



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 089**

**Limited and controlled release of white clover
genetically modified to resist infection by
Alfalfa mosaic virus**

Applicant: Victorian Department of Primary Industries

January 2009

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of white clover genetically modified for resistance to Alfalfa mosaic virus (AMV) into the environment in respect of application DIR 089 from the Victorian Department of Primary Industries (DPI Victoria).

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public¹.

The application

DPI Victoria applied for a licence for dealings involving the intentional release of one line² of GM white clover on a limited scale and under controlled conditions. The GM white clover line has been genetically modified to resist infection by AMV. The release will involve one site in the local government area of Corowa, NSW, on a maximum area of 633 m² per year, between March 2009 and August 2011.

The GM white clover contains a gene from a virus which provides resistance to AMV, as well as an antibiotic resistance gene which was used to identify transformed plants during initial development of the GM plant in the laboratory.

The purpose of the trial is to conduct experiments to evaluate the agronomic performance, including seed yield, of the GM white clover line under field conditions. Some seed would be collected and retained for analysis and possible future trials, subject to further approval(s). The GM white clover will not be used for human food or animal feed.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM white clover line and the introduced genetic materials in the environment that have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including screening protocols, data from previous field trials and unpublished data produced to support weediness and gene flow assessments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

² The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP.

A **hazard** identification process was used in the first instance to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Nine events were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMO in both time and space. This detailed consideration identified one event requiring further assessment. The potential adverse outcome to the environment associated with this event was enhanced spread and persistence (weediness). The remaining eight events were not assessed further as they were considered not to give rise to an identified risk to human health and safety or the environment (refer to Chapter 2 for more information). The principle reasons comprise:

- limits on the size, location and duration of the release proposed by DPI Victoria
- suitability of controls proposed by DPI Victoria to restrict the dissemination or persistence of the GM white clover plants and their genetic material
- none of the GM plant materials or products will be used in human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Risk of weediness

The event that might result in the introduced gene causing greater weediness than the parent non-GM white clover was:

- Expression of the introduced *AMV CP* gene in other white clover plants as a result of gene transfer leading to increased spread and persistence in native plant habitats. (Identified Risk 1).

The consequence and likelihood of harm that might result from the above event was assessed in the context of the current trial. The estimate of risk for the identified risk is **negligible**.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. The level of risk to health and safety of people or the environment for the identified risk was estimated as **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a

range of measures have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

The licence conditions require DPI Victoria to **limit** the release to a total area of 633 m² per year at one site between March 2009 and August 2011. The **control** measures include containment provisions at the trial site, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed³.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of one GM white clover line on a maximum total area of 633 m² per year over two and a half years in the NSW local government area of Corowa, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

³ The licence for DIR 089 is available on the OGTR website via the link to DIR 089 (<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir089-2008>>)

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Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
AMV	Alfalfa mosaic virus
<i>AMV CP</i>	gene encoding Alfalfa mosaic virus coat protein
BLAST	Basic Local Alignment Search Tool
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CaMV	Cauliflower mosaic virus
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
DPI Victoria	Victorian Department of Primary Industries
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
km	kilometre
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
<i>nptII</i>	gene encoding neomycin phosphotransferase type II
OGTR	Office of the Gene Technology Regulator
qPCR	Quantitative Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
TGA	Therapeutic Goods Administration
US FDA	United States Food and Drug Administration
USDA APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service

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Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of white clover genetically modified for resistance to Alfalfa mosaic virus (AMV) into the environment in respect of application DIR 089 from the Victorian Department of Primary Industries (DPI Victoria).

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public⁴.

The application

DPI Victoria applied for a licence for dealings involving the intentional release of one line⁵ of white clover (*Trifolium repens* L.) which has been genetically modified to resist infection by AMV on a limited scale and under controlled conditions. The trial is authorised to take place at one site in the local government area of Corowa, NSW, on a maximum area of 633 m² per year between March 2009 and August 2011.

The GM white clover line proposed for release was produced by transforming plants of the white clover cultivar ‘Irrigation’. The GM plants were then conventionally bred with the white clover cultivar ‘Mink’ and then the white clover cultivar ‘Grasslands Sustain’ to produce the GM white clover proposed for release.

The GM white clover line contains the Alfalfa mosaic virus coat protein (*AMV CP*) gene intended to provide resistance to AMV, and an antibiotic resistance gene (*nptII*) encoding the protein neomycin phosphotransferase type II from *Escherichia coli* which provides resistance to the antibiotic kanamycin, which was used as a marker to select for modified plants in the laboratory.

The purpose of the trial is to conduct further experiments to evaluate the agronomic performance, including seed yield, of the GM white clover line previously released under Licence DIR 047/2003, under field conditions. Some seed would be collected and retained for analysis and possible future trials, subject to further approval(s). The GM white clover will not be used for human food or animal feed.

⁴ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator’s *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

⁵ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

DPI Victoria proposed a number of controls to restrict the dissemination or persistence of the GM white clover line and its genetic material into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including screening protocols, data from previous field trials and unpublished data produced to support weediness and gene flow assessments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment considered information contained in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities on the application (summarised in Appendix B of the RARMP). Submissions received from the public on the consultation RARMP (four submissions), and how they were considered, is summarised in Appendix C of the RARMP. After considering advice on the consultation RARMP, the risk of weediness as a result of gene flow (Event 4; Identified Risk 1) has been considered as an identified risk.

A reference document, *The Biology of Trifolium repens L. (White clover)*, was produced to inform the risk assessment process for licence applications involving GM white clover plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The events that are considered to have the potential to lead to adverse outcomes are assessed further to determine the seriousness of harm (consequence) that could result and how likely it is that the harm would occur. The level of risk is then estimated using the Risk Estimate Matrix (see below and Chapter 2).

		RISK ESTIMATE			
		Low	Moderate	High	High
LIKELIHOOD	Highly Likely	Negligible	Low	High	High
	Likely	Negligible	Low	Moderate	High
	Unlikely	Negligible	Negligible	Low	Moderate
	Highly Unlikely	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
		CONSEQUENCES			

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

Nine events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMO in both time and space. This detailed consideration identified one event requiring further assessment. The potential adverse outcome associated with this event is increased spread and persistence (weediness). The remaining eight events were not assessed further as they were considered not to give rise to an identified risk to human health and safety or the environment (refer to Chapter 2 for more information). The principle reasons comprise:

- limits on the size, location and duration of the release proposed by DPI Victoria
- suitability of controls proposed by DPI Victoria to restrict the dissemination or persistence of the GM white clover plants and their genetic material
- none of the GM plant materials or products will be used in human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

The identified risk was assessed in comparison to the parent non-GM white clover, also taking into account, intended agronomic management practices, and the environmental conditions in the regions approved for the release.

The consequence and likelihood assessments that determined the risk estimates for the identified risk is summarised in Table 1 (the detailed risk assessment is in Chapter 3).

If a risk is estimated to be higher than **negligible**, risk treatment measures may be required to protect the health and safety of people or the environment. However, all risks were estimated to be **negligible** for this release.

Table 1. Summary table for the risk assessment

Event that may give rise to weediness	Consequence assessment	Likelihood	Risk estimate	Does risk require treatment?
<p>Identified Risk 1</p> <p>Expression of the introduced <i>AMV CP</i> gene in other white clover plants as a result of gene transfer leading to increased spread and persistence in native plant habitats.</p>	<p>Minor</p> <ul style="list-style-type: none"> • Non-GM white clover mainly impacts on herbaceous plants in native plant habitats and AMV-resistant white clover is expected to do the same. • Many native plant habitats have no AMV infection and therefore white clover with resistance to AMV will have no selective advantage. • The genetic modification will not extend the range of white clover with resistance to AMV compared to non-GM white clover. • In native plant habitats where AMV is present, the degree to which AMV-resistance white clover may adversely impact native vegetation is uncertain. 	<p>Highly unlikely</p> <ul style="list-style-type: none"> • Outcrossing to other white clover plants would be rare due to containment measures proposed by the applicant, and the small size and short duration of the trial. • The chance of volunteer GM plants arising from seed dispersal finding suitable conditions to establish as weeds would be no greater than for non-GM white clover. • Aphids and AMV must both be present in a native plant habitat and a white clover population must be limited by AMV before a selective advantage could potentially be conferred. • Although resistance to AMV infection may offer a small competitive advantage, abiotic and biotic factors, such as temperature, soil type, water and nutrient availability, are likely to be more important in limiting the spread and persistence of white clover. 	Negligible	No

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. The level of risk to health and safety of people or the environment from the risk identified in the assessment process was estimated as **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

The Acting Regulator has imposed a number of licence conditions including requirements to:

- locate the trial site more than 50 metres away from natural waterways
- surround the release site by both a livestock-proof and a rabbit-proof fence to reduce seed dispersal by grazing animals
- surround the GM white clover plot by a pollen trap, consisting of an inner one metre wide band of non-GM white clover, surrounded by a band of lucerne at least 35 metres wide, and an outer one metre wide band of non-GM white clover

- monitor the GM white clover fortnightly during flowering, and remove flowers from the GM white clover plants in the event of less than 25% of the lucerne plants in the pollen trap flowering at the same time as the GM white clover plants
- monitor the lucerne band monthly and destroy any white clover plants prior to flowering
- monitor monthly for, and destroy any, white clover plants that may occur within 500 metres of the release site
- specific containment, transport and storage conditions in accordance with OGTR guidelines
- destroy bees, honey and pollen from beehives used on the trial site
- destroy all GM plant material not required for testing or future trials
- monitor for, and destroy any, volunteer GM white clover that may occur in the release area for at least five years after completion of the trial.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs*⁶, *Policy on transport and supply of GMOs*⁷). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁸.

As the trial involves early stage research, the applicant does not intend any material from the GM white clover line proposed for release to be used in human food. All genetically modified foods intended for sale in Australia must undergo a safety evaluation by FSANZ. Accordingly, the applicant has not applied to FSANZ to evaluate the GM white clover line. However, in the event of a commercial release, FSANZ approval may need to be obtained before materials or products derived from the GM white clover line could be sold for human consumption.

The APVMA, which has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia, has previously issued a research permit for the small scale use of the AMV CP in GM white clover plants released under DIR 047/2003. DPI Victoria would require a research permit for this proposed release of GM white clover containing the *AMV CP* gene.

⁶ Guidelines for the transport of GMOs

<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>>

⁷ Policy on transport and supply of GMOs

<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/policies-1>>

⁸ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR).

Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of this GM white clover line, or to justify a reduction in containment conditions. This would include:

- additional data on the potential toxicity of plant materials from the GM white clover line
- weediness of the GM white clover under Australian field conditions, including invasiveness and enhanced reproductive capacities
- the degree to which AMV limits white clover spread and persistence outside of pastoral situations, in particular in native plant habitats
- additional data on gene transfer to non-GM white clover.

