence of a high concentration of neomycin or kanamycin. However, antibiotics are not applied to white clover pastures and are not likely to be present in any environment where white clover grows. Thus the expression of NPTII is highly unlikely to confer any selective advantages on the GM white clover.

### **Section 2.3 Potential enhanced weediness of GM white clover**

160. Many of the characteristics associated with weediness are also important agronomic characteristics. Consequently these are assessed as part of the agronomic evaluations during the development of new white clover cultivars, including GM varieties. The site of insertion and levels of expression of the introduced genes could potentially have pleiotropic effects and alter some aspects of the white clover biology that may affect weediness.

161. No unintended or secondary effects on agronomic characteristics, including effects on fertility, have been observed in glasshouse trials of the GM white clover (information supplied by the applicant). The applicant proposes to evaluate agronomic characteristics of the GM white clover under Australian conditions as part of the proposed field trial, including measurements of plant diameter and height, stolon density at plant centre, stolon density at plant edge, and flower numbers.

162. A licence condition is imposed requiring the licence holder to undertake these studies.

163. As discussed in Section 2.2.1, the presence of the AMV CP gene is likely to increase the fitness of the GM white clover in situations where AMV is limiting the growth and

persistence of white clover. This is likely in many pastoral situations but in this environment it would not be considered a problematic weed as white clover is normally a desirable component of grazed pastures. However, there is no information on the incidence of AMV in white clover growing close to pastures, such as roadsides, home gardens and natural environments adjacent to pastures and whether AMV is limiting the growth of white clover. Therefore, it is unknown whether GM white clover would have the potential to become a problematic environmental weed in these situations. In isolated natural environments, such as some subalpine regions, there is little or no AMV (see Section 2.2). Furthermore, very low densities of white clover are reported in subalpine regions (OGTR 2004). However, for white clover populations in isolated natural areas of Australia where information of AMV distribution is not available, it can not be determined if GM white clover will have a selective advantage compared to non-GM white clover.

164. It should be noted that limitations on the establishment and persistence of white clover populations are likely to be due to complex interactions involving one or more diseases (including viral diseases), moisture stress, soil fertility, grazing pressure and/or competition (Latch & Skipp 1987; OGTR 2004).

165. The likelihood of the GM white clover establishing and persisting in various environments within Australia at higher levels than that of non-GM white clover can be divided into three parts:

- the likelihood of the GM white clover having enhanced growth and persistence in pastoral situations where AMV is limiting the growth and persistence of white clover is high, but in this situation, the GM white clover would not be considered to be a problematic weed because enhanced growth and persistence would not be considered an adverse consequence;
- the likelihood of the GM white clover becoming a more problematic environmental weed than non-GM white clover in areas such as roadsides, home gardens and natural environments is uncertain, because it is unknown whether AMV is limiting the growth and persistence of white clover in these areas; and
- the likelihood of the GM white clover becoming a more problematic weed than non-GM white clover in areas with no or low incidence of AMV is very low.

166. More information on the presence of AMV and its role in limiting growth and persistence of white clover populations in the Australian environment, including roadsides, home gardens, natural areas close to pastures, and isolated natural regions would be required for consideration of any larger scale or commercial release of GM AMV resistant white clover (which would require a separate application and assessment process). This information is vital to determining if, and where, the GM white clover may become a more problematic weed than non-GM white clover.

## **Section 2.4 Spread of GM white clover beyond the release site**

### **2.4.1 Spread via plants**

167. The proposed dealings include cultivation of GM white clover, and retention and storage of GM white clover seed for future trials (subject to further approvals). No parts of the GM white clover plants produced in the proposed release will be used for human food or stockfeed.

168. During the proposed field trial, the GM white clover plants will be allowed to flower and set seed. Therefore, there is potential for the seed to be spread due to human and/or animal activities. When working with the GM white clover, seed could be transferred via clothing, footwear, implements and/or machinery. Small amounts of viable white clover seed could also be spread after passing through the digestive tract of animals. For example, seeds can remain viable after passing through the digestive tracts of sheep, cattle and goats several days after consumption (Suckling 1952; Yamada & Kawaguchi 1971; Yamada & Kawaguchi 1972). Viable seed can also be recovered from birds such as sparrows and pigeons (Krach 1959). Dispersal of white clover seed by common Australian animals and birds has not been studied but the attractiveness of white clover seed to these animals in the field is likely to be very low due to the very small size of the seeds. Ingestion of white clover seeds by earthworms does occur and viable seed are found from worm casts (McRill & Sagar 1973), and ants have been shown to carry white clover seeds in Australian pastures (Campbell 1966).

169. White clover seed could also be transferred from the GM trial site via water run-off. However, irrigation of the site or rainfall will produce minimal water run-off as the proposed field trial is on a level site (information supplied by the applicant). Additionally, other plants/crops grown within the outer stock-proof fence will greatly restrict any seed movement from the GM white clover site if any excess water run-off does occur.

170. The scale of the field trial is small with a maximum area of 494 square metres in each of the four planting seasons. The applicant has proposed a number of containment measures to minimise dispersal of the GM white clover. These include:

- surrounding the release site by both a livestock-proof and a rabbit-proof fence to prevent seed dispersal by grazing animals;
- monitoring for, and destroying any, white clover plants that may occur within 500 metres of the release site immediately surrounding the outer limit of the pollen trap during the field trial;
- destroying GM materials not required for subsequent research;
- placing a footbath and washbasin at the gate of the release site to clean all footwear and implements; and
- monitoring for and destroying any volunteer GM white clover that may occur in the release area for five years after completion of the trial.

171. Additional information supplied by the applicant states that regular surveying of roadsides within a one kilometre radius of the research station would be carried out over the duration of the field trial and any white clover plants found would be killed with herbicide.

172. Many of the above conditions proposed by the applicant have been incorporated into the licence to prevent white clover seed escaping into the environment (detailed in Appendix 7 and summarised in Chapter 2). Furthermore, a licence condition has been imposed to limit gene flow by surrounding the GM plot by a bee-proof cage (see Section 2.4.2 of this Appendix). This condition, along with the rabbit-proof fence, will also limit access to the GM white clover plants by many animals, further reducing the likelihood of seed dispersal.

173. Other licence conditions also restrict what plants can be planted and grown at the Location and within the outer stock-proof fence as well as requiring the applicant to monitor for volunteers within the outer fence during the trial. This effectively means that no white

clover plants can be present within the outer fence (placed at a distance of 40 – 90 metres from the Location) with the exception of the GM white clover plants planted at the Location.

174. During the field trial, vegetative material and seed from the GM white clover will be collected and transported to suitable laboratory and storage facilities. The escape of white clover seed during transportation presents an opportunity for the GM white clover to spread in the environment. Specific conditions have been imposed that require harvested material to be securely packed before transporting away from the release sites so as to prevent GM white clover seed escaping into the environment.

#### **2.4.2 Spread via gene flow**

175. Gene flow via cross-pollination with non-GM white clover plants could contribute to potential spread and persistence of the GMO in the environment outside the release site. This issue is discussed in detail in Appendix 4, Section 1.

176. The applicant proposed to limit outcrossing to non-GM white clover using pollen traps and isolation from other white clover plants as well as the removal of mature flower heads during peak flowering time (See Chapter 1 for details).

177. However, because there is uncertainty about the potential for enhanced weediness of GM white clover and the ability of AMV to limit white clover growth and persistence in the area surrounding the trial site, the Regulator considers that a cautious approach should be used with regards to managing potential enhanced weediness through gene flow to non-GM white clover plants in the proposed field trial. Therefore, the licence conditions include a requirement to enclose the GM plot in a bee-proof cage during the flowering period to reduce the likelihood of gene flow, which in turn will minimise the potential for the GM white clover to spread and persist beyond the release site. (See Appendix 4, Section 1.3.1 for more detail).

### **Section 2.5 Persistence of the GM white clover at the release site**

178. During the proposed field trial, the GM white clover plants will be allowed to flower and set seed. Depending on environmental conditions (and genotype) a certain proportion of white clover seed is hard. Any soft seed will germinate with the arrival of favourable soil moisture and temperature conditions (normally autumn and spring) but hard seed is dormant, and may remain in the soil for a long period of time (OGTR 2004). Therefore, there is opportunity for the GM white clover to persist at the release site via the soil seed bank.

179. Germination of any GM white clover seeds present in the soil or regrowth of any white clover plants remaining at the site will produce volunteers. However, the area in which the proposed field trial is to take place is not particularly suitable for growing white clover long-term due to hot summers and lack of moisture (information supplied by applicant) limiting the potential for persistence of GM white clover plants at the release site. Once the trial is complete, the applicant is required to lightly cultivate and irrigate the GM site twice a year to encourage germination of the GM white clover seed bank and destroy any white clover volunteers before flowering.

180. The field trial is small with a maximum area of 494 square metres in each of the four planting seasons. A number of containment measures to minimise persistence of the GM white clover. These include:

- removal of mature flower heads during peak flowering time to reduced the build-up of a seed bank;
- destroying GM materials not required for subsequent research by herbicide application and manually removing whole plants (including roots) before planting the GM white clover for the next growing season and at the completion of the field trial;
- lightly cultivating and irrigating the GM site twice a year to encourage germination of the GM white clover seed bank at the completion of the trial;
- monitoring for, and destroying any, volunteer GM white clover that may occur in the release area for five years after completion of the trial.

### SECTION 3 CONCLUSIONS REGARDING WEEDINESS

181. Non-GM white clover possesses some characteristics commonly associated with weediness, has a close taxonomic affinity to other weedy species and is known to be a problematic weed in some environments in some countries, including Australia. Limitations on the establishment and persistence of white clover populations is likely to be due to complex interactions involving one or more diseases (including viral diseases), moisture stress, soil fertility, grazing pressure and/or competition.

182. GM white clover is likely to be a more problematic weed than non-GM white clover where AMV is limiting its growth and persistence.

183. Therefore, in the absence of isolation/containment measures, the likelihood of the GM white clover establishing and persisting in various environments within Australia at higher levels than that of non-GM white clover can be divided into three parts:

- the likelihood of the GM white clover having enhanced growth and persistence in pastoral situations where AMV is limiting the growth and persistence of white clover is high, but in this situation, the GM white clover would not be considered to be a problematic weed because enhanced growth and persistence would not be considered an adverse consequence;
- the likelihood of the GM white clover becoming a more problematic environmental weed than non-GM white clover in areas such as roadsides, home gardens and natural environments is uncertain, because it is unknown whether AMV is limiting the growth and persistence of white clover in these areas; and
- the likelihood of the GM white clover becoming a more problematic weed than non-GM white clover in areas with no or low incidence of AMV is very low.

184. However, in relation to the proposed trial, it is concluded that the risk of GM virus resistant white clover establishing as a problematic environmental weed is low because:

- the field trial is small with a maximum area of 494 square metres in each of the four planting seasons;
- the area in which the proposed field trial is to take place is not particularly suitable for growing white clover long-term due to hot summers and lack of moisture;
- the GM white clover will not be used as stockfeed which minimises the chances of dispersal; and

- extensive measures to limit seed dispersal and dormancy are proposed by the applicant.

185. It is considered that the risk of the GM virus resistant white clover establishing as a problematic weed can be managed to an acceptable level by imposing the various conditions in the licence to minimise the spread and persistence of the GM white clover in the environment, including the requirement to surround the GM plot by a bee-proof cage during the flowering period, post harvest treatment of the trial site to promote germination of the seed bank, and monitoring for volunteers during and at the completion of the trial. Refer to Chapter 2 and Appendix 7 for the management conditions.

#### **SECTION 4 RESEARCH REQUIREMENTS**

186. The licence holder is required to evaluate agronomic characteristics of the GM white clover under Australian field conditions as part of the proposed field trial, including stolon growth and flower numbers.

187. If the applicant makes any application for future larger scale releases of the GM white clover or reduced containment conditions, data would be required on weediness of the GM white clover under Australian field conditions, including invasiveness, enhanced reproductive capacities, and limitation of white clover persistence by AMV outside of pastoral situations, such as roadsides, home gardens and natural environments.

## APPENDIX 4 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

188. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed through the transfer of the introduced genes from the GM white clover to other organisms.

189. Gene transfer is the movement of genes between individuals. Within a species, genes are routinely exchanged between individuals of successive generations through sexual reproduction. Hybrids can sometimes be produced between closely related species through sexual reproduction although this may require significant assistance. For example, in plants cross pollination of wheat and rye in the laboratory produced triticale. In animals, fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning hybridisation may or may not lead to the introgression of a gene or genes into a population.

190. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except among bacteria. However, transfer of genetic material between sexually incompatible organisms can occur. Detailed examination of DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. However, there seems to have been only very limited transfer of complete genes from plants to other types of organisms.

191. The likelihood of hazards arising from gene transfer is dependent on a number of factors that form a necessary chain, including:

- opportunity for gene transfer to occur such that the recipient organism is exposed to the genetic material in the form of pollen, plant cells or DNA; and
- occurrence of the genetic material being incorporated into the genome of the recipient organism at a site and in a configuration that allows the gene to be functional; and
- persistence of the transferred genetic material such that the recipient organism is able to survive, reproduce and maintain the genetic modification; and
- significance of the transferred genetic material such that its presence and/or expression in the recipient organism will result in an adverse impact on human health and safety, or the environment.

192. For ease of reference, the assessment of gene transfer to other organisms is presented in four sections:

- Section 1 details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM virus resistant white clover to other plants, including other white clover plants;
- Section 2 details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM virus resistant white clover to microorganisms;
- Section 3 details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM virus resistant white clover to animals, including humans; and
- Section 4 draws together the conclusions from these sections.