Suitability of the applicant

The Acting Regulator determined, at the commencement of the assessment process for this application, that DPI Victoria is suitable to hold a DIR licence under the requirements of section 58 of the Act. The Acting Regulator is satisfied that DPI Victoria remains suitable as no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has confirmed its ability to comply with the licence conditions.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of one GM white clover line on a maximum total area of 633 m² per year over two and a half years in the NSW local government area of Corowa, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people or the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁹, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

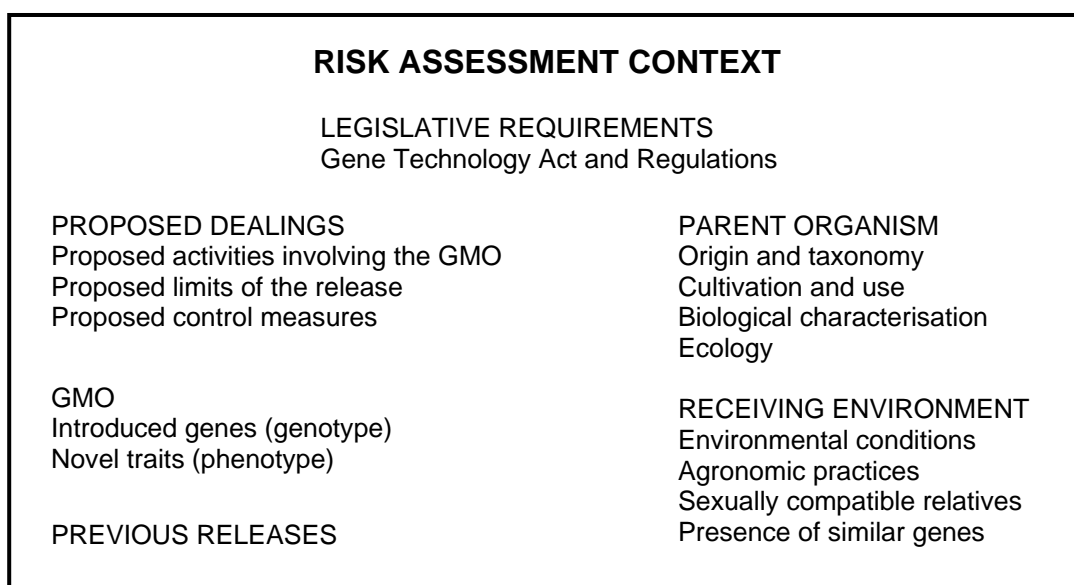


Figure 1. Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:
- the proposed dealings (Section 3.1)
 - the limits proposed by the applicant (Section 3.2)
 - the controls proposed by the applicant (Section 3.3)
 - characteristics of the parent organism (Section 4)
 - the nature and effect of the genetic modification (Section 5)
 - the environmental conditions in the location where the release would occur (Sections 6.1 and 6.2)
 - relevant agricultural practices (Section 6.3)
 - the presence of related plants in the environment (Section 6.4)
 - the presence of the introduced or similar genes in the environment (Section 6.5)
 - any previous releases of this or other GMOs relevant to this application (Section 7).

⁹ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail on the OGTR website at <<http://www.ogtr.gov.au/>> and in the *Risk Analysis Framework* (OGTR 2007) <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom she must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Acting Regulator has considered information provided in the application and is satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, location and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMO and its genetic material in the environment. Those limits and controls are such that the Acting Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Acting Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix B. Four submissions were received from members of the public, and their consideration is summarised in Appendix C.

6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

7. The Victorian Department of Primary Industries (DPI Victoria) proposes to release one white clover line¹⁰ which has been genetically modified (GM) to resist infection by Alfalfa mosaic virus (AMV) into the environment under limited and controlled conditions.

8. Some details of the application, including screening protocols, data from previous field trials and unpublished data produced to support weediness and gene flow assessments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted on the RARMP for this application.

3.1 The proposed activities

9. The applicant has stated that the purpose of the trial is to conduct experiments to evaluate the agronomic performance, including seed yield, of the GM white clover line under

¹⁰ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

field conditions. Some seed would be collected and retained for analysis and possible future trials, subject to further approval(s). The GM white clover will not be used for human food or animal feed.

10. The GM white clover plants would be grown between March 2009 and August 2011. The trial would be carried out in two stages. In the first stage GM white clover seeds would be planted, allowed to establish and evaluated for agronomic traits. In the second stage, a bee hive would be set up in the centre of the field trial to encourage pollination and allow seed yield assessment. The resulting seed would be harvested and any seed not retained for future trials or experiments destroyed.

11. Seed from nine different types of white clover would be planted, including GM and non-GM 'Mink' and 'Grasslands Sustain', as well as five other commercial cultivars which would be used as controls for the assessment of agronomic performance. The trial site would be divided into an array of eight by nine experimental plots, 3 m² in size, giving four to seven replications. GM white clover would be sown in up to 28 of the 72 plots. In addition, some plots would be cross-sown with the non-GM perennial ryegrass cultivar 'Arrow', to enable assessment of the performance of the GM clover in mixed sward plots.

12. At the end of the trial all the plants on the trial site and in the 2 ha pollen trap would be destroyed by application of herbicide and topping (seed head removal). Any remaining plant material not required for further experiments would be destroyed by incineration.

3.2 The proposed limits of the dealings (size, location and duration)

13. The release is proposed to take place at one site in the shire of Corowa, NSW, on a total maximum area of 633 m² per year between March 2009 and August 2011.

3.3 The proposed controls to restrict the dissemination or persistence of the GMO and its genetic material in the environment

14. Only trained and authorised staff will be permitted access to the proposed locations.

15. The applicant has proposed a number of controls to restrict the dissemination or persistence of the GM white clover line and the introduced genetic material in the environment (outlined in Figure 2) including:

- locating the trial site more than 280 metres away from natural waterways
- surrounding the release site by both a livestock-proof and a rabbit-proof fence to reduce 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, surrounded by a band of lucerne at least 35 metres wide, and an outer one metre wide band of non-GM white clover
- monitoring the GM clover fortnightly during flowering, and removing flowers from the GM white clover plants in the event of the lucerne plants in the pollen trap not flowering simultaneously
- monitoring the lucerne band monthly and destroying any white clover plants prior to flowering
- monitoring monthly for, and destroying any, white clover plants that may occur within 500 metres of the release site
- removing mature flower heads during peak flowering time to reduce the build-up of a seed bank
- specific containment, transport and storage conditions in accordance with the Regulator's guidelines

- cleaning the site after trial through herbicide application and seed head removal
- destroy bees, honey and pollen from beehives used on the trial site
- destroying all GM plant material not required for testing or future trials
- monitoring for, and destroying any, volunteer GM white clover that may occur in the release area for five years after completion of the trial.

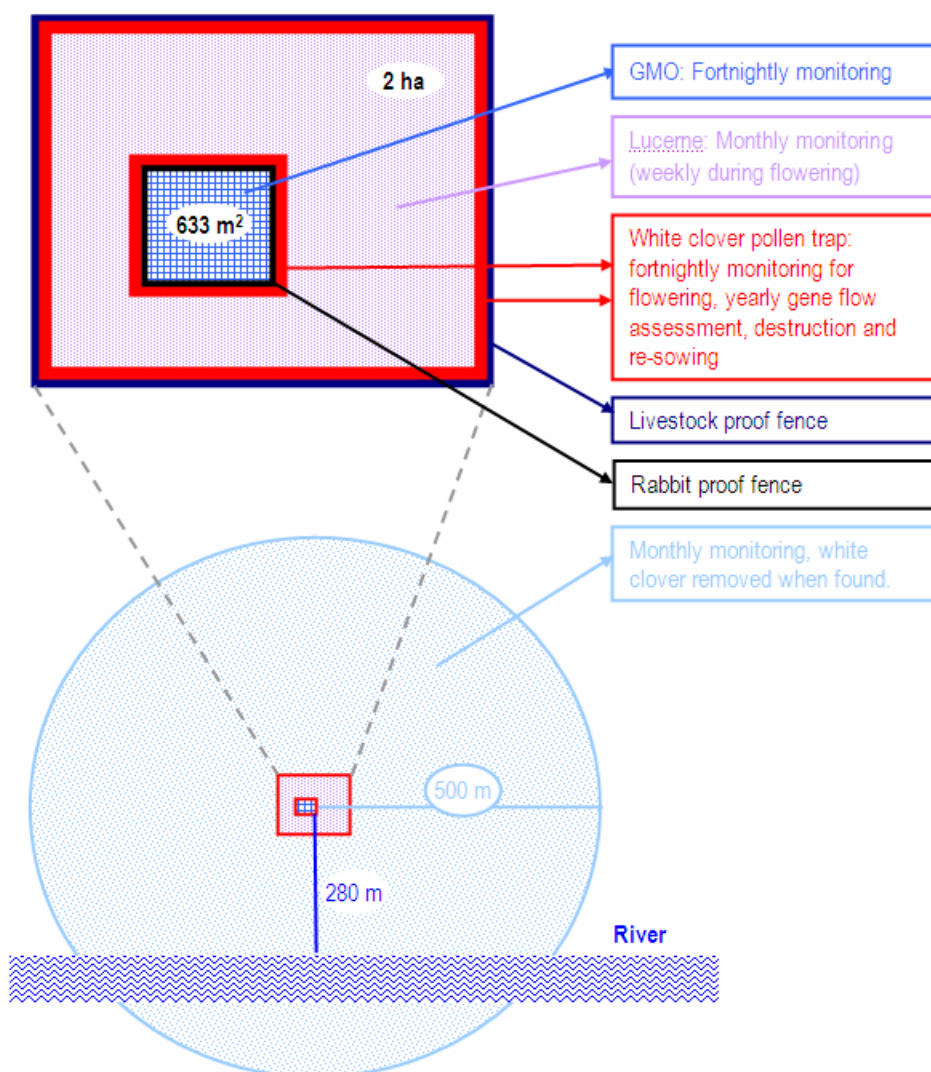


Figure 2. Schematic diagram of some of the proposed containment measures.

16. These controls, and the limits outlined in Chapter 1, Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 4, Section 4.1.1.

Section 4 The parent organism

17. The parent organism is white clover (*Trifolium repens* L.) which is exotic to Australia and is grown as a pasture crop in south-eastern Australia. White clover has been grown in Australia for over 200 years and is highly important in the dairy, meat and wool industries. Further detailed information about the parent organism is contained in a reference document, *The Biology of Trifolium repens L. (White clover)* (OGTR 2008), which was produced in order to inform the risk assessment process for licence applications involving GM white

clover plants. This document is available at
<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

18. The GM white clover line proposed for release was produced by genetic modification of plants of the white clover cultivar ‘Irrigation’ (Oram 1980) as it is relatively easy to transform. The GM plants were then conventionally bred with the white clover cultivar ‘Mink’ (Jahufer et al. 2001) and then the white clover cultivar ‘Grasslands Sustain’ (Caradus et al. 1997) to produce the GM white clover plants proposed for release.

19. The characteristics of white clover cultivar ‘Grasslands Sustain’ include medium-large leaf size, high stolon growing point density and heavy seed set potential (Caradus et al. 1997). Mink has similar characteristics but is more heat tolerant (Jahufer et al. 2001). Both ‘Mink’ and ‘Sustain’ are agronomically superior to ‘Irrigation’.

Section 5 The GMO, nature and effect of the genetic modification

5.1 Introduction to the GMO

20. The GM white clover contains the Alfalfa mosaic virus coat protein (*AMV CP*) gene intended to provide resistance to AMV (Table 2). AMV is a single stranded RNA virus and belongs to the genus *Alfavirus*, family *Bromoviridae* (Büchen-Osmond 2002). 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 many species of AMV infected plants, virions are found in all parts of the plant (Büchen-Osmond 2002).

Table 2. The genes used to modify white clover

Gene	Accession No (Genbank)	Protein produced	Protein involved in	Source	Intended purpose
<i>AMV4</i>	N/A	Alfalfa mosaic virus coat protein	AMV infection, replication and cell to cell movement	Alfalfa mosaic virus isolate WC31	Viral resistance
<i>nptII</i>	AAF65403	neomycin phosphotransferase	Kanamycin resistance	<i>Escherichia coli</i>	Selectable marker

21. In addition, the white clover line contains the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (*nptII*). This gene, encoding the enzyme neomycin phosphotransferase, was derived from *Escherichia coli* and confers kanamycin and neomycin resistance on the GM plant.

22. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genes are also present in the GM white clover line. These are derived from the plant *Pisum sativum* (pea), the plant virus Cauliflower mosaic virus (CaMV), and the bacterium *Agrobacterium tumefaciens*.

5.2 The introduced genes and their encoded proteins

5.2.1 The gene introduced to confer AMV resistance, and its encoded protein

23. 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 (Figure 3). RNAs 1 and 2 encode the viral replicase proteins, P1 and P2 respectively, while RNA3 encodes the coat protein (CP) and the P3 protein, which has a role in viral cell-to-cell movement (Bol 1999).

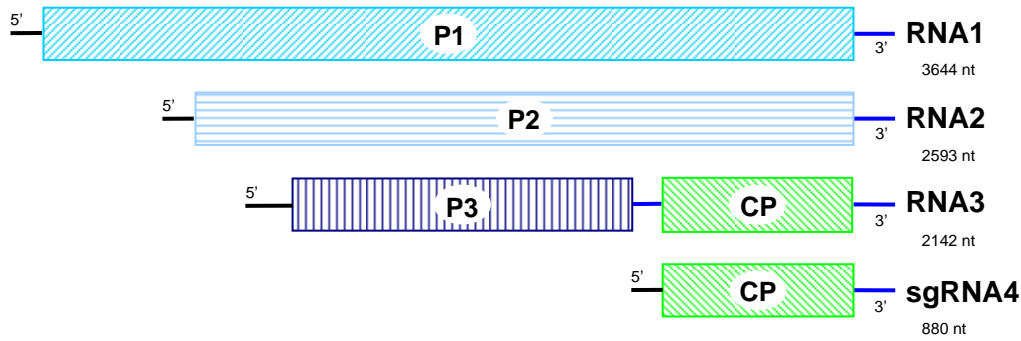


Figure 3. AMV genome structure

24. The *AMV CP* gene, which has been introduced into the white clover plants and 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, the complete coding region of the coat protein (663 bps) and all of the 3' UTR (182 bps). The *AMV CP* gene in the GM white clover is not able to self-replicate nor is it infectious.

25. 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 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' (Smit et al. 1981; Bol 1999). 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).

26. 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.

5.2.2 Toxicity/allergenicity of the introduced gene for AMV resistance

27. AMV infects a wide range of plant species, including peas, beans, lentils and potatoes, and therefore AMV CP occurs naturally in a range of plants widely consumed by people and other organisms (see discussion in Section 6.5 of this chapter). On this basis, people and other organisms have a long history of exposure to the protein encoded by the introduced gene for AMV resistance.

28. AMV CP as well as other viral coat proteins are being investigated for use as carrier molecules for the delivery of vaccines. Antigens from anthrax, 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 are being trialled in mice and humans for immunisations against the above diseases (Yusibov et al. 1997; Belanger et al. 2000; Yusibov et al. 2002; Brodzik et al. 2005). These reports further indicate the lack of toxic or allergenic properties of the AMV CP.

29. 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. Thus, in the US there are proposals to exempt plant-incorporated protectants based on viral coat proteins from both pesticide registration requirements under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) and food tolerance requirements under FFDCAs (Federal Food, Drug, and Cosmetic Act) Acts (US EPA 2007).

30. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequence of the protein encoded by the introduced *AMV CP* gene was compared to a database of known allergens. The results of this analysis did not indicate that AMV CP shared any significant sequence homology with any known allergens (information supplied by applicant).

31. A comprehensive search of the scientific literature also yielded no information to suggest that the encoded protein is toxic or allergenic to people, or toxic to other organisms.

5.2.3 The antibiotic resistance marker gene (*nptII*) and the encoded protein

32. The white clover line contains the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (*nptII*). This gene, encoding the enzyme neomycin phosphotransferase, was derived from *E. coli* and confers kanamycin or neomycin resistance on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plant tissue during initial development in the laboratory of the GM plant line.

33. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, and in more detail in the RARMPs for DIR 070/2006 and DIR 74/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006>) and <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir074-2007>) or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GMOs as not posing a risk to human or animal health or to the environment. The most recent international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority, which concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2007).

5.2.4 The effects associated with the introduced gene for AMV resistance

34. AMV in clover is widespread throughout Australia and has an impact on white clover productivity. AMV can cause severe losses, with reports of up to 60% damage to pasture legumes (Garrett 1991). Incidences of viral infection in stands older than 2 years are commonly 20% or more (Clarke 1999), and in one study greater than 86% infection by AMV was found (Mckirdy & Jones 1995). Glasshouse studies have shown dry mass losses of up to 60% due to AMV (Kalla et al. 2001). In the field, AMV may reduce white clover pasture production by up to 30% through reduced foliage yield and quality, reduced nitrogen fixing capacity and reduced vegetative persistence (Kalla et al. 2001).

Coat protein mediated viral resistance

35. Expression of the *AMV CP* gene in white clover confers resistance to AMV. The exact mechanism of resistance is still unknown but is believed to be protein based. Experiments with GM tobacco (*Nicotiana tabacum*) plants expressing AMV CP have shown that resistance is specific to AMV particles; AMV RNA and unrelated viruses are still capable of causing disease (Loesch-Fries et al. 1987). It has been suggested that high levels of the AMV CP in the GM plants may 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 (Goldbach et al. 2003). This limits damage to the GM plants and also limits further transmission of the virus to other plants. However, later experiments involving other lines of GM tobacco have shown complete protection from AMV RNA as well as AMV particles. This suggests that there may be another mechanism involved in resistance, which has an effect after viral uncoating occurs (Tumer et al. 1991).

36. Experiments on GM tobacco protoplasts expressing AMV CP RNA which had been modified to prevent translation (protein production), showed some protection from AMV infection. This suggests an RNA-dependent mechanism may contribute to the protection provided by the expression of AMV CP (Yusibov & Loesch-Fries 1995). AMV CP is known to bind to the 3' terminal region of sgRNA4, and is necessary for genome activation and infectivity of AMV. AMV CP is involved in dose dependent activation of viral replication, with replication stimulated by low AMV CP concentration and inhibited when AMV CP is present in high concentrations (Guogas et al. 2005). Therefore, high concentrations of AMV CP in the GM white clover lines may inhibit AMV replication.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for expression of the gene for AMV resistance

37. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. The *AMV CP* gene present in the GM white clover is under the control of the enhanced CaMV 35S promoter. The enhanced 35S promoter (35S²) contains two copies of the 35S promoter sequence and produces an approximately ten fold increase in gene expression over the single 35S promoter (Kay et al. 1987). 35S is a constitutive promoter and directs the *AMV CP* gene to be expressed in most plant tissues and throughout the plant lifecycle. Although CaMV is a plant pathogen, the regulatory sequence comprises only a small part of its total genome and is not capable of causing disease.

38. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. 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).

5.3.2 Regulatory sequences for the expression of the *nptII* gene

39. 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* (Bevan 1984). The *nos* promoter is considered a constitutive promoter, which means that genes that are linked to this promoter are generally expressed at relatively high levels throughout the growing season and in most tissues of the plant. Although *A. tumefaciens* is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not capable of causing disease.

5.4 Method of genetic modification

40. The GM white clover line was generated by *A. tumefaciens*-mediated transformation using binary vector pKYLX71:35S²AMV4 (Table 3) and the disarmed *A. tumefaciens* strains Cz707 and AGL1 to transform plant cells in culture (Zambryski 1992). The white clover cultivar 'Irrigation' (Oram 1980) was used as it is readily transformed. However, this cultivar is no longer grown commercially in Australia. Following the transformation process and plant regeneration, screening was performed in the presence of kanamycin to allow the identification of white clover plants containing the introduced gene construct. This method of transformation is used extensively to genetically modify plants (Valentine 2003) and has been discussed in previous RARMPs [most comprehensively for DIR 060/2005 (available at <<http://www.ogtr.gov.au/>> or by contacting the OGTR)].

Table 3. Gene construct used to generate the GM white clover line proposed for release

construct	promoter	gene	terminator	selectable marker cassette
pKYLX71:35S ² AMV4	35S ²	AMV4	rbcSE9pA	nos::nptII_nos

41. The GM plants were then conventionally bred with the white clover cultivar ‘Mink’ (Jahufer et al. 2001) and then the white clover cultivar ‘Grasslands Sustain’ (Caradus et al. 1997) to produce the GM white clover plants proposed for release.

5.5 Characterisation of the GMO

5.5.1 Stability and molecular characterisation

42. The inserted DNA and 315 base pairs of white clover genomic DNA flanking the left border repeat motif has been sequenced, showing that a single copy of the introduced DNA has been incorporated. The inserted genes have been inherited as dominant Mendelian traits over at least four generations of plants grown in the glasshouse and the field.

43. The T₁ generation white clover plants were obtained by crossing T₀ plants with non-GM white clover cultivar ‘Mink’, and the T₂ generation was produced through a diallele cross¹¹ of the T₁ progeny to generate ‘Mink’ type homozygous GM white clover (Figure 4). Southern hybridisation analysis of T₂ plants with both the *AMV CP* and *nptII* genes showed the presence of a single transformation event.

44. The T₂ plants were either (a) bred for a further two polycross¹² generations and selected for AMV resistance and agronomic properties to produce the T₄ ‘Mink’ type plants proposed for release, or (b) crossed with non-GM white clover cultivar ‘Grasslands Sustain’, then diallele crossed to produce homozygous ‘Sustain’ type GM white clover. These were bred for a further two polycross generations and selected for AMV resistance and agronomic properties to produce the T₆ ‘Sustain’ type plants proposed for release (Figure 4).

¹¹ A controlled cross between multiple parent plants in which each parent is mated with every other parent. In a full diallele cross, each parent is represented twice, once as a male and once as a female.

¹² A random cross between multiple parent plants.