## **SECTION 1 GENE TRANSFER FROM GM WHITE CLOVER TO OTHER PLANTS**

### **Section 1.1 Nature of the gene transfer hazard**

193. Transfer of the introduced genes (*AMV CP* and *nptII*) or regulatory sequences to cultivated or naturalised white clover plants would present the same hazards and have the same potential impacts as the presence of the genes in the GM virus resistant white clover (see Appendices 2-6). However, if such a transfer occurred, it would increase the possibility that these genes would further spread and persist in the environment. The flow on impacts of such a transfer could depend on whether the introduced genes conferred any selective advantage to the naturalised white clover in its environment (refer to Section 2.2 for discussion on this issue).

194. If gene transfer to other plants were to occur, the hazards to the environment could be highly varied, broadly depending upon the nature of the species to which transfer occurred and the resulting phenotype of the progeny, such as any alteration in survival or reproductive capacity.

### **Section 1.2 Potential hazards from the introduced genes**

#### **1.2.1 The *AMV CP* (virus resistance) gene**

195. Plants expressing this gene could become resistant to Alfalfa mosaic virus (AMV). This could confer a selective advantage on the plant in the presence of the virus, with increased weediness being the most likely adverse outcome. However, this would only occur if the virus is limiting the growth and/or persistence of the plant.

#### **1.2.2 The *nptII* (antibiotic resistance) gene**

196. Plants expressing this gene could become resistant to the antibiotics, kanamycin and neomycin. This would only have an impact on plant survival if the antibiotics were used on the plants, or otherwise present in the environment of the plant, and were limiting its growth. Antibiotics are not generally applied to crops and would not limit their growth except at very high concentrations not found in the natural or agricultural environment. Expression of the *nptII* marker gene enabled selection of plant cells containing the genetic modification in the laboratory.

#### **1.2.3 Promoters and other regulatory sequences**

197. If these sequences were to be transferred to other plants without the associated genes of the GM white clover, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

198. Some of the introduced regulatory sequences are derived from plant pathogens (Cauliflower mosaic virus and *Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

199. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context occurs naturally in all plant genomes and could also result in unpredictable effects. Thus the potential hazard from the introduced sequences is no different

to that posed by sequence transfer from non-GM plants or sequence transfer occurring within the genome of a plant species.

### **Section 1.3 Likelihood of a hazard arising through gene transfer from GM white clover to other plants**

200. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the characteristics of introduced gene sequences, as well as on the likelihood of transfer itself.

#### **1.3.1 Transfer to cultivated or naturalised white clover**

201. For a detailed consideration of the likelihood of gene transfer occurring, including an overview of the pollination biology of white clover, see the document "The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia", available at [www.ogtr.gov.au](http://www.ogtr.gov.au), that was produced in order to inform the risk assessment process for this licence application involving GM white clover.

202. White clover is an obligate outbreeder (i.e. self incompatible) and pollen transfer is mediated by insect pollinators, in particular honey bees (OGTR 2004). White clover pollen is not easily dispersed by wind and, even if there is airborne pollen, it will not result in fertilisation as mechanical damage has been shown to be important in stimulating pollen germination (Harris 1987). Profuse flowering of white clover throughout spring and summer provides prolonged opportunity for pollen dispersal. Therefore, pollen transfer to cultivated or naturalised white clover would be highly likely to occur given sufficient proximity and exposure.

203. Transfer of the introduced genes to naturalised white clover may increase the likelihood that the genes could spread and/or persist in the environment (away from pastures containing white clover). Transfer of the *AMV CP* gene could result in enhanced weediness where AMV is limiting white clover growth and persistence. Gene transfer to naturalised white clover populations is likely if the physical distances between these naturalised populations and the white clover pastures are close enough for cross-pollination by bees.

204. Although honey bees can travel up to 10 kilometres and distances of 2.5 kilometres are regularly recorded (Beekman & Ratnieks 2000), when there is abundant nectar source, the forage area is a lot smaller (Williams 2001). In a study on honey bee foraging on white clover, 60.9% of flower heads visited were within 10 cm of the previous head visited and only 13.6% were beyond 25 m (Weaver 1965). It has been reported that when a bee visits a pollen donor, that particular pollen is deposited on the next 15 to 20 inflorescences and then sporadically up to the 50<sup>th</sup> inflorescence (Marshall et al. 1999). Bees have been reported to visit an average of 2.5-3.5 white clover florets per inflorescence (Michaelson-Yeates et al. 1997; Weaver 1965) and generally the pollen most effective in fertilisation comes from the last pollen donor visited by the bee (Michaelson-Yeates et al. 1997).

205. There are a number of reports of outcrossing rates for white clover in the scientific literature. Any differences are likely due to the cultivars used, experimental design, differences in the size of the pollen source and pollen sink and their spatial arrangement, local topology and environmental conditions (Eastham & Sweet 2002). Clifford et al. (Clifford et al. 1996) studied gene flow in white clover over two seasons, reporting 0.7% and 1.3% of seed was produced by cross-pollination at 2 metres from a pollen source. At 10 metres, these

figures had dropped to 0.02% and 0.04% (See Table 4.1). Another study on white clover similarly reported outcrossing rates of 0.68% and 0.02% at two and ten metres, respectively (Woodfield et al. 1995). These authors concluded that an isolation distance of 100 metres are adequate to minimise gene flow. When separate plots of white clover were grown various distances apart, pollen transfer between the plots were reported to be high over distances less than one metre but low when distances between pollen sources were greater than two metres (Marshall et al. 1999).

**Table 4.1 Outcrossing rates of white clover**

Table adapted from (Clifford et al. 1996)

Distance from pollen source (m)	% seed produced by pollination from source	
	1st Season	2nd Season
1	-	3
2	0.7	1.3
4	0.12	0.45
6	0.06	0.28
8	0.04	0.14
10	0.02	0.04
12	0.007	0.02
16	0.01	0.09
24	0.036	0.03
32	0.007	0
48	0.007	0
64	0	0
78	-	0
96	0	0.01
112	0.008	-
136	-	0
184	-	0
240	-	0
250	-	0.1

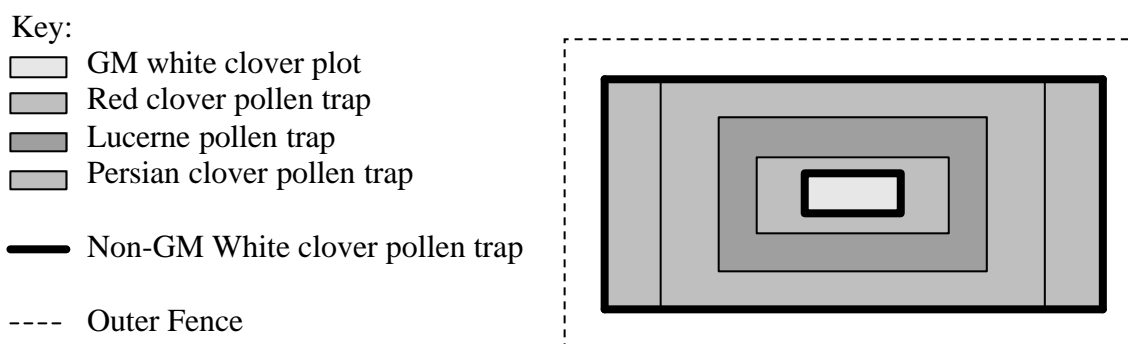
206. Various isolation distances have been applied for the certification of white clover seeds. Under the international AOSCA system, isolation distances (metres) of 302, 151 and 50 for fields of less than five acres are required for foundation, registered and certified seed, respectively (Association of Official Seed Certifying Agencies 2001). The Canadian Food Inspection Agency base their isolation distances for plants with novel traits on the AOSCA scheme (Canadian Food Inspection Agency Plant Products Directorate Plant Biosafety Office). Under the OECD scheme, seed for further multiplication must be isolated from any potential source of possible pollen contamination by 200 metres for fields of two hectares or less, or 100 metres for fields larger than 2 hectares. Seed for fodder production or amenity purposes must be isolated by at least 100 metres for fields of two hectares or less, and 50 metres for fields larger than two hectares (OECD 2003). The State of New South Wales follows the OECD regulations for white clover isolation distances.

207. The applicant proposed a number of containment measures to limit outcrossing to non-GM white clover. These include:

- limiting the size of the field trial to a maximum area of 494 square metres in each of the four planting seasons;

- using a pollen trap consisting of an inner one metre wide band of non-GM white clover, concentric 10 metre wide (minimum) bands of red clover (*T. pratense*), Persian clover (*T. resupinatum*) and lucerne (*Medicago sativa*), side strips of red clover and finally an outer one metre wide band of non-GM white clover (overall the minimum distance from the GM plot to the outer edge of the pollen trap plants would be 40 metres) (see Figure 4.1 for a schematic diagram of the proposed pollen trap);
- excluding all other white clover plants within a 500 metre radius of the trial site;
- removing flower heads from the GM white clover on a weekly basis in the absence of at least one of the pollen trap species flowering; and
- removal of mature flower heads during peak flowering time.

**Figure 4.1 Schematic diagram of the pollen trap proposed by the applicant**



208. Additional information supplied by the applicant states that regular surveying of roadsides within a one km radius of the research station would be carried out over the duration of the field trial and any white clover plants found would be destroyed by herbicide spraying.

209. However, there is the possibility that some gene flow to non-GM white clover could occur using the above containment measures, in particular the pollen trap proposed by the applicant. The reason the particular pollen trap species were selected, is to ensure that at least one species is flowering during the flowering period of GM white clover, especially during the early and late flowering period. Therefore, it is not expected that all pollen trap species flower when the GM white clover flowers. If the pollen trap proposed by the applicant was used, any non-flowering plants could potentially block gene transfer to outer flowering pollen trap species, resulting in the bees ‘missing’ the pollen trap and flying to flowers outside the trial site.

210. A study on white clover growing in a patchy habitat has shown that barley can act as a physical barrier to pollen flow between patches of white clover (Osborne et al. 2001). Hence if only one pollen trap species were to flower and it is ‘blocked’ by another species in the pollen trap, containment of pollen could be inadequate. Furthermore, although honey bee preference can often be attributed to floral resources such as flower numbers and nectar loads (Strickler & Freitas 1999), preference of bees for different flowering species is not well understood. Therefore, there is no guarantee that once a bee has visited the GM white clover flowers that it will forage onto the pollen trap species, especially if there is a relative large distance between the GM plants and the flowering pollen trap species.

211. If the above pollen trap design and isolation zone were applied and some gene flow to non-GM white clover plants did occur, the flow on effect from such gene flow could potentially provide enhanced fitness to white clover plants against AMV infection. As discussed in Appendix 3, there is uncertainty about the potential for AMV to limit white clover growth and persistence in the area surrounding the trial site. Therefore, the Regulator considers that a cautious approach should be used with regards to managing potential enhanced weediness through gene flow to non-GM white clover plants in the proposed field trial.

212. Licence conditions have been imposed to minimise the likelihood of pollen flow to non-GM white clover and the risk of enhanced weediness through gene transfer to white clover plants outside the release site by enclosing the GM plot in a bee-proof cage during the flowering period of the GM white clover and ensuring that the integrity of the cage is maintained. Additionally, any bees used in the pollination of the GM white clover are required to be killed at the end of the pollination period to ensure that no GM pollen is transferred outside the bee cage.

213. If the applicant requested for future larger scale releases of the GM white clover or reduced containment conditions (which would require a separate application and assessment process), additional information would be required on gene flow to non-GM white clover and whether AMV is limiting white clover establishment, growth and/or persistence in specific habitats (See Appendix 3 for greater discussion).

### **1.3.2 Transfer to *Trifolium* and other plant species**

214. Many other *Trifolium* species are also widely distributed throughout temperate and subtropical parts of Australia including species commonly sown in pastures such as subterranean clover (*T. subterraneum*), arrowleaf clover (*T. vesiculosum*), red clover (*T. pratense*), persian clover (*T. resupinatum*) and strawberry clover (*T. fragiferum*) (NSW Agriculture and Grassland Society of NSW Inc 2001). Although many *Trifolium* spp. are cultivated or naturalised in Australia, the likelihood of white clover and other *Trifolium* species forming viable hybrids is extremely low. This is because well-demonstrated genetic differences, such as dissimilar karyotypes, limit gene transfer to other species of *Trifolium*. Viable hybrids between white clover and a few *Trifolium* species have been produced but required human intervention. If hybrids do form naturally by cross-pollination then they tend to be sterile or have developmental abnormalities. Genetic modification of white clover is unlikely to alter this characteristic. For a detailed consideration of the likelihood of hybrids occurring, including an overview of the pollination biology of white clover, see the document "The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia", available at [www.ogtr.gov.au](http://www.ogtr.gov.au).

215. The failure of white clover to successful cross-pollinate with closely related species due to genetic incompatibilities means that white clover is also unable to cross-pollinate with more distantly related plant species. Hence, no gene transfer from GM AMV resistance white clover to other plant species will occur.

## **SECTION 2 GENE TRANSFER FROM GM WHITE CLOVER TO MICROORGANISMS**

### **Section 2.1 Nature of the gene transfer hazard**

216. The transfer of genes from plants to microorganisms cannot occur through cross pollination. Horizontal gene transfer is defined as the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Conner et al. 2003). There is growing evidence that horizontal gene transfer has been a principal force in the evolution of bacteria (Nielsen 1998; Ochman et al. 2000; Smalla et al. 2000; Stanhope et al. 2001).

217. The potential hazards associated with the introduced genes of GM AMV resistant white clover transferring to microorganisms could be highly varied, broadly depending upon the phenotype of the recipient and any changes to its survival, reproductive capacity and/or pathogenicity.

### **Section 2.2 Potential hazards from the introduced genes**

#### **2.2.1 The *AMV CP* (virus resistance) gene**

218. Bacteria expressing this gene would gain no advantage as AMV is not found in bacteria.

219. Viruses expressing this gene may display alterations in pathogenicity, host range, means of transmission or spread. The likelihood of a hazard arising due to transfer or interactions of the introduced *AMV CP* gene (or its product) with viruses is discussed in Appendix 5.

#### **2.2.2 The *nptII* (antibiotic resistance) gene**

220. Microorganisms could become resistant to the antibiotics kanomycin and neomycin. The consequences of this for human health and safety and the environment would depend on other characteristics of the microorganism (for example pathogenicity), the use and significance of the antibiotics in clinical and/or veterinary practice and whether these antibiotics limit growth or survival of the microorganism in other circumstances.

221. Some microorganisms may be limited by antibiotics, either due to the use of antibiotic medicines or in some limited environmental situations where competing microorganisms produce antibiotics. Viruses are not limited by antibiotics.

#### **Section 2.2.3 Promoters and other regulatory sequences**

222. If these sequences were to be transferred to microorganisms without the associated genes of GM AMV resistant white clover, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

223. Some of the introduced regulatory sequences are derived from plant pathogens (Cauliflower mosaic virus and *Agrobacterium tumefaciens*). However, these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants. There is a possibility that, due to sequence similarity, the viral derived regulatory sequences could recombine with the genome of another virus infecting the plants to create a novel recombinant virus. While the likelihood of recombination increases with increasing sequence relatedness, the amount of sequence change in the recipient resulting from the recombination falls. Also

the genes linked to these elements in the GM white clover will not offer any selective advantage to a virus, if transferred along with the homologous sequences.

224. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.

### **Section 2.3 Other sources of the introduced genes in the environment, and their potential for horizontal transfer**

225. Information on other sources of the introduced genes in the environment is discussed here to provide baseline information on the prevalence and transfer of these genes that would happen naturally, irrespective of the GM white clover.

226. Both the introduced genes, *AMV CP* and *nptII*, are already widespread in the environment, being derived from a common virus and a common soil bacteria, respectively. The regulatory sequences are derived from common plant viruses, bacteria or plants.

#### **2.3.1 The *AMV CP* (virus resistance) gene**

227. Many plants can be infected by AMV (at least 599 species including common crop plants and plants in the natural environment) and thus the *AMV CP* gene and the CP itself is widespread and abundant in plants. The *AMV CP* gene is derived from the Victorian AMV white clover isolate WC31, and has been shown to exhibit over 92% identity with CPs from other known AMV isolates (information supplied by applicant). Therefore, any organism that consumes or comes into contact with a cell of a plant infected by AMV will have high exposure to the *AMV CP* gene.

228. Degradation of infected plants and incorporation into the soil also potentially exposes soil dwelling microorganisms to the *AMV CP* gene.

#### **2.3.2 The *nptII* (antibiotic resistance) gene**

229. The *nptII* gene was originally isolated from mobile genetic elements (transposons) found in the plasmids and chromosomes of common bacteria. Transposons are readily transferable between bacterial species in nature. The *nptII* gene is associated with transposon Tn5 and is observed in gram negative bacteria and *Pseudomonas sp.* While it is widely dispersed in the environment, other genes that also confer resistance to neomycin and kanamycin are more common, and also readily transferable among bacterial species (Belgian Biosafety Server 1999; Smalla et al. 1994).

### **Section 2.4 Likelihood of a hazard arising through gene transfer from GM white clover to microorganisms**

230. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the characteristics of introduced gene sequences, as well as on the likelihood of the transfer itself.

231. Most gene transfers have been identified through analyses of gene sequences (Ochman et al. 2000; Worobey & Holmes 1999). In general, gene transfers are detected over

evolutionary time scales of millions of years (Lawrence & Ochman 1998). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000).

232. In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses have been exceedingly rare (Aoki & Syono 1999; Greene & Allison 1994; Harper et al. 1999; Mayo & Jolly 1991; Nielsen et al. 1998; Nielsen et al. 2000; Pittard 1997; Schoelz & Wintermantel 1993; Worobey & Holmes 1999). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Greene & Allison 1994; Nielsen et al. 1998; Nielsen et al. 2000; Pittard 1997; Schoelz & Wintermantel 1993; Worobey & Holmes 1999). However, in all cases this was achieved only under controlled conditions with the presence of related gene sequences (homologous recombination), and using powerful selection methods to detect extremely rare gene transfer events.

### 2.4.1 Bacteria

233. Mechanisms of conjugation (gene transfer between bacteria) and transduction (gene transfer from bacterial viruses to bacteria) will not be considered here as both these mechanisms are one step removed from the only possible route of plant to bacteria DNA transfer - natural transformation in the environment.

234. Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1999) and is the mechanism that is most likely to contribute to a horizontal gene transfer from GM plants to bacteria (Smalla et al. 2000). Natural transformation enables competent bacteria to generate genetic variability by taking up and integrating free DNA that is present in their surroundings. This uptake of DNA does not necessarily depend on DNA sequence, thus indicating the potential for gene transfer from divergent donor organisms (Nielsen 1998).

235. A number of steps and conditions would need to be fulfilled for functional natural transformation to occur (Bertolla & Simonet 1999), many of which are highly unlikely, making the overall likelihood of gene transfer, and of resulting hazard, extremely low:

- release of the DNA molecules from plant cells into the environment;
- persistence of the free DNA in the environment;
- presence of bacterial genotypes capable of developing competence for natural transformation;
- appropriate biotic and abiotic conditions for the development of the competent state;
- uptake of DNA fragments;
- chromosomal integration via recombination or autonomous replication of the transforming DNA;
- expression of the genes by the recipient bacterium; and
- selective advantage to fix (maintain) the transferred DNA in the gene pool of the recipient species.

236. Thus horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (Coghlan 2000; Schlüter et al. 1995). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous

sequences and under conditions of selective pressure (De Vries & Wackernagel 1998; De Vries et al. 2001; Gebhard & Smalla 1998; Mercer et al. 1999) and even then only at a very low frequency.

#### **2.4.2 Viruses**

237. There is a theoretical possibility of recombination between sequences that have been introduced into the genome of GM plants and the genome of viruses that infect the plants (Ho et al. 2000; Hodgson 2000b; Hodgson 2000a). Recombination between viral genomes and plant DNA has only been observed at very low frequencies, and only between homologous sequences under conditions of selective pressure, for example regeneration of infectious virus through complementation of a defective virus by viral sequences introduced into a GM plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999). With homologous sequences the consequent risk of adverse effects arising from gene transfer is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties is low. Refer to Appendix 5 for more detail on viral recombination.

238. The hazard of viral recombination is considered in detail in Appendix 5. The risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.3).

#### **2.4.3 Fungi**

239. Fungi are known to be transformable, and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by *Aspergillus niger* (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

240. Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.3).

### **SECTION 3 GENE TRANSFER FROM GM WHITE CLOVER TO ANIMALS**

#### **Section 3.1 Nature of the gene transfer hazard**

241. The potential hazards associated with the introduced genes in GM AMV resistant white clover transferring to animals, including humans, could be highly varied, broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny.

#### **Section 3.2 Potential hazards from the introduced genes**

##### **3.2.1 The AMV CP (virus resistance) gene**

242. The expression of this gene in animals would not be expected to lead to any significant effects, since animals are not susceptible to infection by AMV and therefore such a transfer would not confer any survival or reproductive advantage to the animal.

### 3.2.2 The *nptII* (antibiotic resistance) gene

243. Animal cells could gain the ability to degrade the corresponding antibiotics. If the transfer occurred to humans or other animals treated with these antibiotics, this may affect antibiotic treatment. However the gene product, the NPTII enzyme, would only be active within the transformed animal cell, where appropriate conditions and co-factors for activity exist, therefore interference with any antibiotic treatment is unlikely. Neomycin and kanamycin are no longer used to any significant extent as medical, veterinary, agricultural or aquaculture treatments, because they have severe side effects and many bacteria are already resistant to them (Flavell et al. 1992) so alternative antibiotics are used. Animals are not controlled by antibiotics, so no selective advantage would result.

### 3.2.3 Promoters and other regulatory sequences

244. If these sequences were to be transferred to animals without the associated genes of GM virus resistant white clover, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on the resulting phenotypic change induced.

245. Some of the introduced regulatory sequences are derived from plant pathogens (Cauliflower mosaic virus and *Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

246. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the nature of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.

## Section 3.3 Likelihood of hazard arising through gene transfer from GM white clover to animals (including humans)

247. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the likelihood of transfer itself, as well as on the characteristics of introduced gene sequences, as discussed in previous sub-sections.

248. The most significant route for entry of foreign DNA into animals, including humans, would be through food, as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract is exposed to foreign DNA released from food. Microorganisms colonise the whole length of the gastrointestinal tract, aiding the digestive process. However, the proportion of DNA derived from the introduced genes of GM plants in the animal diet is extremely low. For example, Beaver and Kemp (2000) estimate that in a diet comprising 40% GM maize, the introduced genes would represent 0.00042% of total dietary DNA intake.

249. The fate of DNA in the digestive tract of various animals has been studied and is discussed in detail in the risk assessments for DIR 021/2002 and DIR 22/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and no greater than the likelihood of transfer from other sources of the introduced genes in the environment (see Section 2.3).

250. No products from the GM white clover in the proposed field trial will be used for human food or livestock feed. Thus, the likelihood of gene transfer to animals, including humans, is negligible.

## **SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS**

### **Section 4.1 Conclusions regarding gene transfer to non-GM white clover plants**

251. Transfer of the *AMV CP* gene to non-GM white clover could potentially confer a selective advantage that results in enhanced weediness in situations where the virus is limiting the spread and persistence of white clover.

252. Gene transfer from GM virus resistant white clover to cultivated or naturalised white clover is highly likely in the absence of isolation/containment measures. However, the risks posed by the trial are low because the field trial is small with a maximum area of 494 square metres in each of the four planting seasons;

253. In addition, the Regulator has imposed licence conditions to minimise the risk of enhanced weediness through gene transfer to white clover plants outside the release site, including enclosing the GM plot in a bee-proof cage during the flowering period of the GM white clover and ensuring that the integrity of the cage is maintained (See Chapter 2 and Appendix 7).

### **Section 4.2 Conclusions regarding gene transfer to other plant species**

254. It is considered that the risk of gene transfer from GM AMV resistant white clover to other *Trifolium* species is negligible, because:

- genetic incompatibility prevents the production of fertile hybrids.

255. It is considered that the risk of gene transfer from GM AMV resistant white clover to other plant genera is negligible, because:

- well established genetic incompatibility prevents successful cross pollination with other plant species.

### **Section 4.3 Conclusions regarding gene transfer to microorganisms**

256. It is considered that the risk of a hazard arising through transfer of the introduced genes from GM AMV resistant white clover to microorganisms is negligible, because:

- the introduced genes in GM AMV resistant white clover are already widespread in the environment, and are available for transfer from these sources via demonstrated natural mechanisms; and
- gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.

### **Section 4.4 Conclusions regarding gene transfer to animals, including humans**

257. It is considered that risks through transfer of the introduced genes from the GM white clover to animals, including humans, are negligible because:

- the introduced genes in the GM white clover are already widespread in the environment;
- the GM AMV resistant white clover will not be fed to livestock nor enter the human food supply;
- the probability of interaction, uptake and integration of intact plant DNA by other organisms occurring is extremely low, especially if it involves unrelated sequences (non-homologous recombination); and
- natural events of horizontal gene flow from plants to distantly related organisms are extremely rare;
- in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely affected.

## **SECTION 5 RESEARCH REQUIREMENTS**

258. If the applicant applied for future larger scale releases of the GM white clover or reduced containment conditions, detailed information would be required on gene flow to non-GM white clover.

## APPENDIX 5 INTERACTIONS BETWEEN INTRODUCED VIRAL GENE AND VIRUSES

259. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers the potential hazards that may be posed through the interaction of the introduced viral gene and/or its product in the GM white clover with viruses that are naturally present in the plant or in the environment. These interactions may result in the modification of viral properties, which may in turn lead to increased disease burden in white clover and/or other plants.

260. The exchange of viral genes is an important evolutionary process for viruses (Gibbs et al. 1997; Keese & Gibbs 1993). In contrast, incorporation of non-viral genes into viral genomes is very rare (Mayo & Jolly 1991) and has not played an important role in the development of new variants of viruses. Therefore, the focus of this Appendix is on the potential for the introduced viral gene, *AMV CP*, or its product, to interact with viruses and lead to the adverse consequence of increased disease burden in white clover and/or other plants.

### SECTION 1 NATURE OF THE POTENTIAL HAZARD ARISING FROM INTERACTIONS BETWEEN INTRODUCED VIRAL GENE AND VIRUSES

261. The GM virus resistant white clover differs from non-GM white clover in the expression of two additional proteins. These are the Alfalfa mosaic virus (AMV) coat protein (CP) and the neomycin phosphotransferase type II (NPTII) protein. The expression of the introduced genes are controlled by regulatory elements derived from viruses, bacteria and plants. See Appendix 1 for details.

262. The *AMV CP* gene is controlled by the constitutive CaMV 35S promoter in the GM white clover. This results in expression of the *AMV CP* gene in most cells throughout the plant's lifecycle. This suggests that any viruses infecting the GM white clover will have the opportunity to interact with the introduced *AMV CP* gene and or its product.

263. White clover is known to be susceptible to at least 45 different viruses representing all major plant virus groups (Brunt et al. 1996), and a number of these viruses have been found to infect white clover in Australia (Garrett 1991; Norton & Johnstone 1998; Coutts & Jones 2002; Jones 1996). Additionally, white clover can be infected simultaneously by more than one virus (Campbell & Moyer 1984; Barnett & Gibson 1975; Barnett & Gibson 1977). Therefore, there is potential for the introduced viral gene in the GM white clover to interact with a number of different viruses.

264. Viruses that infect a GM plant with an introduced viral gene may include:

- **homologous virus** – the same species from which the introduced viral gene was derived;
- **heterologous virus** – unrelated or distantly related to the introduced viral gene; and
- **non-host virus** - a virus that may or may not multiply when injected into a cell, but is unable to spread and initiate a general infection.