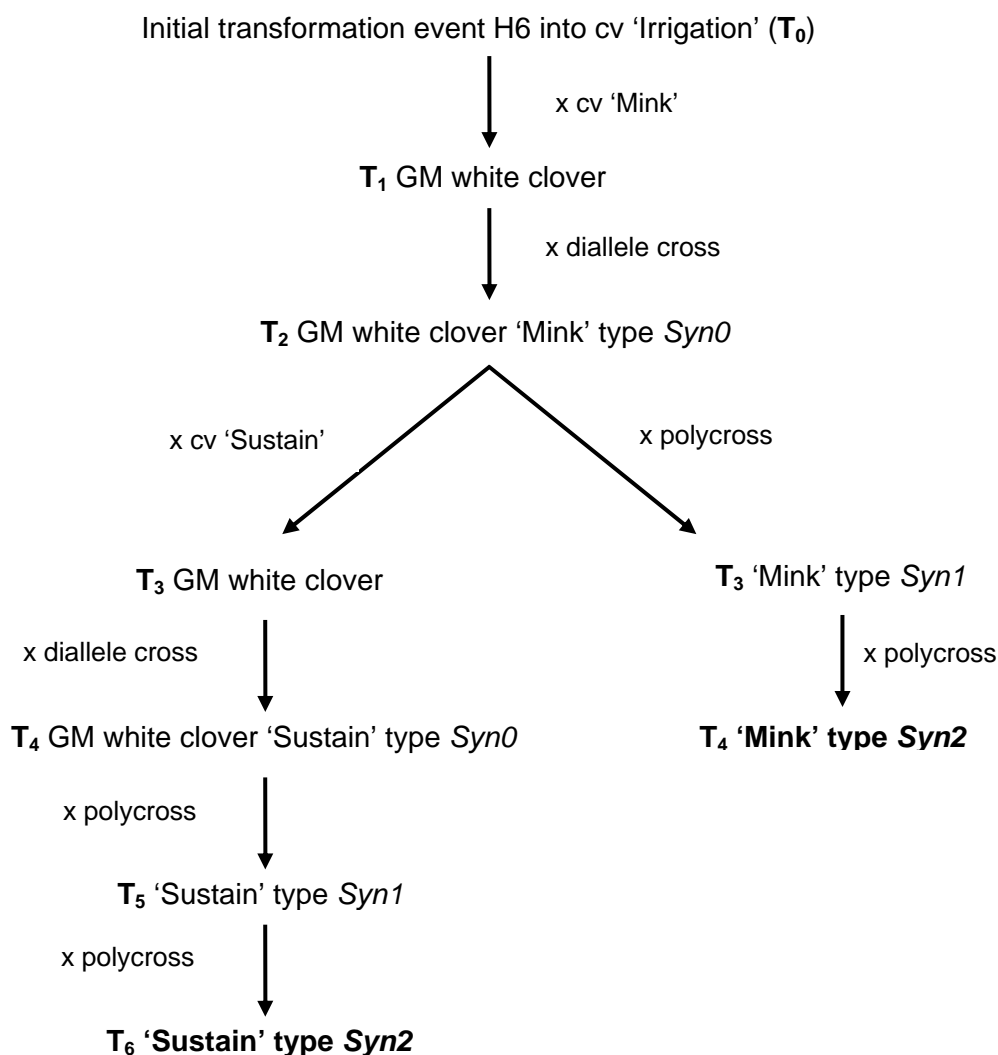


Figure 4. Flow chart indicating crosses used to obtain 'Mink'-type and 'Sustain'-type Syn2 generations (in bold) for use in the proposed field trial.

5.5.2 Expression of the introduced genes in the GM white clover

45. Expression of the introduced genes *AMV CP* and *nptII* has been assessed in field and glasshouse grown 'Mink' type *Syn0* and 'Sustain' type *Syn0* plants.

46. Expression data indicates that both of the introduced genes are expressed in the leaves, stolons and inflorescences of GM white clover plants. However, no mRNA transcripts for either of the introduced genes were detected in the pollen of the GM white clover plants. *AMV CP* transcripts were also detected in the leaves and stolons of non-GM white clover infected with *AMV*. Low levels of *AMV CP* protein were also detected in GM white clover leaves. However, it was present at a much lower level than that seen in leaves from non-GM white clover infected with *AMV* (data supplied by applicant).

47. The applicant has also developed screening methods to detect the presence of both inserted genes in genomic DNA obtained from various plant tissues including leaves, seeds and pollen, and also in clover hay. These methods can be used to identify GM white clover and also to detect the presence of GM pollen in honey. These detection methods are sensitive enough to detect the presence of one GM seed in 1000 non-GM seeds.

5.5.3 Characterisation of the phenotype of the GM white clover

48. One of the aims of the proposed trial is to compare agronomic performance of the GM white clover line and the non-GM parent under field conditions. The intended effect is improved AMV resistance in the field without unacceptable impacts on agronomic characteristics. Comparison between GM and non-GM plants in a variety of glasshouse and field conditions have shown no evidence that the introduced genes have affected the growth habit or persistence of the GM white clover in the absence of AMV. The GM white clover plants have been selected for 'Mink' and 'Sustain' like characteristics.

49. The GM white clover plants have also been assessed for resistance to AMV and were shown to have a stable virus resistant phenotype in both the homozygous and heterozygous state, in all three cultivars used to create the GMO proposed for release.

50. Results of virus infectivity tests where the GM plants are inoculated with the target virus in the presence of control non-GM parent plants are presented in Figure 5. These showed no AMV infection in Winter and early Spring, but by October the non-GM plants were infected whereas the T₀ GM plants were resistant to AMV.

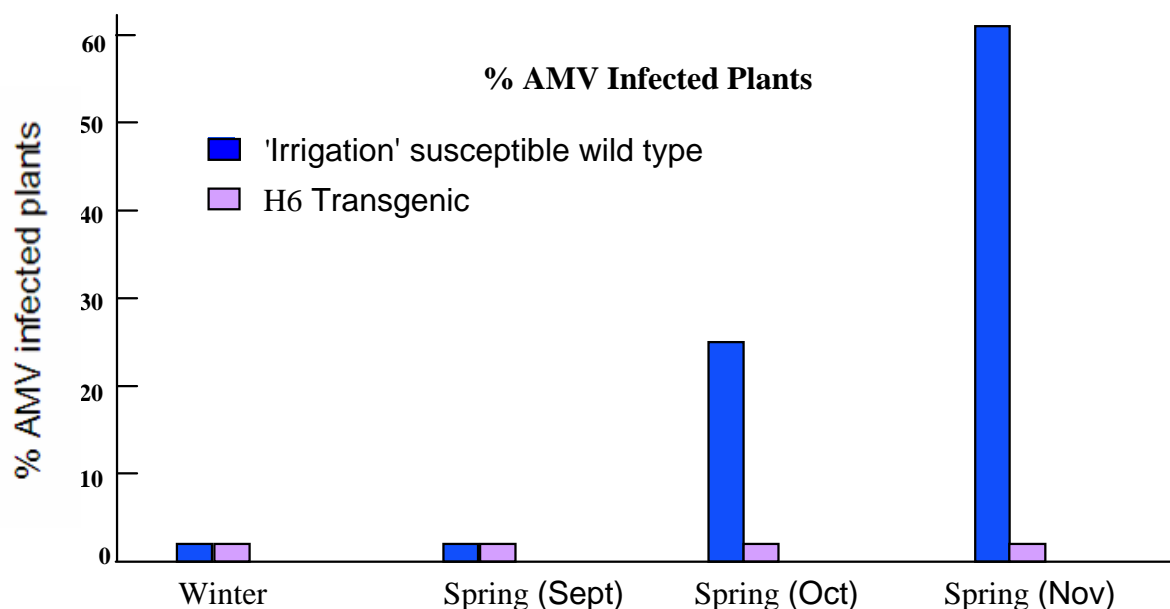


Figure 5. Field resistance of GM white clover primary transformants to AMV.

The proportion of infected wild-type (cultivar 'Irrigation') was compared to the proportion of infected GM lines (H6 transgenic event, Irrigation background) in winter and spring of 1998.

51. White clover can potentially be toxic to grazing animals if ingested in large quantities or under particular situations, for example when an animal is deficient in iodine, 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 2008). The levels of these compounds have been determined in samples of field and glasshouse grown white clover. Biochemical studies have shown that the levels of the three classes of natural toxicants in leaves of the GM plants are likely to be within the range seen in wild-type plants grown under field or glasshouse conditions (see Figure 6).

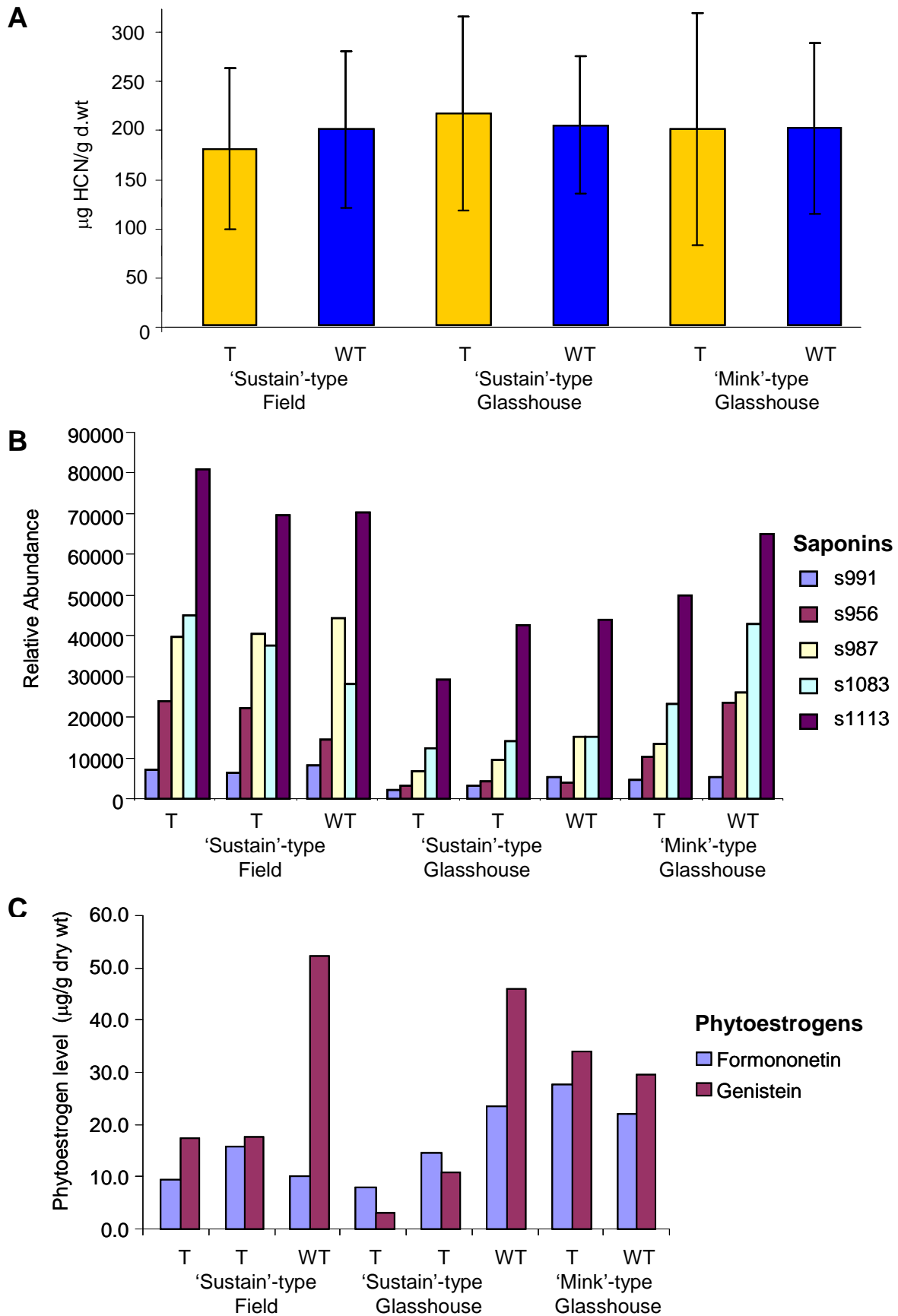


Figure 6. Mean levels of cyanogenic glycosides (A), saponins (B), and phytoestrogens (C), in GM (T) and non-GM white (WT) white clover grown under Australian field and glasshouse conditions. (Figure adapted from DIR 089 application)

52. Levels of cyanogenic glycosides in the GM white clover and non-GM white clover, under both field and glasshouse conditions did not differ significantly (Figure 6A).

53. The relative abundance of five different saponins was assessed in GM and non-GM white clover under field and glasshouse conditions in the two genetic backgrounds (Figure 6B). No consistent differences in saponins were seen as a result of the genetic modification.

54. Similarly, there was considerable variation in the levels of the two phytoestrogens detected, and no consistent differences were seen as a result of the genetic modification (Figure 6C).