265. Therefore, the introduced *AMV CP* gene in the GM white clover could interact with AMV or another virus potentially resulting in a change to the properties of the infecting virus. Modified properties of a virus can be a permanent phenomenon due to genetic changes via recombination or selection of AMV variants capable of overcoming the AMV resistance of the GM white clover. Alternatively, the modification may be short-term and/or localised due to transient changes such as complementation, transcapsidation, synergism or resurgence of a non-target virus. These mechanisms are discussed in the sections below.

266. Modification of viral properties could potentially result in increased disease burden in white clover and/or other plants. Increased disease burden can result from increased pathogenicity, a change in host range, increased viral spread, higher viral production in cells or plants, or a new means of transmission of the modified virus. The pathogenicity of a virus is a combination of infectivity or aggressiveness (the ability to infect and multiply in the host) and virulence (the degree of damage)(Bos 1999).

### **Section 1.1 Recombination**

267. Recombination is where genetic information is exchanged between two viral sequences. Persistence of a recombined viral genome is dependent on its fitness, ability to replicate, transmissibility and ability to spread systemically within the host plant (OECD 1996). Recombination between the introduced *AMV CP* gene and AMV (known as homologous recombination) should result in little alteration of viral properties but recombination with a distantly or non-related virus (heterologous recombination) would have more unpredictable effects and therefore be more likely to result in adverse consequences. Recombination could alter pathogenicity, viral spread or host range and, because recombination involves a genetic change which is passed onto progeny viruses, it has a greater potential for persistent effects compared to non-genetic changes.

### **Section 1.2 Selection of AMV variants**

268. Expression of the introduced viral gene in the GM white clover could lead to selection of naturally occurring AMV variants capable of overcoming the GM white clover's resistance to AMV (Matthews 1991). If selection pressure is maintained, a population of AMV capable of overcoming the GM plants resistance could become established and the disease burden could persist. This process is dependent on both a genetic and ecological (i.e. selection pressure) change. This will alter the balance of viral diversity but the impact is likely to be restricted to the GM crop.

### **Section 1.3 Complementation**

269. Viral genes express proteins with various functions that may complement or enhance the properties of another virus. The AMV CP is a structural protein but is also involved in multiple steps of the replication cycle and cell-to-cell movement of AMV (Bol 1999). Therefore, the AMV CP expressed in the GM white clover could potentially assist viruses that infect the GM plants in a number of different ways. Complementation could result in increased pathogenicity but, because it is only a transient change and it can only occur in the GM plant, it will not have a long-term negative impact on the environment.

## Section 1.4 Transcapsidation

270. Transcapsidation is where the genome of one virus becomes encapsidated (i.e. packaged) by the coat protein of another virus (or becomes encapsidated by CPs of both viral strains). Transcapsidation of the Cucumber mosaic virus has been shown to occur in tobacco plants expressing AMV CP. Transcapsidation may be a necessary process for virus survival/function, as for umbraviruses that rely on other viruses to provide the coat protein necessary for viral vector transmission (Taliensky & Robinson 2003), or it may offer no advantage to the encapsidated virus. AMV is transmitted from plant to plant by certain species of aphid. The aphid recognises and specifically interacts with the AMV CP as a necessary step in the transmission process (Matthews 1991). Therefore, transcapsidation of a virus by the AMV CP expressed in the GM white clover could result in transmission by an alternative vector which in turn can lead to an increased disease burden if the vector is more efficient at spreading the virus, or feeds on a different or wider range of plants with the potential of increasing the host range of the virus. However, transcapsidation is transient as it is not maintained in subsequent rounds of viral infection and therefore long-term adverse impacts are unlikely to occur.

## Section 1.5 Synergism

271. Synergism is when two or more viruses interact to produce a greater effect than the sum of their individual effects. Characteristics that could be enhanced include plant host symptoms, and/or viral levels. An example of an introduced gene having a synergistic effect on infecting viruses is the Tobacco mosaic virus movement protein on several tobamoviruses, where symptom development was accelerated, severity of the symptom formation was enhanced, and/or viral accumulation of these viruses was increased (Cooper et al. 1995). The expression of the AMV CP could also potentially have a synergistic effect on some viruses possibly by altering the metabolism or reducing the fitness of white clover, which may allow infecting viruses to have a greater impact on the GM plant compared to non-GM white clover plants. Synergism is a transient change in the GM plants and is unlikely to pose a long-term hazard.

## Section 1.6 Resurgence of a non-target virus

272. Exclusion of AMV may allow other viruses, that would not normally become established when AMV is present, to become established in the GM white clover plants. This would produce a transient ecological change, requiring the continued presence of the GM white clover, and is unlikely to pose a long-term hazard.

## SECTION 2 LIKELIHOOD OF THE HAZARD OCCURRING

273. In assessing the likelihood of the hazard of increased disease burden arising through interactions between the introduced viral gene (or its product) and viruses, a number of factors were considered including:

- the likelihood of interactions between viruses occurring naturally in the environment leading to persistent increased disease burden;
- other sources of the *AMV CP* gene or its product in the environment and the potential for interactions to produce increased disease burden;

- the likelihood of interactions between the introduced *AMV CP* gene or its product in the GM white clover and viruses leading to increased disease burden in white clover and/or other plants.

## **Section 2.1 Likelihood of viral interactions naturally occurring in the environment leading to increased disease burden**

274. The likelihood of naturally occurring viral interactions leading to increased disease burden is discussed here to establish a baseline for comparison with interactions involving the introduced *AMV CP* gene (or its product) being considered in this risk assessment.

275. Viral interactions that produce permanent genetic changes, i.e recombination and selection of AMV variants (see above in Section 1), are the most likely interactions to cause persistent adverse effects (Richards & Scown 2002). The other interactions discussed in Section 1 of this Appendix (i.e complementation, transcapsidation, synergism, and resurgence of a non-target virus) are transient effects, and therefore the likelihood that these interactions could lead to long-term adverse impacts is very low.

276. Although the occurrence of viral strains capable of overcoming the resistance of the GM plants is a natural genetic change, the establishment of new viral variants is dependent on continuous selection pressure. Because the proposed field trial of GM AMV resistant white clover is small and only for a short time period (four planting seasons), the likelihood of new AMV variants arising that overcome the CP-mediated resistance of GM white clover during the trial is very low. Furthermore, even if new AMV variants do occur, it is unlikely that severity of AMV infection will be different to existing AMV variants. This issue is discussed further in Appendix 6.

277. Therefore, only the likelihood of recombination between viral sequences in the natural environment and the potential for recombination to lead to increased disease burden will be discussed below as this interaction is the most likely to result in adverse impacts when considering the current application (Richards & Scown 2002). Consideration is given to natural recombination levels between two viruses as well as between viral sequences within a plant genome and an infecting virus.

278. Homologous recombination is the most likely type of recombination to occur in nature. Homologous recombination occurs naturally within viral populations (Bruyere et al. 2000; Candresse 1997) whenever there is a viral infection of a plant, but survival, competitiveness and multiplication are constrained by the natural selection of emerging recombinants.

279. However, even if homologous recombination does occur between viral sequences, most homologous exchanges give rise to a progeny virus with highly similar or identical gene sequences and therefore likely to result in similar virus properties. Therefore, the likelihood that this type of recombination event resulting in increased disease burden is very low.

280. Heterologous recombination is an important natural evolutionary process for viruses (Gibbs et al. 1997) and is more likely to generate significant changes in viral properties such as pathogenicity, host range or vector specificity than is homologous recombination. However, it tends to occur at a lower frequency than homologous recombination. For example, in the Brome mosaic bromovirus, heterologous recombination occurs 5-10 times less frequently than homologous recombination (Figlerowicz & Bujarski 1998). Additionally, heterologous recombination is much more likely to produce less competitive recombinants

rather than viable recombinants capable of increasing disease burden in plants. Heterologous recombinants that have a selective advantage and give rise to an increased disease burden have occasionally been observed in nature (Pita et al. 2001).

281. Endogenous viral sequences are already present within plant genomes (Harper et al. 2002) and, like introduced viral genes in GM plants, these sequences have the potential to undergo recombination with infecting viruses. However, these endogenous viral sequences appear to be involved in disease resistance and have not been reported to contribute to new viral infections in nature (Mette et al. 2002).

## **Section 2.2 Other sources of the *AMV CP* gene or its product in the environment and the potential for interactions to produce increased disease burden**

282. As discussed in Appendix 2, Section 2.3, *AMV CP* is naturally produced by *AMV* which is a virus widespread in the environment, being reported to infect at least 599 plant species in 245 genera in 68 families (Edwardson & Christie 1986). Species commonly infected by *AMV* include pasture plants such as white clover and lucerne, food crops such as tomatoes, beans, peas and capsicums, and flowering garden plants such as sweet william (*Dianthus barbatus*) and snapdragon (*Antirrhinum majus*). Therefore, the *AMV CP* gene and its product are widely available in the natural environment, providing opportunities for interactions with viruses. Nevertheless, the likelihood of naturally occurring *AMV CP* gene (or its product) leading to increased disease burden due to interactions with viruses is very low (refer to Section 2.1 for detailed discussion on likelihood of interactions between viruses producing increased disease burden).

## **Section 2.3 Likelihood of increased disease burden arising as a result of interactions between the introduced *AMV CP* gene (or its product) in the GM white clover and viruses**

283. As discussed above in Section 2.1 of this appendix, the likelihood of increased disease burden arising and persisting long-term due to interactions between the introduced *AMV CP* gene (or its product) and infecting viruses in the GM white clover plants is negligible for the interactions involving complementation, transcapsidation, synergism, and resurgence of a non-target virus. The likelihood of selection of *AMV* variants arising that can overcome the *AMV* resistance of the GM white clover is discussed in Appendix 6.

284. Therefore, only likelihood of increased disease burden arising and persisting long-term due to recombination between the introduced *AMV CP* gene (or its product) and infecting viruses is discussed in detail below.

285. Homologous recombination between introduced viral genes and infecting viruses has been shown to occur in GM plants (Adair & Kearney 2000; Greene & Allison 1994; Schoelz & Wintermantel 1993) although it appears that the frequency of homologous recombination varies for different regions of the virus genome (Bruyere et al. 2000). For example, the absence of the 3' untranslated region from an introduced *CP* gene of Cowpea chlorotic mottle virus in a GM plant was found to discourage homologous recombination (Greene & Allison 1996).

286. It is uncertain whether the presence of the *AMV CP* gene in the GM white clover plant will alter the frequency of natural recombination with viruses. Potentially, recombination frequencies between the *AMV CP* gene and viruses could be greater in the GM white clover

plants than would naturally occur. Firstly, all the GM white clover plants express the *AMV CP* gene and the gene is expressed in most cells throughout the plant, hence there is the potential for greater exposure of the gene to infecting viruses than might occur in non-GM white clover plants. Secondly, the *AMV CP* gene expressed in the GM white clover consists of the complete coding region as well as the 3' and 5' untranslated region of the gene, whereas if the 3' untranslated region had been removed the potential for recombination could possibly have been reduced (Greene & Allison 1996).

287. Conversely, recombination between the *AMV CP* gene and viruses could be lower than those naturally occurring in the environment. Firstly, RNA expression levels of the *AMV CP* gene are several-fold lower in GM white clover plants than in infected non-GM white clover plants. Secondly, the AMV virus cannot establish itself in the GM white clover (results from previous glasshouse and field trials) so the opportunity for homologous recombination between the introduced *AMV CP* gene and AMV is very low.

288. Even if the frequency of recombination between the introduced *AMV CP* gene and a virus is higher than recombination levels that would normally occur in nature, the likelihood that recombination will lead to increase in disease burden needs to be considered. The presence of the complete *AMV CP* gene, which encodes a functional CP, could potentially increase the likelihood that recombination could alter the properties of an infecting virus. This is because the AMV CP has many functions in AMV and its presence in another virus could potentially alter or enhance the pathogenicity of that virus (Bol 1999). However, as discussed in Section 2.1, recombination between introduced viral genes in GM plants and viruses has not resulted in increased disease, and endogenous viral sequences already present within plant genomes do not appear to contribute detectably to new viral infections in nature. Therefore, the likelihood of GM white clover giving rise to an increased disease burden due to recombination between the introduced viral gene and infecting viruses is likely to be very low, and similar to the likelihood from natural recombination events in non-GM white clover.

289. Furthermore, the likelihood of GM white clover giving rise to an increased disease burden due to recombination between the AMV CP gene and viruses during the proposed release is very low because the field trial is small (an area of 494 square metres at any one time) and it will be conducted for a short period of time (i.e four planting seasons).

290. As a licence condition, the applicant is required to examine the disease status of the GM white clover plants during the trial to determine whether novel viruses (which includes new AMV variants) emerge due to recombination (or other interactions).

### SECTION 3 CONCLUSIONS

291. The likelihood of the hazard of increased disease burden arising and persisting long-term due to modified viral properties caused by interactions between the introduced *AMV CP* gene (or its product) and infecting viruses in the GM white clover plants is very low. This is because:

- the proposed field trial is small (an area of 494 square metres at any one time) and for only for a short period of time (four planting seasons);
- the *AMV CP* gene (and its product) is naturally widespread in the environment;
- many interactions between viral genes produce transient changes and therefore are unlikely to have a persistent adverse impact;

- the likelihood of homologous recombination between the introduced *AMV CP* gene and infecting AMV is uncertain; but even if homologous recombination does occur, the likelihood that it will have an adverse impact is very low;
- natural heterologous recombination rarely produces adverse effects; and
- the likelihood of heterologous recombination between the introduced *AMV CP* gene and infecting viruses in the GM white clover is uncertain; but if heterologous recombination does occur, the likelihood that it will have an adverse impact is very low.

#### **SECTION 4 RESEARCH REQUIREMENTS**

292. As a licence condition, the applicant is required to examine the disease status of the GM white clover plants during the trial to determine whether novel viruses emerge.

## APPENDIX 6 ANTI-VIRAL RESISTANCE

293. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. This Appendix considers hazards that may be posed to the environment through the potential for new Alfalfa mosaic virus (AMV) variants to arise that overcome AMV coat protein (CP)-mediated resistance of the GM white clover, and causing increased disease burden in white clover or other plant species.

### SECTION 1 REGULATION OF AGRICULTURAL CHEMICALS IN AUSTRALIA

294. Regulation of agricultural chemicals is principally the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the *Agricultural and Veterinary Chemicals Code Act 1994* (the Ag Vet Code Act). The viral CP produced by the GM white clover proposed for release falls under the Ag Vet Code Act definition of an agricultural chemical product, due to its anti-viral action, and is thus subject to regulation by the APVMA.

295. The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to the use of a product that is already on the market must also be referred to the APVMA. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits for experimental work that allow restricted use of an agricultural chemical, for example, for a limited period of time or for a limited area.

296. In considering applications for registration or permits, as well as considering potential health and environmental impacts, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as efficacy and the trade implications of residues. The hazard of development of resistance to agricultural chemicals is part of the APVMA's assessment of agricultural chemical use. The APVMA can impose conditions on the use of chemical products in both registrations and permits. These conditions can include restrictions on use, implementation of a resistance management plan, and ongoing reporting on compliance.

297. The *Gene Technology Act 2000* requires the regulator to consult the APVMA in relation to the assessment of licence applications involving intentional release of GMOs to the environment. The *Gene Technology (Consequential Amendments) Act 2000* places a reciprocal obligation upon the APVMA to consult the Gene Technology Regulator in relation to certain decisions regarding registrations and permits for an agricultural chemical that is or contains a genetically modified product.

298. The APVMA and the OGTR work closely together to ensure thorough coordinated assessments of parallel applications are undertaken and, wherever possible, that the timing of assessments and decisions by both agencies coincide. Further information about the APVMA's assessment and approval processes can be obtained from [www.apvma.gov.au](http://www.apvma.gov.au).

## **SECTION 2 NATURE OF THE POTENTIAL HAZARD OF ANTI-VIRAL RESISTANCE DEVELOPMENT**

299. Expression of the introduced viral gene (*AMV CP*) in the GM white clover could enhance the rate of selection of naturally occurring AMV variants capable of overcoming the CP-mediated resistance of GM white clover (Matthews 1991). If selection pressure is maintained, a population of new AMV variants with the ability to overcome the resistance of GM white clover plants to infection by AMV could become established and the disease burden could persist.

## **SECTION 3 LIKELIHOOD OF THE HAZARD OCCURRING**

300. Due to the small scale and limited duration of the proposed release, the likelihood of new AMV variants arising that overcome the CP-mediated resistance of GM white clover is very low. Furthermore, even if new AMV variants do arise, it is unlikely that severity of AMV infection will be different to that of existing AMV variants.

301. DPI (Victoria) has submitted an application to the APVMA for a research permit for the use of the viral CP gene in GM white clover for the proposed release. The hazard of anti-viral resistance development will also be assessed by the APVMA in considering DPI (Victoria)'s research permit application. The APVMA would impose conditions if it considered this necessary to manage any risks.

## **SECTION 4 CONCLUSIONS REGARDING ANTI-VIRAL RESISTANCE**

302. The risk of AMV variants arising that overcome the CP-mediated resistance of GM white clover and cause increased disease burden in white clover or other plant species is negligible. This is due to the small scale and limited duration of the proposed release and because the severity of AMV infection is unlikely to be different to that of existing AMV variants. This hazard will be assessed by the APVMA in considering DPI (Victoria)'s permit application for the use of the viral CP as an agricultural chemical product in the GM white clover.

303. Therefore, the Regulator has not imposed specific conditions in relation to management of AMV variants arising that overcome the CP-mediated resistance of GM white clover. However, the requirement to comply with any conditions imposed by the APVMA has been noted in the licence.

## APPENDIX 7 LICENCE CONDITIONS

The *Gene Technology Act 2000* (Cth) and corresponding state and territory legislation form a substantial part of a range of integrated regulatory measures relevant to controlling genetically modified organisms (GMOs) and their use.

The Gene Technology Regulator is required to consult with, and take into account advice from, a range of regulatory authorities on risks to human health and safety and the environment in assessing applications for dealings involving the intentional release of GMOs into the Australian environment.

### **Note in relation to Anti-viral Resistance Management**

The *Gene Technology (Consequential Amendments) Act 2000* (Cth) requires the Australian Pesticides and Veterinary Medicines Authority (APVMA) to consult the Gene Technology Regulator for the purposes of making certain decisions regarding registration or issuing a permit for a chemical product that is or contains a genetically modified product.

The GMOs referred to in this licence falls under the *Agricultural and Veterinary Chemicals Code Act 1994* (Cth) definition of agricultural chemical product, due to the production of a viral coat protein which confers resistance to infection by Alfalfa mosaic virus, and is therefore subject to regulation by the APVMA. The APVMA assesses the hazard of development of anti-viral resistance as part of its evaluation process and would impose conditions to manage any risks. Therefore, the conditions of this licence do not relate to management of anti-viral resistance, and do not displace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to dealings with the GMOs covered by this licence.

## SECTION 1 INTERPRETATIONS AND DEFINITIONS

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

- (a) Words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;
- (b) Words importing a gender include any other gender;
- (c) Words in the singular include the plural and words in the plural include the singular;
- (d) Words importing persons include a partnership and a body whether corporate or otherwise;
- (e) References to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
- (f) Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning; and
- (g) Specific conditions prevail over standard conditions to the extent of any inconsistency.

In this licence:

**‘Act’** means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law;

**‘Autumn’** means the period 1 March to 31 May;

**‘Clean’** (or **‘Cleaned’**), as the case requires, means:

- (a) in relation to a Location or other area, the Destruction of the plants in that Location or area, to the reasonable satisfaction of the Regulator; or
- (b) in relation to Equipment, the removal and Destruction of plants or plant material from the Equipment, to the reasonable satisfaction of the Regulator;

**‘Cultivate’** means plough to a depth of not more than 25mm;

**‘Destroy’**, (or **‘Destroyed’** or **‘Destruction’**) means, as the case requires, killed by one or more of the following methods:

- (a) stalk pulling; or

- (b) uprooting by ploughing; or
- (c) burning; or
- (d) treatment with herbicide; or
- (e) hand weeding;

*Note: ‘As the case requires’ has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.*

**‘Equipment’** includes machinery, harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), clothing and tools used in connection with this licence;

**‘GM’** means genetically modified;

**‘GMOs’** means the genetically modified organism or organisms authorised for release by this licence;

**‘Inner Fence’** means the rabbit-proof fence surrounding the Location with the GPS corner coordinates set out at Attachment C;

**‘Location’** means the area of land where the GMOs are planted and grown;

**‘Material from the GMOs’** means genetically modified material, including parts of GMOs that are derived from or produced by the GMOs;

**‘Natural Waterways’** means waterways other than irrigation channels, holding dams or storage ponds used to collect water runoff from irrigated areas;

**‘OGTR’** means the Office of the Gene Technology Regulator;

**‘Outer Fence’** means the stock-proof fence around the Location with the GPS corner coordinates set out at Attachment C;

**‘Regulator’** means the Gene Technology Regulator;

**‘Sign-off’** means a notice in writing from the Regulator, in respect of a place, that inspection conditions no longer apply in respect of that place;

**‘Spring’** means the period 1 September to 30 November;

**‘Volunteer plants’** means progeny of the GMOs GM or non-GM White Clover;

**‘White Clover’** means *Trifolium repens* L.

## **SECTION 2 GENERAL CONDITIONS**

### **Duration of Licence**

1. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with GMOs are authorised during any period of suspension.

### **Holder of Licence**

2. The holder of this licence ('the licence holder') is the Victorian Government Department of Primary Industries.

### **Project Supervisor**

3. The Project Supervisor in respect of this licence is identified at Attachment A.
4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

### **No dealings with GMOs except as authorised by this licence**

5. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

### **GMOs covered by this licence**

6. The GMOs covered by this licence are described at Attachment B.

### **Permitted dealings**

7. The permitted dealings with the GMOs are to plant and grow 4 crops of the GMOs and to conduct experiments on the GMOs that are grown. The permitted dealings includes the possession, storage, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings.

### **Persons covered by this GMO licence**

8. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs grown in a Location pursuant to this Licence.

### **Informing people of their obligations**

9. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:
  - (a) the particular condition (including any variations of it);
  - (b) the cancellation or suspension of the licence;
  - (c) the surrender of the licence.

10. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

#### **Licence holder to notify of circumstances that might affect suitability**

11. The licence holder must immediately, by notice in writing, inform the Regulator of:
  - (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
  - (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;
  - (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of his licence to meet the conditions in it.

#### **Additional information to be given to the Regulator**

12. It is a condition of a licence that the licence holder inform the Regulator if the licence holder:
  - (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
  - (b) becomes aware of any contraventions of the licence by a person covered by the licence; or
  - (c) becomes aware of any unintended effects of the dealings authorised by the licence.

#### **People dealing with GMOs must allow auditing and monitoring of the dealing**

13. If a person is authorised by this licence to deal with GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

#### **Remaining an accredited organisation**

14. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

### **SECTION 3      SPECIFIC CONDITIONS**

#### **Permitted period during which dealings may be undertaken**

1. The permitted dealings with the GMOs (other than disposal of the GMOs) may be only undertaken during the permitted period.
2. The permitted period commences on the date of issue of this licence and concludes on 30 April 2007.

#### **One crop at a time to a maximum of 4 crops**

3. Only 1 crop of the GMOs may be grown at a time. Not more than 4 crops of the GMOs may be grown during the permitted period.

#### **Location and restrictions on the size of trial**

4. The permitted dealings may be undertaken at a single Location on land at the Department of Primary Industries field station on Mount Napier Road, Hamilton, Victoria.
5. The Location must not be larger than 494 square metres (or 26m x 19m).
6. The Location must not be within 50m of a Natural Waterway.
7. The licence holder must be able to access and control a Location to the extent necessary to comply with this licence, for the duration of the life of the licence.

#### **Notification of planting of the GMOs**

8. The licence holder must provide a notice in writing to the Regulator each time a crop of the GMOs are planted at the Location.
9. The notice must set out:
  - (a) the date on which planting of the GMOs commenced;
  - (b) the period during which the licence holder considers the GMOs are likely to flower;  
and
  - (c) the period during which the licence holder considers the Location will be Cleaned (after the GMOs have been grown there).
10. The notice must be provided to the Regulator within 14 days of the date on which planting of the GMOs commenced.

#### **Notice of Cleaning of Location**

11. The licence holder must provide a notice in writing to the Regulator when the Location is Cleaned pursuant to this licence.

12. The notice must be provided to the Regulator within 14 days of the date on which Cleaning the Location concluded.

**Location must be within a bee cage while GMOs are flowering**

13. Whenever there are flowers on the GMOs, the Location must be inside a bee-proof cage.
14. The bee-proof cage must be lockable and must be kept closed and locked at all times other than when there are people working inside it.
15. When people are working inside the bee-proof cage, the door on the cage must be kept closed.
16. Bees may be placed inside the bee-proof cage.
17. If there are live bees inside the bee-proof cage, reasonable steps must be taken to prevent the bees from escaping from the bee-proof cage.
18. The bee-proof cage cannot be moved, taken down or otherwise interfered with unless all the bees inside it have been killed.

**Seed and GM material may be collected for research and stored for the purposes of this licence**

19. Seed and Material from the GMOs may be collected from the Location for the purpose of conducting experiments on the seed and Material from the GMOs.
20. Seed and Material from the GMOs that is collected from the Location may only be transported off the Location to the extent necessary to transfer it to a facility certified by the Regulator to physical containment level PC2.
21. Seed and Material from the GMOs that is collected from the Location may be stored to the extent necessary to enable other permitted dealings contemplated by this licence to be undertaken. Where seed or Material from the GMOs is stored, it must be stored in a sealed container, within a locked facility that is signed so as to indicate that GM seed or Material from the GMOs is stored within the facility.

**Conditions in relation to the Cleaning of Location after each crop of GMOs is grown**

22. After each crop of the GMOs is grown, the Location must be Cleaned.

**General conditions in relation to the Cleaning of all other places and Equipment used in connection with this licence**

23. If:
  - (a) an area or place other than the Location is used in connection with this licence; or
  - (b) Equipment is used in connection with the GMOs or Material from the GMOs;then that area, place or Equipment must also be Cleaned.

24. Cleaning must occur immediately or as soon as practicable after the use and before it is used for any other purpose.
25. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned. (It is not necessary for Equipment to be Cleaned only at a Location.)
26. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with these Cleaning conditions.

#### **Inspections that must take place while a bee proof cage is over the location**

27. Commencing on the day that a bee proof cage is placed over the Location, the following places must be inspected for the existence of Volunteer plants:
  - (a) the area between the Inner Fence and the Outer Fence;
  - (b) irrigation channels and drains through which water flows to and from the Location;
  - (c) any areas used to Clean Equipment.
28. Whenever a bee proof cage is in place over the Location, the structural integrity of the cage must be inspected weekly. Any damage to the cage that could result in the escape of bees from the cage must be immediately:
  - (a) repaired; and
  - (b) reported to the Regulator in writing.

#### **Inspections that must take place while there is no bee-proof cage in place over the Location**

29. The following places must be inspected for the existence of Volunteer plants during the permitted period unless there is a bee-proof cage in place over the Location:
  - (a) the Location;
  - (b) the area between the Inner Fence and the Outer Fence;
  - (c) irrigation channels and drains through which water flows to and from the Location;
  - (d) any areas used to Clean Equipment.