Section 6 The receiving environment

55. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO are assessed. This includes the geographic region where the release would occur and any relevant biotic/abiotic properties of this location; the intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007).

6.1 Relevant abiotic factors

56. White clover is grown on six million hectares of improved pastures throughout south-eastern Australia and in the wetter parts of Western Australia, is the dominant pasture clover for the Australian dairy industry and is a preferred source of pollen and nectar for bees. White clover is sensitive to high temperatures, and requires annual rainfall in excess of 750 mm extending over summer or is grown under irrigation.

57. The abiotic factors relevant to the growth and distribution of commercial white clover in Australia are discussed in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008).

58. The release is proposed to take place at one site in southern NSW. The research farm is located 30 km west of Albury on the banks of the Murray River at Howlong in southern NSW. The farm covers 70 hectares in total with about 30 hectares of this used for pasture grass and legume breeding trials and has an annual rainfall of 580-600 mm. The soil type is poorly structured coluvial loam with moderate fertility. The applicant states that the field trial site is at least 280 metres from the river, has no history of flooding and is approximately eight metres above the level of the river.

59. Although climatic data for Howlong NSW is not available, representative data from the three closest weather stations is provided in Table 4.

Table 4. Climatic data near the GM white clover trial site

	Albury	Corowa	Rutherglen
Average daily max/min temperature (Summer*)	30.3°C/15.3°C	31.3°C /15°C	30.4°C/12.9°C
Average daily max/min temperature (Winter*)	14°C/3.4°C	13°C /3.3°C	13.2°C/2.5°C
Average monthly rainfall (Summer*)	39.3 mm	37.2 mm	38.3 mm
Average monthly rainfall (Winter*)	67.6 mm	54.8 mm	60.2 mm

Source: <<http://www.bom.gov.au>>

* Summer averages were based on December to February and winter averages were based on June to August.

6.2 Relevant biotic factors

60. Research on a variety of forage species is carried out at the research farm including Italian, hybrid and perennial ryegrass, Mediterranean and continental tall fescue, cocksfoot, white clover, red clover, chicory, lucerne, forage cereals (oats, barley, triticale), milling wheat, durum wheat, malting barley and turf grasses.

61. Clover seed production within Australia generally takes place in the south-east part of South Australia, north-east along the Murray River within Victoria and from Howlong to Corowa and up to Forbes within New South Wales. The applicant states that subterranean clover and lucerne are grown in preference to white clover in the area surrounding the farm. However, there is a possibility that white clover is naturalised near the site, mostly as roadside volunteers.

62. The biotic factors pertaining to the growth and distribution of commercial white clover in Australia are discussed in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008). Of relevance to this proposed release are the following points:

- white clover may be grown both commercially and for research purposes in the region surrounding the trial site
- invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products. In particular:
 - aphids and other sucking insects may spread AMV within the site of the trial
 - native and honey bees may consume and disperse pollen and nectar from the GM white clover and non-GM pollen trap plants
 - birds and mammals (either introduced or native) may visit the proposed release site.

6.3 Relevant agricultural practices

63. It is not anticipated that the agronomic practices for the cultivation of the GM white clover by the applicant will be significantly different from conventional practices for white clover growing, with the exception that the applicant will not allow animal grazing to occur on the GM white clover. Conventional cultivation practices for white clover are outlined below and discussed in more detail in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008).

64. Management practices for the research farm are typical of that on Australian farms and it is run under environmental conditions similar to those on commercial farms including extreme summer temperatures, varying levels of moisture stress and physically difficult soils with moderate fertility.

65. There are a number of pests and diseases of white clover (see OGTR 2008 for further details), which may require management (eg application of pesticides such as herbicides or insecticides) during the growing season. Weed control using specific classes of herbicides may involve a pre- or post-emergence application.

66. In Australia, white clover varieties are commonly grown as part of a mixed grazing pasture, often alongside grasses such as perennial ryegrass, phalaris, cocksfoot, fescue, paspalum, Italian ryegrass and kikuyu. However, white clover has a high water requirement and is generally not tolerant of high temperatures, and so may require careful management during summer (Betts & Ayres 2004). The applicant has indicated the GM white clover line will be irrigated with a fixed system to ensure a high level of consistency between plots.

67. The applicant intends to trial the GM white clover alongside the non-GM white clover parents and other cultivars from major white clover growing regions in Australia. The GM white clover will also be grown in combination with the non-GM perennial ryegrass cultivar ‘Arrow’, to enable assessment of the performance of the GM clover in mixed sward plots. Mowers may also be used to trim the white clover and perennial ryegrass plants to maintain separation of plots within the trial site.

68. The applicant has indicated that the GM white clover plants will be destroyed at the end of the trial through the use of herbicides and incineration. All equipment used will be cleaned to remove GM and non-GM plant material after use.

6.4 Presence of related plants in the receiving environment

69. White clover is an obligate outcrosser so viable seed and fertile progeny would be produced from cross-pollination of white clover plants, irrespective of whether the white clover plants are in the semi-cultivated environment of a pasture or in other areas such as roadsides or native grasslands. White clover is grown on a commercial scale in Australia. The applicant has indicated that there will be at least 500 m between the GM white clover and any other planting of white clover.

70. There are no closely related native plant species in Australia. However, many introduced *Trifolium* species are 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). White clover can not cross pollinate with these *Trifolium* species (Chen & Gibson 1971; Williams 1987).

71. Hybrids of white clover and other clover species do not occur naturally (Burdon 1983). White clover has been crossed with ball clover (*T. nigrescens*) (Chen & Gibson 1972; Williams 1987; Marshall et al. 1995), oneflower clover (*T. uniflorum*) (Pandey et al. 1987), Moroccan clover (*T. isthmocarpum*), western clover (*T. occidentale*) (Gibson & Reinhart 1969; Pederson & McLaughlin 1989), sharp-tooth clover (*T. argutum*) and Caucasian clover (*T. ambiguum*) (Williams & Verry 1981; Meredith et al. 1995) but most hybrids were generated through tissue culture methods and many were sterile or showed abnormal development. Of the species that can form viable hybrids, *T. nigrescens*, *T. uniflorum* and *T. ambiguum* have been found at one or more sites in Australia (Australia's Virtual Herbarium) with *T. ambiguum* being a relatively common clover in pastures of NSW (NSW Agriculture 2002). However, due to the difficulties of forming hybrids with these species, it is extremely unlikely that *T. repens* would form viable competitive hybrids with other *Trifolium* species in nature.

72. The interspecific crossing potential of white clover is discussed in more detail in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008). No intergeneric hybrids involving white clover have been documented.

6.5 Presence of the introduced genes or similar genes and encoded proteins in the environment

73. Both of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

74. 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.

75. 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.

76. The *nptII* gene is derived from the common gut bacteria *E. coli* which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected humans routinely encounter the encoded protein through contact with plants and food.

Section 7 Australian and international approvals

7.1 Australian approvals of GM white clover

7.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator

77. The Regulator has previously issued a licence (DIR 047/2003) to DPI Victoria for the conduct of a field trial (on 494 m² in Victoria) of GM white clover involving the GMO in this current application.

78. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been four field trials of GM white clover with the same disease virus resistance trait, including the line proposed for release in this 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 investigated. 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 Corowa, NSW.

79. There have been no reports of adverse effects on human health and safety or the environment resulting from the releases.

7.1.2 Approvals by other Australian government agencies

80. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS), Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 4.

81. As the trial involves early stage research, the applicant does not intend any material from the GM white clover line proposed for release to be used in human food. All genetically modified foods intended for sale in Australia must undergo a safety evaluation by Food Standards Australia New Zealand (FSANZ). Accordingly, the applicant has not applied to FSANZ to evaluate the GM white clover line. However, in the event of a commercial release, FSANZ approval may need to be obtained before materials or products derived from the GM white clover line could be sold for human consumption.

82. The APVMA, which has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia, has previously issued a research permit for the small scale use of the AMV CP in GM white clover plants released under DIR 047/2003. DPI Victoria would require a research permit for this proposed release of GM white clover containing the *AMV CP* gene.

7.2 International approvals of GM plants expressing viral coat proteins

83. 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 trialled 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 United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS), concluded that the GM alfalfa and tobacco have no significant impact on the environment (USDA-APHIS 1988; USDA-APHIS 1994). The USDA APHIS has not yet published an environmental assessment of the GM pea.

84. GM plants with other introduced viral coat protein genes have also been field trialled 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 trialled. Other countries have also trialled 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).

85. Five GM crops expressing viral coat proteins have been approved for commercial release in the US. One of these crops, Potato Virus Y-Protected Potato Lines RBMT15-101, SEMT15-02 and SEMT15-16, has also been approved for human consumption in Australia (FSANZ 2001a; FSANZ 2001b), as well as in Canada, Japan, Korea, Mexico and the Philippines (AGBIOS 2008).

86. No adverse effects from the field trials or commercial release of GM plants containing viral CPs have been reported.

Chapter 2 Risk assessment

Section 1 Introduction

87. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 7) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

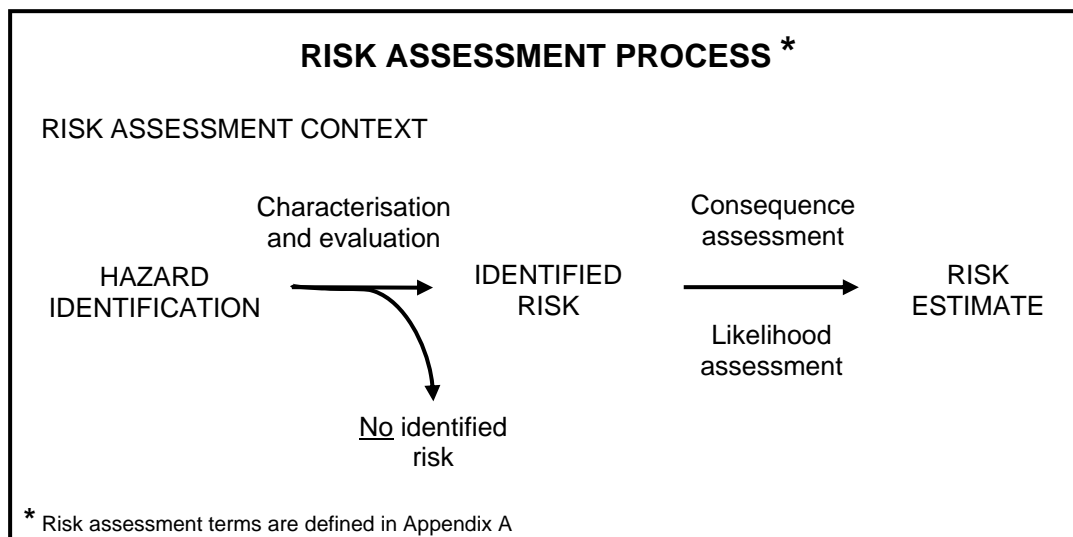


Figure 7. The risk assessment process.

88. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

89. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2007).

90. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

91. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

92. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation and the identification of risk

93. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

94. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2007). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

95. The following factors are taken into account during the analysis of events that may give rise to harm:

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the non-GM parent
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs.

96. The GMO in this application has previously been approved for trial under limited and controlled conditions under Licence DIR 047/2003.

97. Events considered during the risk assessment for this application that are discussed in detail later in the Section are summarised in Table 5. Events that share a number of common features are grouped together in broader hazard categories. Nine events were characterised, one of which (Event 4) was considered to lead to an identified risk that required further assessment (see Chapter 3).

98. As discussed in Chapter 1, Section 5.2.3, the GM white clover contains the antibiotic resistance selectable marker gene, *nptII*. The *nptII* gene, encoding neomycin phosphotransferase type II, has already been considered in detail in the RARMP prepared for DIR 070/2005 and by other regulators and was found to pose no risks to either people or the environment. Therefore the potential effects of the *nptII* gene will not be further assessed for this application.

Table 5. Summary of events that may give rise to an adverse outcome through the expression of the introduced gene for AMV resistance.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing the protein encoded by the introduced gene.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The encoded protein is widespread in the environment and is unlikely to be toxic/allergenic to people or toxic to other organisms. The limited scale, short duration and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced gene.
Section 2.2 Spread and persistence of the GM white clover line in the environment	2. Expression of the introduced <i>AMV CP</i> gene improving the survival of GM white clover plants at the trial site.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Many factors other than AMV resistance are expected to limit the spread and persistence of white clover in the area proposed for release. The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> As discussed in Event 1, the encoded protein is already widespread in the environment and unlikely to be toxic or allergenic. The proposed limits and controls would minimise dispersal, such as locating the site at least 50 m away from natural waterways and surrounding the site by both a rabbit-proof and a stock-proof fence.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced gene or regulatory sequences in other white clover plants.	Weediness; allergic reactions in people or toxicity in people and other organisms	Yes	<ul style="list-style-type: none"> See Chapter 3, Identified Risk 1.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genetic material in other viruses as a result of gene transfer.	Increased pathogenicity or host range of viruses	No	<ul style="list-style-type: none"> The <i>AMV CP</i> gene is already widespread in the environment and available for transfer via demonstrated natural mechanisms.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	6. Presence of the introduced gene, in other organisms as a result of gene transfer.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The introduced gene and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Events 1 – 4 associated with expression of the introduced gene did not constitute identified risks for people or the environment.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	7. Changes to biochemistry, physiology or ecology of the GM white clover line resulting from expression, or random insertion, of the introduced gene.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process
	8. Effects of <i>AMV CP</i> expression on viruses.	Transiently increased pathogenicity or host range of viruses	No	<ul style="list-style-type: none"> The AMV CP is already widespread in the environment and available for interaction with viruses Any novel virus characteristics will be transient The limited scale and short duration further reduce exposure of other viruses to the introduced gene.
Section 2.6 Unauthorised activities	9. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

99. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

100. Allergenicity is the potential of a protein to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

101. A range of organisms may be exposed directly or indirectly to the protein encoded by the introduced gene for AMV resistance. Workers cultivating the white clover would be exposed to all plant parts. Organisms may be exposed directly to the protein through biotic

interactions with GM white clover plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM white clover plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1. Exposure to GM plant material containing the protein encoded by the introduced gene

102. Expression of the introduced gene for AMV resistance could potentially result in the production of novel toxic or allergenic compounds in the GM white clover line, or alter the expression of endogenous white clover proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

103. Non-GM white clover contains a number of natural toxicants including cyanogenic glycosides, phytoestrogens and saponins (OGTR 2008). These can be toxic to grazing animals under particular situations, or if ingested in large quantities. Cyanogenic glycosides produced by white clover under certain conditions are unlikely to be at levels which are directly toxic, but the glycosides can interfere with selenium metabolism and cause conditions such as nutritional myopathy and goitre in ruminants (Lehmann et al. 1991; Gutzwiller 1993). Although clover contains phytoestrogens, the levels in white clover are low (0.01%-0.06% of dry matter) (Saloniemi et al. 1993; Saloniemi et al. 1995). Triterpenoid saponins and flavonoids are found in white clover and can potentially reduce the digestibility of forage proteins, affecting feed intake, growth and reproduction in ruminants, although some beneficial effects have also been reported (Francis et al. 2002; Oleszek & Stochmal 2002). The high protein and low condensed tannin content of actively-growing white clover herbage can lead to pasture bloat, a potentially lethal condition, in ruminants. More information is available in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008).

104. Biochemical studies have shown that the levels of natural toxicants (cyanogenic glucosides, five classes of saponins and the phytoestrogens formononetin and genistein) in the leaves of the GM white clover plants are within the range of the non-GM parent plants (Chapter 1, Section 5.5.3).

105. Humans do not generally eat white clover plant material. However, there is reference to white clover seeds being used as flour, leaves in salad and flowers in tea (Peterson & Peterson 1978). Additionally, bee pollen (which may contain clover pollen (Somerville 2001)) is sold as a nutritional supplement (Somerville 2000).

106. White clover flowers are an important source of nectar and pollen for commercial honey production by honey bees (Somerville 1999; Malone 2002). As honey usually contains some pollen grains (MAFF 1997), there is the potential for honey produced in the beehives at the GM trial site to contain GM white clover pollen. The average pollen content of sieved honey is normally less than 0.1% (Agrifood Awareness Australia 2001). Pollen grains contain protein and, therefore, may also contain the introduced AMV CP. However, no expression of the *AMV CP* gene or protein could be detected in pollen from the GM white clover plants (information supplied by applicant) and it is not known whether AMV CP can be detected in the pollen of AMV infected non-GM white clover plants. AMV virus particles have been observed in pollen from infected alfalfa plants (Pesic et al. 1988; Pesic & Hiruki 1998) and pollen transmission of AMV has also been demonstrated in *Medicago polymorpha* plants (Pathipanawat et al. 1995). Although it has not been shown, it is possible that pollen from other AMV infected species could also contain AMV particles. Therefore, bees could have been exposed to AMV CP in the pollen of a number of plant species infected by AMV, and AMV CP could also be present in honey produced by bees feeding on AMV infected plants.

107. AMV infection has been shown to alter the feeding preference of spotted alfalfa aphids (*Therioaphis trifolii*) as these aphids were shown to prefer to feed on lucerne cv Siriver plants infected with AMV rather than uninfected cv Siriver plants. However, this response was cultivar specific as the spotted alfalfa aphids did not discriminate between AMV infected and uninfected cv Hunter River plants (Garran & Gibbs 1982). AMV infection of soybean has been shown to affect the growth and survival of the soybean aphid (*Aphis glycines*) with aphids avoiding infected plants and showing a 20% reduction in population growth when fed solely on infected plants compared with aphids fed on uninfected plants (Donaldson & Gratton 2007). The mechanism behind these effects is unknown and therefore expression of the AMV CP in the GM white clover could potentially affect aphid feeding behaviour at the site.

108. In terms of allergenicity, white clover pollen is not considered to be an airborne allergen for humans as clover pollen is generally transported by insects and is not wind-borne (OGTR 2008). An extensive literature search has not revealed any published information on white clover pollen allergenicity.

109. Although no toxicity studies have been performed on the GM white clover plant material or encoded protein, the introduced *AMV CP* gene is isolated from a naturally occurring organism that is already widespread and prevalent in the environment. It is not expected that any novel products would be produced as a result of the expression of the introduced gene as the gene is homologous to the gene present in AMV which is found in infected non-GM white clover and other AMV infected plants (see Chapter 1, Section 6.5).

110. No information was found to suggest that the protein encoded by the introduced gene is toxic or allergenic to people or to other organisms (Chapter 1, Sections 5.1 and 5.2.2) or could affect the production of endogenous white clover toxins and therefore exposure to the GM plant materials is not expected to adversely affect the health of humans or other organisms.

111. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. The proposed trial site will be surrounded by two fences. The central plot site planted with the GM white clover will be surrounded by a rabbit-proof fence and the whole two hectare release site will be surrounded by a stock-proof fence with a locked gate. Only approved staff with appropriate training will have access to the site. This will reduce inadvertent access by humans and prevent grazing livestock and rabbits from entering the site, which minimises exposure of the public and animals to the GM plant material. Livestock would not be intentionally exposed as the GM plant material will not be used as feed. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material, or products from the on-site beehives will be used as animal feed or human food. The short duration (2009-2011) and small size (633 m² per year) of the proposed trial would also limit the potential for exposure to the GM plant material.

112. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced gene is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM white clover line in the environment

113. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM white clover plants in particular, is given in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008). In summary, white clover shares some characteristics with known weeds, such as weedy relatives, high seed output, hard seeds that form a persistent seed bank, sexual and asexual reproduction, rapid adaptation to environmental conditions and profuse flowering throughout spring and summer providing

prolonged opportunity for pollen dispersal. In addition, white clover has been identified as a weed of natural and agricultural systems although it is not classed as a weed of national significance (Department of the Environment and Heritage 2006). White clover does lack other characteristics that are common to many weeds, such as wind pollination, ability to self-pollinate, rapid growth to flowering, and long-distance seed dispersal (OGTR 2008).

114. Scenarios that could lead to increased spread and persistence of the GM white clover line include expression of the introduced gene conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins.

Event 2. Expression of the introduced AMV CP gene improving the survival of the GM white clover plants at the trial site

115. If the GM white clover line was to establish or persist at the trial site it could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of exposure to GM plant materials or the encoded protein has been considered in Event 1 and was not considered an identified risk.

116. If the expression of the introduced gene for AMV resistance was to provide the GM white clover plants with a significant selective advantage over non-GM white clover plants and they were able to establish and persist in favourable agricultural environments this may give rise to lower abundance of desirable species.

117. The impact of the genetic modification on survival of the GM white clover line is not completely characterised under field conditions. Data collected during previous field releases showed no evidence that the introduced gene in this GM white clover line mediates any change in growth habit or persistence in the absence of AMV (information supplied by applicant). However, the applicant states that the introduced gene has demonstrated the capacity to produce an AMV-resistant phenotype in the GM white clover line grown in glasshouse experiments and in previous field releases. In an environment in which infection by AMV was the main factor limiting the spread and persistence of white clover, expression of the gene for AMV resistance could result in increased weediness of the GM white clover line relative to non-GM white clover.

118. AMV infection in non-GM white clover is likely to reduce foliage yield and quality, nitrogen fixing capacity and vegetative persistence in an agricultural setting. (Kalla et al. 2001). This reduced growth is also likely to impact on seed set.

119. GM AMV-resistant white clover may have altered invasiveness potential by having increased vegetative reproductive capacity, and could have an impact on other plant species by reducing the establishment or amount of desired plants/vegetation or by increasing nutrient levels in the soil which may affect other vegetation. However, in an environment in which AMV infection is not present, or does not adversely affect the survival or persistence of the white clover plants, the introduced gene will have no effect. In this case, GM white clover plants will behave in the same manner as uninfected non-GM white clover plants. This means that the potential invasiveness and impact of GM white clover with resistance to AMV in the Australian environment is the same as uninfected non-GM white clover in areas free of AMV.

120. There are a number of factors required in the casual pathway for the GM white clover to become a weed at a particular location, including the presence of white clover populations, aphid vector populations and AMV as well as conditions conducive to viral spread. Increased

weediness is also only likely if AMV is the factor limiting the spread and persistence of non-GM white clover at the location. In pastoral situations, aphid numbers and the incidence of AMV infection can be extremely high (refer to DIR 047/2003 for details).

121. However, it is likely that the survival of the GM white clover plants would still be limited by high temperatures, low water availability, nutrient availability and other environmental factors that normally limit the spread and persistence of white clover plants in Australia (OGTR 2008).

122. Therefore, the expression of the introduced gene for AMV resistance is only expected to provide the GM white clover plants with a significant selective advantage over non-GM white clover plants where AMV is main limiting factor for the spread and persistence of white clover.

123. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM white clover line proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures including removal of mature flower heads during peak flowering time to reduce the build-up of a seed bank, destruction of all plant materials not required for further analysis, and post harvest monitoring of the proposed site (and destroying any germinated seed) for at least five years.

124. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced gene for AMV resistance improving the survival of the GM white clover line at the trial site is **not an identified risk** and will not be assessed further.

Event 3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions

125. If the GM white clover line was to be dispersed from the release site it could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of exposure to the GM white clover line have been assessed in Event 1 and were not an identified risk.

126. White clover seed may be dispersed by birds, grazing animals, worms, ants and to a small extent by wind. 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, pigeons, pheasants and rooks (Krach 1959) but the seed is relatively unattractive to birds due to its very small size. Ingestion of white clover seeds by earthworms does occur and viable seed are found in worm casts (McRill & Sagar 1973). Distribution by common Australian animals and birds has not been studied. However, ants have been shown to carry white clover seeds in Australian pastures (Campbell 1966).

127. Dispersal and digestibility characteristics are not expected to be altered in the GM white clover line compared to non-GM parental white clover line. However, seed production may be increased compared to the AMV-infected non-GM parental white clover line but is expected to be similar to uninfected non-GM white clover.

128. White clover is capable of asexual reproduction through the production of stolons. There is an inverse relationship seen between stolon branching and flowering vigour (Thomas 1987), thus profusely flowering plants do not produce many stolons. If stolons are detached from the parent plant prior to rooting there is no evidence that they can establish under natural conditions.

129. Control measures have been proposed by the applicant to minimise dispersal of the GM white clover. The proposed release site will be surrounded by two fences. The central plot site

planted with the GM white clover will be surrounded by a rabbit proof fence and the whole two hectare release site will be surrounded by a stock proof fence with a locked gate. This will reduce inadvertent access by humans and prevent grazing livestock and rabbits from entering the site, thus limiting dispersal of the GM plant material. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing. Finally all GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise the opportunity to disperse the GM material.

130. 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 site is on a level site (information supplied by applicant). Additionally, the possibility of seed dispersal by excessive runoff of irrigation water is minimized by the planting of pollen trap crops (white clover and lucerne) around the GM white clover plot. The applicant also intends to remove mature flower heads during peak flowering time to reduce the build-up of a seed bank and proposes to monitor the lucerne pollen trap monthly and destroy any white clover plants prior to flowering. Furthermore, the applicant proposes to perform post harvest monitoring of the site for five years and destroy any volunteer plants found in either the site or the pollen trap. This would ensure any remaining GM white clover seeds, or plants that had dispersed into these areas would be destroyed.

131. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal. These include locating the proposed release site at least 280 m away from natural water ways to prevent dispersal in the event of flooding, and surrounding the site with a fine mesh rabbit-proof fence which would prevent strong winds dispersing plant parts.

132. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials from the trial site through various means including animals and extreme weather conditions is **not an identified risk** and will not be assessed further.

2.3 Vertical transfer of gene or genetic elements to sexually compatible plants

133. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful crosspollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

134. Baseline information on vertical gene transfer associated with non-GM white clover plants can be found in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008). In summary, white clover plants are primarily out-crossing with most pollination effected by bees. There are some species in the *Trifolium* genus (for example, *T. nigrescens*) which are sexually compatible with white clover and are known to form hybrids under natural conditions. However, many hybrids formed have sterile pollen, chlorophyll deficiencies or produce non-viable seeds (Chen & Gibson 1972; Williams 1987; Marshall et al. 1995) and therefore do not persist. As a consequence, transfer of the *AMV CP* gene to other plant species is extremely unlikely (refer to Chapter 1, Section 6.4 as well as the RARMP prepared for DIR 047/2003) and will not be considered further here.

Event 4. Expression of the introduced gene and regulatory sequences in other white clover plants

135. Transfer and expression of the introduced gene for AMV resistance and regulatory sequences in other white clover plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

136. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to the white clover plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. However, the impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

137. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM white clover plants by the introduced gene or regulatory sequences. This will be the same if the introduced gene is expressed in other white clover plants.

138. Transfer and expression of the introduced *AMV CP* gene to other white clover plants via vertical gene transfer could result in these plants becoming resistant to AMV. This in turn could confer a fitness advantage in environments where white clover plants are limited by AMV.

139. White clover is generally classified as a weed in most States of Australia (Groves et al. 2003). Groves et al. 2003 produced a weed ratings scheme, with a rating given to various plants based on their distribution and the extent of the problem in both agricultural and natural environments for each State of Australia. In agricultural systems, Queensland has the highest rating as white clover is recognised as a weed in turf. A rating of 3 is given by New South Wales, Victoria and Western Australia, which means white clover is naturalised and known to be a minor problem warranting control at four or more locations within a State or Territory. The other states rate it as a less important agricultural weed.

140. The impact of GM white clover in the agricultural setting and non-native plant habitats such as roadsides is not considered an identified risk as control options for white clover are readily available and relatively easily applied in these situations. For example, white clover can be controlled by a number of herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) (Rolston 1987; Riffkin et al. 2005). In high nitrogen soils, white clover is not as competitive as in soils where its nitrogen-fixing ability gives it an advantage; hence application of nitrogen fertiliser has also been suggested as a control measure. Non-chemical measures that focus on reducing the competitiveness of white clover relative to grasses, such heavy grazing or close mowing, are also effective. Hence, any increased fitness advantage would have to be very large to have a serious adverse impact in these areas.

141. However, a risk is identified for increased weediness in native plant habitats as a result of gene transfer of the *AMV CP* gene into non-GM white clover plants. The level of risk of weediness from this event is estimated in **Chapter 3** as **Identified Risk 1**.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

142. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the

recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

143. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

144. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Event 5. Presence of the introduced genetic material in other viruses as a result of gene transfer

145. If the GM white clover lines were to be infected by viruses other than AMV, the transfer of the introduced genetic material into these viruses by recombination could potentially occur. This could result in the production of novel viruses with the potential for increased pathogenicity, altered host range or altered vector specificity.

146. If the whole *AMV CP* gene was recombined into a virus and expressed, the novel viruses produced could potentially result in similar adverse outcomes to those resulting from viral synergy or transcomplementation (discussed in Section 2.8, Event 8) that may persist through viral replication. Such effects could include changes in virus transmission as a result of encapsidation by the *AMV CP*, or changes in virus pathogenicity as a result of interaction between *AMV CP* and other proteins of the virus.

147. If recombination occurred between a portion of the *AMV CP* gene and a coding or regulatory sequence from a virus, novel protein sequences or expression patterns could result. This could potentially result in adverse effects such as increased virus-related losses of white clover production due to increased infectivity or virulence of recombinant viruses, or virus-related losses of other plant species as a result of altered host range or vector specificity of recombinant viruses.

148. Genetic information can be exchanged between two RNA molecules by recombination. This can occur between two viruses co-infecting a plant cell, or between plant mRNAs (including those from transgenes) and viruses, and most frequently occurs between similar nucleotide sequences. Recombination between the highly similar sequences of virus-derived plant transgenes and their viruses of origin has been reported for a variety of CP-expressing GM plants (Tepfer 2002; reviewed by Fuchs & Gonsalves 2007). This has typically been in experimental situations of strong selective pressure, such as requiring recombination to restore normal function to mutant viruses. Compared to recombination between two viruses, recombination between a virus-derived transgene and an infecting virus is thought to result in highly similar populations of recombinants. However, they occur more commonly in the GM situation (Turturo et al. 2008). This higher frequency of recombination in GM plants expressing viral sequences is thought to be because an infecting virus has a greater likelihood of interacting with a virus-derived transgene expressed in every plant cell rather than with a second infecting virus present only in some plant cells.

149. In addition to sequence similarity and selection pressure, a further factor thought to influence recombination frequency between viruses and virus-derived transgenes is the inclusion in transgenes of nucleotide sequences from which viral replication is initiated. Some plant viruses, including AMV, have sequences in their 3' untranslated regions (UTRs) at which viral RNA-dependent RNA polymerases (RdRps) bind and initiate replication (Bol

1999). When expressed as a part of plant transgenes, these sequences are known to function as replication initiation points for RdRps from a range of related viruses (Teycheney et al. 2000). Greene and Allison (1996) found that recombination between Cowpea chlorotic mottle bromovirus (CCMV) and a CCMV CP transgene in *Nicotiana benthamiana* was only detectable if the transgene contained the full RdRp binding site in the 3'UTR, and not if it was truncated. Equivalent results have been reported for Plum pox virus in *N. benthamiana* (Varrelmann et al. 2000).

150. A number of factors influence the likelihood of recombination between viruses and virus-derived transgenes and the potential outcomes that may result. Compared to natural viral recombination events, the expression of the *AMV CP* gene in white clover is considered likely to increase the frequency of recombination because of transgene expression in every plant cell, and the inclusion of the RdRp recognition sequence. However, it is considered unlikely that recombinant viruses that might occur in the *AMV CP*-expressing GM white clover would differ significantly from those arising from recombination between infecting viruses and *AMV* occurring naturally in white clover. Further, the emergence of recombinant viruses as a result of the GM white clover is considered no more likely than the baseline situation, given that there is no increase in selection for recombinant viruses. This conclusion is supported by a range of field studies which have not been able to detect recombinant viruses in commercial releases of a variety of virus CP-expressing GM plants (reviewed by Fuchs & Gonsalves 2007).

151. The likelihood of any novel viruses emerging as a result of recombination with the *AMV CP* transgene is minimised by the proposed limits and controls outlined in Chapter 1, Section 3.3. In particular, the proposed release is limited in size, duration and location.

152. **Conclusion:** The potential for an adverse outcome as a result of viral recombination of the introduced *AMV CP* gene with other viruses is **not an identified risk** and will not be assessed further.

Event 6. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

153. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material. Risks that might arise from HGT from a GMO to another organism have been recently reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

154. HGT could result in the presence of the introduced *AMV CP* gene in bacteria, plants, animals or other eukaryotes. However, *AMV* infects a wide range of plant species (See Chapter 1, Section 5.1) and thus the *AMV CP* gene sequence is widespread in the environment and already available for transfer via demonstrated natural mechanisms.

155. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced viral CP gene rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced gene or its end product is not associated with any risk then even in the unlikely event of HGT occurring, it should not pose any risk to humans, animals or the environment. Conclusions reached for Events 1 - 4 associated with the expression of the introduced *AMV CP* gene did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

156. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unintended changes in biochemistry, physiology or ecology

157. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹³ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

158. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, altered pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

Event 7. Changes to biochemistry, physiology or ecology of the GM white clover line resulting from expression or random insertion of the introduced gene

159. There are no observed phenotypic differences between the GM white clover plants and the non-GM parental line under glasshouse and field release conditions (information supplied by applicant). Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced gene, have already been discussed in Events 1 - 3, and were not considered identified risks.

160. Various biochemical pathways of the GM white clover plants could be changed by the expression of the *AMV CP* gene, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM white clover can be toxic to animals if consumed in large quantities or under particular environmental conditions (due to the presence of cyanogenic glycosides, triterpenoid saponins and phytoestrogens). However, chemical analysis has shown that the expression of these compounds in the GM white clover line remains within the same range as the non-GM parent cultivars (see Chapter 1, Section 5.5.3). For further discussion regarding the toxicity and allergenicity of non-GM white clover see *The Biology of Trifolium repens L. (White clover)* (OGTR 2008).

161. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

¹³ Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

162. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2 and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. The proposed trial sites will be surrounded by both a rabbit proof and a stock fence, with access to the trial site being via locked gates, which limits exposure of the public, and some animals to the GM plant material. Livestock will not be exposed as the GM plant material will not be used as feed.