#### **Inspections that must take place after fourth crop of GMOs is Cleaned**

30. Following Cleaning of fourth crop of the GMOs at the Location, the following places must be inspected for the existence of Volunteer plants:
  - (a) the Location;
  - (b) the area between the Inner Fence and the Outer Fence;
  - (c) irrigation channels and drains through which water flows to and from the Location;

(d) any areas used to Clean Equipment.

31. If:

- (a) inspections in respect of a place have been routinely completed for a period of at least 5 years; and
- (b) inspection records for that place show that no Volunteers have been observed in the most recent 12 month inspection period;

then the licence holder may make written application to the Regulator that these inspection conditions no longer apply in respect of that place.

**General conditions that apply wherever inspections must be undertaken for the existence of Volunteer plants**

- 32. The following conditions apply wherever this licence requires inspections to be undertaken in connection with the possible existence of Volunteer plants.
- 33. Inspection must be performed by a person who is able to recognise Volunteer plants.
- 34. The results of inspection activities must be recorded in a logbook. The logbook must be available on request for examination or photocopying by the OGTR. The findings of the inspections as recorded in the logbook must be included in the licence holder's annual report to the Regulator. The logbook must contain at least the following:
  - (a) details of the areas inspected;
  - (b) details of the date of inspection;
  - (c) the names of the person or persons who undertook the monitoring and details of the experience, training or qualification that enabled them to recognise Volunteer plants;
  - (d) the number of Volunteer plants observed, if any;
  - (e) details of the development stages reached by the Volunteer plants, if any; and
  - (f) details of methods used to Destroy Volunteer plants, if any.
- 35. Any Volunteer plant identified must be Destroyed prior to the plant flowering.
- 36. Unless this licence provides otherwise, a place must be inspected at least once every month, either until this licence expressly provides that inspections are no longer required, or until the Regulator has issued a sign-off.
- 37. Inspection conditions do not apply in respect of a place if the Regulator has issued a sign-off in respect of that place.

**Restrictions in relation to areas and plants while GMOs are being grown**

- 38. From the date of commencement of this licence until the day the last crop of the GMOs is Cleaned:

- (a) no plants other than White Clover may be planted in the Location;
  - (b) no plants may be grown between the Inner Fence and the Outer Fence unless the licence holder has received prior written approval from the Regulator to grow the plants; and
  - (c) plants, or parts of plants, from the Location or from between the Inner and the Outer Fence (including Material from the GMOs), must not be used as animal feed and must not enter the human food supply.
39. Without limiting condition 38(c) above, honey from bees that have had access to the GMOs must not be fed to animals or humans.

#### **Restrictions in relation to areas and plants after the fourth crop of GMOs is grown**

40. Subject to the requirements in this licence for shallow cultivation of certain areas once the fourth crop of the GMOs has been grown, from the day the last crop of the GMOs is Cleaned until the licence holder has received a sign-off:
- (a) no plants of any kind may be planted in the Location;
  - (b) no plants may be grown between the Inner Fence and the Outer Fence unless the licence holder has received prior written approval from the Regulator to grow the plants; and
  - (c) plants, or parts of plants, from the Location or from within the Outer Fence (including Material from the GMOs), must not be used as stockfeed and must not enter the human food supply.

#### **Requirement to manage White Clover seed bank by shallow Cultivation and irrigation in Spring and Autumn**

41. Commencing 1 September 2007, the licence holder must Cultivate the Location each Spring and each Autumn until such time as the licence holder has received a Sign-off in connection with the Location. In the 14 days following Cultivation of the Location, the Location must receive either 25mm of rain or an equivalent level of combined irrigation and rainfall.

#### **Transportation of the GMOs and Material from GMOs**

42. Subject to the condition immediately below in respect of transportation, the GMOs and Material from the GMOs must be transported in accordance with the OGTR Guidelines for the Transport of GMOs (June 2001) issued by the Regulator.
43. Every container used to transport the GMOs or Material from the GMOs must be labelled:
- (a) to indicate that it contains GM White Clover; and
  - (b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.

## Contingency Plans

44. Within 30 days of the date of the commencement of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs or Material from the GMOs outside an area that must be inspected.
45. The Contingency Plan must include details of procedures to:
  - (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;
  - (b) destroy any of the GMOs or Material from the GMOs; and
  - (c) inspect and Destroy any Volunteer plants that may exist as a result of the event.
46. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs or Material from the GMOs is discovered outside an area that must be inspected.

## Compliance Management Plan

47. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with these conditions and document that compliance.

## Reporting

48. The licence holder must provide the Regulator with a written report within 90 days of each anniversary of this licence, in accordance with any Guidelines issued by the Regulator in relation to annual reporting. This report must include information on any adverse impacts on human health and safety or the environment, caused as a result of the GMOs or Material from the GMOs.

## Research requirements

49. The licence holder must, in consultation with the OGTR, develop an agreed research program to collect information regarding:
  - (a) expression levels of the proteins of the introduced Alfalfa mosaic virus coat protein (*AMV CP*) and neomycin phosphotransferase type II (*nptII*) genes in different parts of the plants under Australian field conditions;
  - (b) agronomic characteristics of the GMOs, with a view to seeking to determine the potential weediness of the GMOs under Australian field conditions;
  - (c) the disease status of the GMOs during the course of this licence, with a view to seeking to determine whether novel viruses emerge.
50. In accordance with any Guidelines issued by the Regulator in relation to annual reporting, the licence holder must provide the Regulator with a written report of the

progress and results of the research program. This report must accompany the annual report to be sent to the Regulator.

### **Testing methodology**

51. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence (at Attachment B) in a recipient organism. The instrument must be provided within 12 months of the issuing of this licence.

## **APPENDIX 8 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES**

### **SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA**

304. *The Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

305. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

306. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Australian Government regulatory agency located within the Health and Ageing portfolio.

307. The Act prohibits persons from dealing with GMOs unless the dealing is exempt, a Notifiable Low Risk Dealing, on the Register of GMOs, or licensed by the Regulator (see section 31 of the Act).

308. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans (RARMPs) are discussed in detail in Division 4, Part 5 of the Act and summarised below.

309. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website ([www.ogtr.gov.au](http://www.ogtr.gov.au)).

### **SECTION 2 THE LICENCE APPLICATION**

310. Licence applications for dealings involving the intentional release (DIR) of a genetically modified organism into the environment must be submitted in accordance with the requirements of section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:

- the parent organism;
- the GMOs;
- the proposed dealing with the GMOs;
- interaction between the GMOs and the environment;
- risks the GMOs may pose to the health and safety of people;
- risk management;
- previous assessments of approvals; and
- the suitability of the applicant.

311. The application must also contain:

- additional information required for a GMO that is:
  - a plant;
  - a micro-organism (not living in or on animals and not a live vaccine);
  - a micro-organism that lives in or on animals;
  - a live vaccine for use in animals;
  - a vertebrate animal;
  - an aquatic organism;
  - an invertebrate animal;
  - to be used for biological control;
  - to be used for bioremediation; and
  - intended to be used as food for human or vertebrate animal consumption;
- supporting information from the Institutional Biosafety Committee.

312. A preliminary screening of an application is undertaken by OGTR staff to determine whether it complies with the Act and the Regulations, by containing the required information. If this information is provided in the application, the Regulator may then accept the application for formal consideration. section 43 of the Act provides that the Regulator is not required to consider an application if the application does not contain the required information.

313. After accepting an application for consideration, the Regulator must decide to issue, or refuse to issue, a licence. The decision must be taken following an extensive consultation and evaluation process, as detailed in sections 3-6 of this Appendix. Regulation 8 of the Regulations prescribe a period of 170 working days within which this decision must be taken. This period does not include weekends or public holidays in the Australian Capital Territory. Also, this period does not include any days in which the Regulator is unable to progress the application because information sought from the applicant in relation to the application has not been received.

### **SECTION 3 THE INITIAL CONSULTATION PROCESSES**

314. In accordance with section 50 of the Act, the Regulator must seek advice in preparing a RARMP from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);
- prescribed Australian Government agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
- the Australian Government Minister for Environment and Heritage; and
- relevant local council(s) where the release is proposed.

315. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and

safety of people or to the environment, the Regulator must publish a notice (in national and regional news papers, in the *Gazette* and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

316. As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application's receipt on the OGTR website and directly advising those on the OGTR mailing list. Copies of applications are available on request from the OGTR.

## SECTION 4 THE EVALUATION PROCESSES

317. The risk assessment process is carried out in accordance with the *Act* and *Regulations*, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Australian Government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

318. In undertaking a risk assessment, the following are considered and analysed:

- the data presented in the proponent's application;
- data provided previously to GMAC, the interim OGTR or the OGTR in respect of previous releases of relevant GMOs;
- submissions or advice from States and Territories, Australian Government agencies and the Australian Government Minister for Environment and Heritage and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

319. In considering this information and preparing the RARMP, the following specific matters are taken into account, as set out in section 49 and required by section 51 of the Act:

- the risks posed to human health and safety or to the environment;
- the properties of the organism to which the dealings relate before it became a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred on the properties of the organism;
- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings;
- any likely impacts of the proposed dealings on the health and safety of people.

320. In accordance with Regulation 10 of the Regulations, the following are also taken into account:

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
  - be harmful to other organisms;
  - adversely affect any ecosystems;
  - transfer genetic material to another organism;
  - spread, or persist, in the environment;
  - have, in comparison to related organisms, a selective advantage in the environment;
  - and
  - be toxic, allergenic or pathogenic to other organisms.
- the short and long term when taking these factors into account.

## SECTION 5 FURTHER CONSULTATION

321. Having prepared a risk assessment and a risk management plan, the Regulator must, under section 52 of the Act, seek comment from stakeholders, including those outlined in section 3 and the public.

322. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. Section 56 of the Act requires that these be taken into account in making a decision on whether or not to issue a licence for the proposed release.

323. Comments received in written submissions on this RARMP are very important in shaping the final RARMP and in informing the Regulator's decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the final RARMP.

324. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to **protect human health and safety and the environment**. Matters in submissions that do not address these issues and/or concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

## SECTION 6 DECISION ON LICENCE

325. Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (section 55 of the Act). The Regulator must not issue the licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.

326. The Regulator must also be satisfied, under section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence e.g.:

- any relevant convictions;
- any relevant revocations or suspensions of a licences or permits; and
- the capacity of the person or company to meet the conditions of the licence.

327. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

328. The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.

329. If a licence is issued, the Regulator may impose licence conditions (section 62 of the Act). For example, conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- specify waste disposal methods;
- manage risks posed to the health and safety of people, or to the environment;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur;
- require contingency planning in respect of unintended effects of the dealings; and
- limit the dissemination or persistence of the GMO or its genetic material in the environment.

330. It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (section 63 of the Act). Access to the site of a dealing must also be provided to persons authorised by the Regulator for the purpose of auditing and monitoring the dealing and compliance with other licence conditions (section 64 of the Act). It is a condition of any licence that the licence holder inform the Regulator of:

- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
- any contraventions of the licence by a person covered by the licence; and
- any unintended effects of the dealings authorised by the licence.

331. It should be noted that, as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to enforce compliance with licence conditions, and indeed, to direct a licence holder to take any steps the

Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors trial sites to determine whether the licence holder is complying with the licence conditions, or whether there are any unforeseen problems.

## APPENDIX 9 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

**Submission from: I:** individual

**Issues raised/consideration:** **App:** appendix; **EN:** environmental risks; **ETH:** ethical concerns; **FS:** feed safety; **H:** human health and safety; **HR:** herbicide resistance; **IR:** insecticide resistance; **M:** markets; **NA:** not applicable; **OSA:** outside scope of the assessment.

Sub. No.	Type	Summary of issues raised	Issue	Consideration of issue
1	I	There is no proof and insufficient testing of the long term effects of GM white clover on animal and human health.	H, EN	App 2
		It is false to suggest that the clover release may be harmless because there were no reports of adverse effects on human health and safety or the environment from the four previous releases of GM white clover resistant to AMV". These releases were not designed to show up any such effects.	H, EN	App 2-6
		This release is for the purpose of assessing commercial viability and practicability of the GM white clover, and not for the purpose of investigating effects on health and safety.	H	App 2
		It may take many years for genetic instability of GM white clover to be linked to epidemiological evidence of harm to humans or the environment.	H, U	App 1
2	I	As advised previously, holds concerns about the introduction of GMOs into the environment of a particular region (although not the region where the current trial is proposed). These concerns include:		Noted
		insufficient buffer areas between GM plants and normal crops (due to pollen capable of travelling several kilometres);	G	App4
		the potential to breed a poison-resistant weed;	HR	NA
		the potential to breed pesticide-resistant bugs;	IR	NA
		potential health concerns stemming from what is not known of the effect on the environment and the food chain from these newly developed plants;	D, EN, FS, H	App 2-6
		the detrimental effect of cross-breeding or cross-contamination of crops on existing and future export markets; and	M	OSA
		feels there are ethical consequences of biotechnology that have yet to be addressed by industry or Government.	ETH	OSA
		Does not wish to see trials of GM plants occurring in a particular region of Australia (although not the region where the current trial is proposed).		Noted
3	I	Objects to the application and feels the trial should be disallowed because the trial is to determine agronomic performance and should be preceded by tests for health and safety and ecological consequences.	H, E, D	App 2-6

## APPENDIX 10 REFERENCES

- Adair, T.L., Kearney, C.M. (2000). Recombination between a 3-kilobase tobacco mosaic virus transgene and a homologous viral construct in the restoration of viral and nonviral genes. *Archives of Virology* **145**: 1867-1883.
- ANZFA (2000). Draft risk analysis report, application A355: Food produced from glyphosate-tolerant cotton line 1445. Australia New Zealand Food Authority, Canberra, Australia. pp 1-78.
- ANZFA (2001a). Final assessment report. Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and Oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines MS1, MS8, RF1, RF2 and RF3. 05/02, Australia New Zealand Food Authority, Canberra, Australia. pp 1-88.
- ANZFA (2001b). Food derived from glyphosate-tolerant cotton line 1445 - A safety assessment. Australia New Zealand Food Authority, Canberra, Australia.
- Aoki, S., Syono, K. (1999). Horizontal gene transfer and mutation: *Ngrol* genes in the genome of *Nicotiana glauca*. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 13229-13234.
- Association of Official Seed Certifying Agencies Genetic and crop standards of the AOSCA. (2001) Available from: <ftp://www.aosca.org/opandcs.pdf>
- Astwood, J.D., Leach, J.N., Fuchs, R.L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotechnology* **14**: 1269-1273.
- Baker, H.G. (1965). The genetics of colonizing species: Characteristics and modes of origin of weeds. In "The Genetics of Colonizing Species", HG Baker, GL Stebbins, eds. Academic Press, New York and London. pp 147-172.
- Barnett, O.W., Gibson, P.B. (1977). Effect of virus infection on flowering and seed production of the parental clones of tillman white clover (*Trifolium repens*). *Plant Disease* **61**: 203-207.
- Barnett, O.W., Gibson, P.B. (1975). Identification and prevalence of white clover viruses and the resistance of *Trifolium* species to these viruses. *Crop Science* **15**: 32-37.
- Bazzaz, F.A. (1986). Life history of colonizing plants: some demographic, genetic and physiological features. In "Ecology of Biological Invasions", HA Mooney, JA Drake, eds. Springer-Verlag, New York. pp 96-110.
- Beachy, R.N. (1997). Mechanisms and applications of pathogen-derived resistance in transgenic plants. *Current Opinion in Biotechnology* **8**: 215-220.
- Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. (1982). Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* **19**: 327-336.

- Beekman, M., Ratnieks, F.L.W. (2000). Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology* **14**: 490-496.
- Beever, D.E., Kemp, C.F. (2000). Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts and Reviews, Series B: Livestock Feeds and Feeding* **70**: 175-182.
- Belanger, H., Fleysh, N., Cox, S., Bartman, G., Deka, D., Trudel, M., Koprowski, H., Yusibov, V. (2000). Human respiratory syncytial virus vaccine antigen produced in plants. *FASEB Journal* **14**: 2323-2328.
- Belgian Biosafety Server Types of antibiotics and related resistance genes. (1999) Available from: <http://www.antibioresistance.be/ARmenu.html>
- Berberich, Leimgruber, and Regan (1993). Preparation and verification of dose for a mouse acute oral toxicity study with neomycin phosphotransferase II protein (NPTII), study ML-91-409. Unpublished. Monsanto Company. Monsanto Report No. MSL:13277.
- Bergelson, J., Purrington, C.B., Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* **395**: 25.
- Bertolla, F., Simonet, P. (1999). Horizontal gene transfers in the environment: natural transformation as a putative process for gene transfers between transgenic plants and microorganisms. *Research in Microbiology* **150** (6): 375-384.
- Bevan, M. (1984). Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research* **12**: 8711-8721.
- Bol, J.F. (1999). Alfalfa mosaic virus and ilarviruses: involvement of coat protein in multiple steps of the replication cycle. *Journal of General Virology* **80**: 1089-1102.
- Bos, L. (1999). Viruses as disease incitants. In "Plant viruses, unique and intriguing pathogens - a textbook of plant virology". Backhuys Publishers, Leiden, The Netherlands. pp 21-42.
- Brimecombe, M.J., De Leij, F.A., Lynch, J.M. (2001). The effect of root exudates on rhizosphere microbial populations. In "The rhizosphere: biochemistry and organic substances at the soil-plant interface", R Pinton, Z Varanini, P Nannipieri, eds. Marcel Dekker, Inc., New York, USA. pp 95-140.
- Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L., and Zurcher, E.J. Plant viruses online: Descriptions and lists from the VIDE database. (1996) [cited 20 March 2003]. Available from: <http://biology.anu.edu.au/Groups/MES/vide/>
- Bruyere, A., Wantroba, M., Flasiński, S., Dzianott, A., Bujarski, J.J. (2000). Frequent homologous recombination events between molecules of one RNA component in a multipartite RNA virus. *Journal of Virology* **74**: 4214-4219.
- Bryngelsson, T., Gustafson, M., Green, B., Lind, C. (1988). Uptake of host DNA by the parasitic fungus *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **33**: 163-171.

Büchen-Osmond, C. The Universal Virus Database of the International Committee on Taxonomy of Viruses. (2002) Available from: <http://www.ncbi.nlm.nih.gov/ICTVdb/>

Buhariwalla, H., Mithen, R. (1995). Cloning of a *Brassica* repetitive DNA element from resting spores of *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **47**: 95-101.

Campbell, C.L., Moyer, J.W. (1984). Yield responses of six white clover clones to virus infection under field conditions. *Plant Disease* **68**: 1033-1035.

Campbell, M.H. (1966). Theft by harvesting ants of pasture seed broadcast on unploughed land. *Australian Journal of Experimental Agricultural and Animal Husbandry* **6**: 334-338.

Candresse, T. (1997). Systematic search for recombination events in plant viruses and viroids. In "Virus-resistant transgenic plants: potential ecological impact", M Tepfer, E Balázs, eds. Springer, Berlin, Germany. pp 20-25.

Caradus, J.R., Clifford, P.T.P., Chapman, D.F., Cousins, G.R., Williams, W.M., Miller, J.E. (1997). Breeding and description of 'Grasslands Sustain', a medium -large- leaved white clover (*Trifolium repens* L.) cultivar. *New Zealand Journal of Agricultural Research* **40**: 1-7.

Clifford, P.T.P., Baird, I.J., Grbavac, N., Sparks, G.A. (1990). White clover soil seed loads: effect on requirements and resultant success of cultivar-change crops. *Proceedings of the New Zealand Grassland Association* **52**: 95-98.

Clifford, P. T. P., Sparks, G. A., and Woodfield, D. R. (1996). The intensifying requirements for white clover cultivar change. In "*White clover: New Zealand's competitive edge. Joint symposium*", Agronomy Society of New Zealand, Christchurch. pp. 19-24.

Coghlan, A. (2000). So far so good: for the moment, the gene genie is staying in its bottle. *New Scientist* **2231**: 4.

Conner, A.J., Glare, T.R., Nap, J.P. (2003). The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. *The plant journal* **33**: 19-46.

Cooper, B., Lapidot, M., Heick, J.A., Dodds, J.A., Beachy, R.N. (1995). A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility. *Virology* **206**: 307-313.

Coruzzi, G., Brogue, C., Edwards, C., Chua, N.H. (1984). Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. *EMBO Journal* **3**: 1671-1679.

Coutts, B.A., Jones, R.A.C. (2002). Temporal dynamics of spread of four viruses within mixed species perennial pastures. *Annals of Applied Biology* **140**: 37-52.

Davies, J.E. (1986). Aminoglycoside-aminocyclitol antibiotics and their modifying enzymes. In "Antibiotics in laboratory medicine", V Lorian, ed Ed. 2. Williams and Wilkins, Easton, MD. USA. pp 790-809.

De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly

depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.

De Vries, J., Wackernagel, W. (1998). Detection of *nptII* (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. *Molecular and General Genetics* **257**: 606-613.

Eastham, K., Sweet, J. (2002). Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. Environmental issue report No. 28, European Environment Agency (EEA), Copenhagen, Denmark, [http://reports.eea.eu.int/environmental\\_issue\\_report\\_2002\\_28/en](http://reports.eea.eu.int/environmental_issue_report_2002_28/en) pp 1-75.

Edwardson, J.R., Christie, R.G. (1986). Alfalfa Mosaic Virus. In "Viruses infecting forage legumes", Florida Agricultural Experiment Station, ed Ed. Vol I Monogram series 14. University of Florida, Gainesville, Florida. pp 21-57.

EPA (1994). Neomycin phosphotransferase II; tolerance exemption. *Federal Register* **59**: 49351-49353.

EPA Fall 2003 Regulatory Agenda. (2003) Available from: <http://www.epa.gov/EPA-GENERAL/2003/December/Day-22/g28903.htm>

FAO and WHO Safety aspects of genetically modified foods of plant origin. (2000) Available from: [http://www.who.int/fsf/GMfood/FAO-WHO\\_Consultation\\_report\\_2000.pdf](http://www.who.int/fsf/GMfood/FAO-WHO_Consultation_report_2000.pdf)

FDA (1994). Secondary food additives permitted in food for human consumption; food additives permitted in feed and drinking water of animals; aminoglycoside 3'-phosphotransferase II; final rule. 59, United States Food and Drug Administration, Washington, USA. pp 26700-26711.

FDA (1998). Guidance for Industry: Use of antibiotic resistance marker genes in transgenic plants. U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Premarket Approval, <http://vm.cfsan.fda.gov/~dms/opa-armg.html>.

Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.

Figlerowicz, M., Bujarski, J.J. (1998). RNA recombination in brome mosaic virus, a model plus strand RNA virus. *Acta Biochimica Polonica* **45**: 847-868.

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

Frick, O.L. (1995). The potential for allergenicity in transgenic foods. In "Genetically Modified foods; Safety Aspects", KH Engel, GR Takeoka, R Teranishi., eds. American Chemical Society, Washington DC.

Fuchs, M., Tricoli, D.M., Carney, K.J., Schesser, M., McFerson, J.R., Gonsalves, D. (1998). Comparative virus resistance and fruit yield of transgenic squash with single and multiple coat protein genes. *Plant Disease* **82**: 1350-1356.

- Fuchs, R.L., Astwood, J.D. (1996). Allergenicity assessment of foods derived from genetically modified plants. *Food Technology* **50**: 83-88.
- Fuchs, R.L., Berberich, S.A., Serdy, F.S. (1993a). Safety evaluation of genetically engineered plants and plant products: insect resistant cotton. In "Biotechnology and Safety Assessment", JA Thomas, LA Myers, eds. Raven Press Ltd, New York, pp 199-212.
- Fuchs, R.L., Heeren, R.A., Gustafson, M.E., Rogan, G.J., Bartnicki, D.E., Leimgruber, R.M., Finn, R.F., Hershman, A., Berberich, S.A. (1993b). Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. *Biotechnology (NY)* **11**: 1537-1542.
- Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruber, R.M., Berberich, S.A. (1993c). Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Bio/Technology* **11**: 1543-1547.
- Garrett, R.G. (1973). Non-persistent aphid-borne viruses. In "Viruses and Invertebrates", AJ Gibbs, B Amundsen, eds. North-Holland. pp 473-492.
- Garrett, R. G. (1991). Impact of viruses on pasture legume productivity. In "*Proceedings of Department of Agriculture Victoria White Clover Conference*", pp. 50-56.
- Garrett, R. G. and Chu, P. W. G. (1997). White clover expressing the coat protein of alfalfa mosaic virus: field trial issues. McLean, G. D., Waterhouse, P. M., Evans, G., and Gibbs, M. J. eds, Bureau of Resource Sciences, Canberra. pp. 125-136.
- Garrett, R.G., McLean, G.D. (1983). The epidemiology of some aphid-borne viruses in Australia. In "Plant Virus Epidemiology". Blackwell Scientific, Oxford. pp 199-209.
- Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.
- Gibbs, M. J., Waterhouse, P. M., and Weiller, G. F. (1997). Analysis of natural viral recombination may assist the design of new virus resistance traits. In "*Commercialisation of transgenic crops: risk, benefit and trade considerations*", McLean, G. D., Waterhouse, P. M., Evans, G., and Gibbs, M. J. eds, Cooperative Research Centre for Plant Science and Bureau of Resource Sciences, Canberra. pp. 159-172.
- Gibson, P.B., Barnett, O.W., Burrows, P.M., King, F.D. (1982). Filtered-air enclosures exclude vectors and enable measurement of effects of viruses on white clover in the field. *Plant Disease* **66**: 142-144.
- Gibson, P.B., Barnett, O.W., Skipper, H.D., McLaughlin, M.R. (1981). Effects of three viruses on growth of white clover. *Plant Disease* **65**: 50-51.
- Godfree, R.C., Chu, P.W.G., Woods, M.J. (2004). White clover (*Trifolium repens*) and associated viruses in the subalpine region of southeastern Australia: implications for GMO risk assessment. *Australian Journal of Botany* **In press**:
- Greene, A.E., Allison, R.F. (1996). Deletions in the 3' untranslated region of cowpea chlorotic mottle virus transgene reduce recovery of recombinant viruses in transgenic plants. *Virology* **225**: 231-234.

- Greene, A.E., Allison, R.F. (1994). Recombination between viral RNA and transgenic plant transcripts. *Science* **263**: 1423-1425.
- Harper, G., Hull, R., Lockhart, B., Olszewski, N. (2002). Viral sequences integrated into plant genomes. *Annual Review of Phytopathology* **40**: 119-136.
- Harper, G., Osuji, J.O., Heslop-Harrison, J.S., Hull, R. (1999). Integration of banana streak badnavirus into the *Musa* genome: Molecular and cytogenetic evidence. *Virology* **255**: 207-213.
- Harris, W. (1987). Population dynamics and competition. In "White Clover", MJ Baker, WM Williams, eds. CAB International, Wallingford. pp 203-278.
- Hill, M.J., Donald, G.E. (1998). Australian Temperate Pastures Database. National Pastures Improvement Coordinating Committee/CSIRO Animal Production, published as a compact disc.
- Ho, M.W., Ryan, A., Cummins, J. (2000). Cauliflower mosaic viral promoter - a recipe for disaster? *Microbial Ecology in Health and Disease* **11**: 194-197.
- Hodgson, J. (2000a). Reply to hazardous CaMV promoter? *Nature Biotechnology* **18**: 363.
- Hodgson, J. (2000b). Scientists avert new GMO crisis. *Nature Biotechnology* **18**: 13.
- Hoffman, T., Golz, C., Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* **27**: 70-76.
- Jones, R.A.C. (1996). Virus diseases of Australian pastures. In "Pasture and Forage Crop Pathology". American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, Wisconsin, USA. pp 303-322.
- Kalla, R., Chu, P., Spangenberg, G. (2001). Molecular breeding of forage legumes for virus resistance. In "Molecular breeding of forage crops. Proceedings of the 2nd International Symposium, Molecular Breeding of Forage Crops, Lorne and Hamilton, Victoria, Australia, 19-24 November, 2000", G Spangenberg, ed. Kluwer Academic Publishers, Dordrecht. pp 219-237.
- Kawchuk, L.M., Lynch, D.R., Martin, R.R., Kozub, G.C., Farries, B. (1997). Field resistance to the potato leafroll luteovirus in transgenic and somaclone potato plants reduces tuber disease symptoms. *Canadian Journal of Plant Pathology* **19**: 260-266.
- Kay, R., Chan, A., Daly, M., McPherson, J. (1987). Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. *Science* **236**: 1299-1302.
- Keese, P., Gibbs, A. (1993). Plant viruses: master explorers of evolutionary space. *Current Opinion in Genetics & Development* **3**: 873-877.
- Kimber, I., Kerkvliet, N.L., Taylor, S.L., Astwood, J.D., Sarlo, K., Dearman, R.J. (1999). Toxicology of protein allergenicity: prediction and characterization. *Toxicological Sciences* **48**: 157-162.

- Klee, H.J., Rogers, S.G. (1989). Plant gene vectors and genetic transformation: plant transformation systems based on the use of *Agrobacterium tumefaciens*. *Cell Culture and Somatic Cell Genetics of Plants* **6**: 1-23.
- Krach, K.E. (1959). Excretion of undigested seeds of clover, grasses and weeds of birds and effect of passage through stomach and intestine on their viability. *Zeitschrift für Acker- und Pflanzenbau* **107**: 405-434.
- Lai, M.M.C. (1992). RNA recombination in animal and plant viruses. *Microbiological Reviews* **56** (1): 61-79.
- Latch, G.C.M., Skipp, R.A. (1987). Diseases. In "White clover", MJ Baker, WM Williams, eds. CAB International, Wallingford. pp 421-460.
- Lawrence, J.G., Ochman, H. (1998). Molecular archaeology of the *Escherichia coli* genome. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 9413-9417.
- Levy, S.B., Marshall, B., Schluederberg, S., Rowse, D., Davis, J. (1998). High frequency of antimicrobial resistance in human fecal flora. *Antimicrobial Agents and Chemotherapy* **32**: 1801-1806.
- Marshall, A., Michaelson-Yeates, T., Williams, I. (1999). How busy are bees - modelling the pollination of clover. IGER Innovations, pp 18-21.
- Matthews, R.E.F. (1991). Plant Virology. Academic Press, New York.
- Mayo, M.A., Jolly, C.A. (1991). The 5'-terminal sequence of potato leafroll virus RNA: evidence of recombination between virus and host RNA. *Journal of General Virology* **72**: 2591-2595.
- Mckirdy, S.J., Jones, R.A.C. (1995). Occurrence of alfalfa mosaic and subterranean clover red leaf viruses in legume pastures in Western Australia. *Australian Journal of Agricultural Research* **46**: 763-774.
- McRill, M., Sagar, G.R. (1973). Earthworms and seeds. *Nature* **244**: 482.
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.
- Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews in Food Science and Nutrition* **36(S)**: S165-S186.
- Mette, M.F., Kanno, T., Aufsatz, W., Jakowitsch, J., van der Winden J., Matzke, M.A., Matzke, A.J. (2002). Endogenous viral sequences and their potential contribution to heritable virus resistance in plants. *The EMBO Journal* **21**: 461-469.
- Michaelson-Yeates, T.P.T., Marshall, A.H., Williams, I.H., Carreck, N.L., Simpkins, J.R. (1997). The use of isoenzyme markers to determine pollen flow and seed paternity mediated

- by *Apis mellifera* and *Bombus* spp. in *Trifolium repens*, a self-incompatible plant species. *Journal of Apicultural Research* **36**: 57-62.
- Morelli, G., Nagy, F., Fraley, R.T., Rogers, S.G., Chua, N.H. (1985). A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose-1,5-bisphosphate carboxylase small subunit of pea. *Nature* **315**: 200-204.
- Neeleman, L., Bol, J.F. (1999). Cis-acting functions of alfalfa mosaic virus proteins involved in replication and encapsidation of viral RNA. *Virology* **254**: 324-333.
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *APMIS* **106**: 77-84.
- Nielsen, K.M., Bones, A.M., Smalla, K., van Elsas, J.D. (1998). Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? *FEMS Microbiology Reviews* **22** (2): 79-103.
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.
- Noble, I.R. (1989). Attributes of invaders and the invading process: terrestrial and vascular plants. In "Biological Invasions: a Global Perspective", JA Drake, HA Mooney, F di Castri, RH Groves, FJ Kruger, M Rejmanek, M Williamson, eds. John Wiley & Sons, Chichester. pp 301-313.
- Norton, M.R., Johnstone, G.R. (1998). Occurrence of alfalfa mosaic, clover yellow vein, subterranean clover red leaf, and white clover mosaic viruses in white clover throughout Australia. *Australian Journal of Agricultural Research* **49**: 723-728.
- NSW Agriculture and Grassland Society of NSW Inc (2001). Pasture grass, legume and herb varieties used in NSW 2002-2003. NSW Agriculture, Dubbo. pp 1-36.
- Ochman, H., Lawrence, J.G., Grolsman, E. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**: 299-304.
- OECD (1996). Consensus document of general information concerning the biosafety of crop plants made virus resistant through coat protein gene-mediated protection. 5, OECD, Paris. pp 1-54.
- OECD (2003). OECD Seed Schemes 2003. C(2000)146/FINAL incl. 2003 amendments, Organisation for Economic Cooperation and Development, Paris, [www.oecd.org/agr/seed](http://www.oecd.org/agr/seed). pp 1-221.
- OGTR The Biology and Ecology of White Clover (*Trifolium repens* L.) in Australia. (2004) Available from: [www.ogtr.gov.au](http://www.ogtr.gov.au)
- Osborne, J. L., Williams, I. H., Marshall, A. H., and Michaelson-Yeates, T. P. T. (2001). Pollination and gene flow in white clover, growing in a patchy habitat. Benedek, P. and Richards, K. W. eds, ISHS, International Society for Horticultural Science, Leven, Belgium. pp. 35-40.

- Panetta, F.D. (1993). A system of assessing proposed plant introductions for weed potential. *Plant Protection Quarterly* **8**: 10-14.
- Pheloung, P.C. (1995). Determining the weed potential of new plant introductions in Australia. Department of Agriculture, Perth, Australia.
- Pheloung, P.C., Williams, P.A., Halloy, S.R. (1999). A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management* **57**: 239-251.
- Pita, J.S., Fondong, V.N., Sangare, A., Otim-Nape, G.W., Ogwal, S., Fauquet, C.M. (2001). Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* **82**: 655-665.
- Pittard, A. J. (1997). The use of antibiotic resistance markers in transgenic plants and microorganisms which are to be released into the environment. In *Proceedings of Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations Conference*, Canberra. pp. 173-178.
- Randall, R.P. (2002). A global compendium of weeds. R.G. & F.J. Richardson, Meredith, Victoria.
- Richards, A., Scown, J. (2002). Environmental risks associated with viral recombination in virus resistant transgenic plants. CSIRO Entomology and Environment Australia, pp 1-77.
- Roy, J. (1990). In search of the characteristics of plant invaders. In *Biological Invasions in Europe and the Mediterranean Basin*, F di Castri, AJ Hansen, M Debussche, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 335-352.
- Sanders, P.R., Sammons, B., Kaniewski, W., Haley, L., Layton, J., Lavalley, B.J., Delannay, X., Tumer, N.E. (1992). Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes. *Phytopathology*, **82**: 683-690.
- Schlüter, K., Fütterer, J., Potrykus, I. (1995). Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs - if at all - at an extremely low frequency. *Bio/Technology* **13**: 1094-1098.
- Schoelz, J.E., Wintermantel, W.M. (1993). Expansion of viral host range through complementation and recombination in transgenic plants. *The Plant Cell* **5**: 1669-1679.
- Smalla, K., Borin, S., Heuer, H., Gebhard, F., van Elsas, J. D., and Nielsen, K. (2000). Horizontal transfer of antibiotic resistance from transgenic plants to bacteria - are there data to fuel the debate? Fairbairn, C., Scoles, G., and McHughen, A. eds, University Extension Press, Saskatchewan, Canada. pp. 146-154.
- Smalla, K., Gebhard, F., van Elsas, J. D., Matzk, A., and Schiemann, J. (1994). Bacterial communities influenced by transgenic plants. Jones, D. D. eds, Division of Agriculture and Natural Resources, University of California, Oakland, USA. pp. 157-167.
- Smit, C.H., Roosien, J., Van Vloten-Doting, L., Jaspars, E.M.J. (1981). Evidence that alfalfa mosaic virus infection starts with three RNA-protein complexes. *Virology* **112**: 169-173.

- Stanhope, M.J., Lupas, A., Italia, M.J., Koretke, K.K., Volker, C., Brown, J.R. (2001). Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates. *Nature* **411**: 940-944.
- Strickler, K., Freitas, S. (1999). Interactions between floral resources and bees (*Hymenoptera: Megachilidae*) in commercial alfalfa seed fields. *Environmental Entomology* **28**: 178-187.
- Suckling, F.E.T. (1952). Dissemination of white clover (*Trifolium repens*) by sheep. *New Zealand Journal of Science and Technology* **A33**: 64-77.
- Syvanen, M. (1999). In search of horizontal gene transfer. *Nature Biotechnology* **17**: 833.
- Taliansky, M.E., Robinson, D.J. (2003). Molecular biology of umbraviruses: phantom warriors. *Journal of General Virology* **84**: 1951-1960.
- Taylor, S. (1995). Evaluation of the allergenicity of foods developed through biotechnology. In "Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms", University of California, Division of Agriculture and Natural Resources, Oakland, California, USA.
- Taylor, S. (2000). Joint FAO/WHO expert consultation on foods derived from biotechnology - Topic 13 Allergenicity. Food and Agriculture Organisation of the United Nations and World Health Organization,
- Taylor, S.L., Lehrer, S.B. (1996). Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition* **36**: S91-S118.
- Teycheney, P. Y. and Tepfer, M. (1999). Gene flow from virus-resistant transgenic crops to wild relatives or to infecting viruses. In "Gene flow and agriculture: Relevance for transgenic crops, British Crop Protection Council Symposium Proceedings No. 72", pp. 191-196.
- Timmerman-Vaughan, G.M., Pither, J.M.D., Cooper, P.A., Russell, A.C., Goulden, D.S., Butler, R., Grant, J.E. (2001). Partial resistance of transgenic peas to alfalfa mosaic virus under greenhouse and field conditions. *Crop Science* **41**: 846-853.
- Tomassoli, L., Ilardi, V., Barba, M., Kaniewski, W. (1999). Resistance of transgenic tomato to cucumber mosaic cucumovirus under field conditions. *Molecular Breeding* **5**: 121-130.
- USDA-APHIS (1988). Environmental assessment and finding of no significant impact (permit number 88-028-01). <http://www.isb.vt.edu/biomon/relea/8802801r.eaa>
- USDA-APHIS (1994). USDA/APHIS permit 94-027-03 for field testing genetically engineered alfalfa plants. <http://www.isb.vt.edu/biomon/relea/9402703r.eaa>
- Wang, K., Herrera-Estrella, L., Van Montagu, M., Zambryski, P. (1984). Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from *Agrobacterium* to the plant genome. *Cell* **38**: 455-462.
- Weaver, N. (1965). Foraging behavior of honeybees on white clover. *Insectes Sociaux* **12**: 231-240.

- Williams, I.H. (2001). Bee-mediated pollen and gene flow from GM plants. *Acta Horticulturae* **561**: 25-33.
- Williamson, M.H., Fitter, A. (1996). The characters of successful invaders. *Biological conservation* **78**: 163-170.
- Woodfield, D. R., Clifford, P. T. P., Baird, I. J., and Cousins, G. R. (1995). Gene flow and estimated isolation requirements for transgenic white clover. In "*Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms*", Jones, D. D. eds, The University of California, Division of Agriculture and Natural Resources, Oakland. pp. 509-514.
- Worobey, M., Holmes, E.C. (1999). Evolutionary aspects of recombination in RNA viruses. *Journal of General Virology* **80**: 2535-2543.
- Xu, D., Collins, G.B., Hunt, A.G., Nielsen, M.T. (1998). Resistance to alfalfa mosaic virus in transgenic burley tobaccos expressing the AMV coat protein gene. *Crop Science* **38**: 1661-1668.
- Yamada, T., Kawaguchi, T. (1971). Dissemination of pasture plants by livestock I. Recovery and viability of some pasture plant seeds passed through digestive tract of goats. *Journal of Japanese Society of Grassland Science* **17**: 36-47.
- Yamada, T., Kawaguchi, T. (1972). Dissemination of pasture plants by livestock. 2. Recovery, viability and emergence of some pasture plant seeds passed through the digestive tract of the dairy cow. *Journal of Japanese Society of Grassland Science* **18**: 8-15.
- Yusibov, V., Modelska, A., Steplewski, K., Agadjanyan, M., Weiner, D., Hooper, D.G., Koprowski, H. (1997). Antigens produced in plants by infection with chimeric plant viruses immunize against rabies virus and HIV-1. *Proceedings of the National Academy of Science of the United States of America* **94**: 5788.
- Yusibov, V., Hooper, D.C., Spitsin, S.V., Fleysh, N., Kean, R.B., Mikheeva, T., Deka, D., Karasev, A., Cox, S., Randall, J., Koprowski, H. (2002). Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* **20**: 3155-3164.
- Zambryski, P. (1992). Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annual Review Plant Physiology and Plant Molecular Biology* **43**: 465-490.