163. **Conclusion:** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

Event 8. Effects of AMV CP expression on viruses

164. If the GM white clover plants were to be infected by a virus other than AMV, interactions (direct or indirect) between the infecting virus and the AMV CP could potentially occur. These interactions could transiently enhance or modify the pathogenicity of the infecting virus, potentially increasing the susceptibility of the GM white clover to infection and altering other viral properties. Alternatively, the presence of the AMV-resistant white clover could have an impact on the naturally occurring AMV population. There are a variety of potential interactions and impacts which may occur, for example:

- Increased disease burden in the GM white clover from non-target viruses as a result of decreased AMV viral load (discussed in DIR 047/2003 RARMP)
- Changes in the target virus as a result of strong selection for AMV variants able to overcome AMV resistance of the GM white clover (discussed in DIR 047/2003 RARMP)
- Increased susceptibility of the GM white clover to viruses whose pathogenicity is supported or enhanced by interaction with the AMV CP, resulting in, for example, stronger viral symptoms or increased viral replication in infected GM white clover (discussed below)
- Altered vector specificity of viruses infecting the GM white clover as a result of transencapsidation by AMV CP, potentially resulting in spread of viruses by new vectors to new susceptible plant populations, in the worst case causing virus epidemics (discussed below).

165. The functions of viral proteins can contribute to or enhance the activity of other viruses co-infecting the same cells. Interactions are described as synergistic if they enhance viral infection, or as transcomplementation where infection by a dependent virus is supported by proteins from a helper virus. These types of interactions occur naturally between a great variety of plant viruses, and also between plant-expressed virus-derived proteins and infecting viruses. A variety of outcomes can result, including acceleration of infection, increased viral titre, enhanced movement and increased host range (reviewed by Latham & Wilson 2008).

166. As discussed in Chapter 1, Section 5.2.1, the AMV CP is involved in a number of processes required for viral reproduction, including genome activation and RNA synthesis. A closely related genera, the Ilarviruses, have similar genome structures to AMV including similar coat protein functions, and it has been shown that AMV CP can transcomplement for Ilarvirus CP in activation of the genome of several Ilarviruses (reviewed by Bol 1999; Bol 2005).

167. One particular instance of transcomplementation is transencapsidation, also known as heteroencapsidation or heterologous encapsidation, which is the encapsidation (packaging) of the genetic material of one virus in the coat protein of another virus. This may occur naturally in cells infected simultaneously by two viruses, or in GM plants expressing a viral CP while

infected by another virus. The phenomenon has been reported in a variety of situations, including the encapsidation of Cucumber mosaic virus by AMV CP expressed by GM tobacco plants (Candelier-Harvey & Hull 1993). Transencapsidation can change the properties of the encapsidated virus which are determined by its CP, including pathogenicity or vector specificity, for example, enabling aphid transmission of a virus not normally spread by aphids (Lecoq et al. 1993) or transmission to a host not normally susceptible to infection (Spitsin et al. 1999).

168. While it is theoretically possible that synergism and transcomplementation (including transencapsidation) could result in new virus epidemics as a result of virus transmission to new host populations, this is not expected given that the effects of transencapsidation are for only a single viral passage. This is because transencapsidation occurs without the transfer of the gene for the transencapsidating CP, so there is no means to produce the new CP units required for perpetuation of the novel viral characteristics. In comparison to the natural situation of interactions between AMV and other viruses in white clover, interaction between the GM AMV CP and other viruses infecting white clover does not present any new risks, particularly considering the limited size, duration and location of the proposed release. This conclusion is further supported by many field studies of CP-expressing GM plants, which have repeatedly failed to detect novel viral traits (Fuchs & Gonsalves 2007).

169. **Conclusion:** The potential for an adverse outcome as a result of interactions between the AMV CP with other viruses is **not an identified risk** and will not be assessed further.

2.6 Unauthorised activities

Event 9. Use of GMOs outside the proposed licence conditions (non-compliance)

170. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM white clover line outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

171. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

172. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

173. One event from the hazard identification process (Identified Risk 1 in Table 4) is considered to lead to an identified risk with the adverse outcome of weediness.

174. Chapter 3 gives detailed consideration to the consequences and likelihood of this identified risk in order to obtain estimates of the level of risk. The risk is assessed against baselines established by reference to the characteristics of the parent organism and aspects of the receiving environment (including agronomic practices).

175. Information contained in the application (including information required by the Act and the Regulations on the GMOs, the parent organism, the proposed dealings and potential impacts on the health and safety of people and the environment), current scientific knowledge, and submissions received during consultation with experts, agencies and

authorities (summarised in Appendix B) as well as with the public (summarised in Appendix C) were also considered.

176. The consequence assessment considers the seriousness of the harm that could potentially result from an event, while the likelihood assessment considers the chance of the event resulting in harm. Consequence and likelihood assessments are then combined to give an overall risk estimate using the Risk Estimate Matrix (Figure 8). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty that arise from a lack of data.

		RISK ESTIMATE			
		Low	Moderate	High	High
LIKELIHOOD	Highly Likely	Low	Moderate	High	High
	Likely	Negligible	Low	High	High
	Unlikely	Negligible	Low	Moderate	High
	Highly Unlikely	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
		CONSEQUENCES			

Figure 8. The OGTR Risk Estimate Matrix (OGTR 2007)

Risk Estimate Matrix: A *negligible* risk is considered to be insubstantial with no present need to invoke actions for mitigation. A *low* risk is considered to be minimal but may invoke actions for mitigation beyond normal practices. A *moderate* risk is considered to be of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. A *high* risk is considered to be unacceptable unless actions for mitigation are highly feasible and effective

177. Definitions of risk analysis terms used by the Regulator can be found in Appendix A.

178. After an estimate is obtained for an identified risk, risks higher than negligible are evaluated to determine if risk treatment measures are required to mitigate potential harm (see Chapter 4 - Risk Management).

Chapter 3 Risk estimates for weediness

179. This Chapter estimates the risk associated with an event that could lead to the adverse outcome of increased weediness in native plant habitats arising from this proposed release. The risk estimate is based on the consequence and likelihood assessment for the event.

Section 1 Background

180. As discussed in Chapter 2, Section 2.2, weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens and give rise to negative impacts for people and the environment.

181. Negative characteristics of weeds may include competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. In addition, the spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative environmental impacts such as reduced biodiversity, interference with the intended use of the land they occupy, or degradation of landscape or ecosystems.

182. The first step in this pathway, the spread and persistence is determined by complex interactions between a plant and its environment (including availability of water, nutrients and light). A number of measurable properties of plants that may influence spread and persistence or competitiveness include germination, survival and reproduction under a wide range of environmental conditions, rates of seedling growth, rates of growth to reproductive stage, degree of self-pollination, use of non-specialist pollinators or wind when out-crossing, period of seed production and seed output, degree of seed dispersal, longevity of seed and degree of dormancy, allelopathy (effect on the germination and/or growth of neighbouring plants through chemical exudates) and resistance to pests or pathogens.

183. In the risk assessment, consideration is given to characteristics that may be expected to be altered as a result of the genetic modification and that may increase the spread and persistence of the GMOs, or of sexually compatible relatives that may receive the introduced gene(s). Alterations in these characteristics may indicate potential for weediness.

184. The GM white clover line proposed for release expresses two proteins as a result of the genetic modification. Events that may give rise to weediness were considered in Chapter 2. Expression of the NPTII protein in the GM white clover line is not expected to have any impact on the weediness of the GM white clover. The resistance to AMV conferred by the AMV CP may lead to increased weediness of the GM white clover line.

185. The risk of increased weediness as a result of expression of the *AMV CP* gene in GM white clover has previously been assessed in the RARMP prepared for DIR 047/2003. This document is available at <<http://www.ogtr.gov.au>>. This risk assessment concluded that expression of the protein enhancing the weediness potential of GM white clover plants (in comparison to non-GM white clover plants) in the clover growing regions of Australia poses a low risk.

186. Potential weediness of the GM white clover line at the trial site and dispersal of the GM white clover line itself was discussed in Chapter 2, Events 2 and 3 of this RARMP and no risk was identified. Therefore, this chapter will be limited to assessing the risk of weediness in native plant habitats as a result of transfer and expression of the *AMV CP* gene in non-GM white clover.

Section 2 Consequence and likelihood assessments

187. Consideration is given to the identified risks in Chapter 2 (Hazard identification) that may give rise to weediness. For each of the identified risks the level of risk is estimated from

assessments of the seriousness of harm (consequence—ranging from marginal to major) and the chance of harm (likelihood—ranging from highly unlikely to highly likely).

188. The Regulator is required to consider risks posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMO is considered relative to the baseline of weediness of the non-GM parent and the environment in which the GM white clover line is proposed for release. Therefore, other sources of the introduced genes or similar genes in the environment and the agronomic practices proposed by the applicant are relevant to the risk estimate.

2.1 Identified Risk 1: Expression of the introduced *AMV CP* gene in other white clover plants as a result of gene transfer leading to increased spread and persistence in native plant habitats

189. The potential for the introduced *AMV CP* gene to lead to increased spread and persistence compared to non-GM white clover depends on a number of factors. These include issues such as the weediness of the parent plant in native plant habitats and the presence of the *AMV*.

Weediness of non-GM white clover in natural environments

190. Despite widespread use of white clover in agriculture, it is generally classified as a weed in most States of Australia (Groves et al. 2003). Groves et al. 2003 produced a weed ratings scheme, with a rating given to various plants based on their distribution and the extent of the problem in both agricultural and natural environments for each State of Australia. White clover has been given a rating acknowledging that it is naturalised and known to be a major problem at 3 or fewer locations within the State or Territory (Groves et al. 2003). It is therefore recognised as a naturalised non-native species in natural ecosystems.

191. White clover is distributed in many temperate areas of Australia. These areas include potentially sensitive ecosystems such as woodlands and grasslands of montane and subalpine regions, of which some may be areas of national environmental significance and therefore included in the Environment Protection and Biodiversity Conservation Act (1999). However, to date there is limited information on the impact of white clover on native flora and fauna. White clover tends to establish in areas that have been disturbed and where there is minimal competition from other plant species, such as roadsides and freshly excavated areas (Godfree et al. 2004).

192. In lowland grasslands of Victoria, white clover is limited by both low rainfall and low soil fertility. It is common along roadsides, but tends to be restricted to the shoulder of the road where the ground is disturbed and is not found on the adjacent native grassland verge (Garrett & Chu 1997).

193. In the Bogong high plains in Victoria, white clover is found in clearings at low densities (less than 1% of ground cover) and in moist flushes on ridges, slopes and streamside flats. It persists in the moist flushes of ski slopes, where it has been previously sown and it has been found in recently disturbed areas around ski lodges. It does not establish under closed shrub canopies (Garrett & Chu 1997). In subalpine regions in NSW and the ACT, white clover was found in *Poa* grasslands and *Eucalyptus-Poa* woodlands and typically constitutes 0.5-5% of total cover (Godfree et al. 2004). Another study in a national park in the ACT found that white clover generally comprised less than 0.25% of the cover in most grassland and woodland plots (Godfree et al. 2006). It is not generally found in areas with native trees and is more common in communities with a higher cover of native herbs, suggesting that the distribution of white clover is controlled by the structure and composition of the existing plant community (Godfree et al. 2004).

194. White clover is also found in mesic (where adequate moisture is present) grassland communities. In one 1 m² plot in a mesic grassland community, white clover constituted over 40% of total herb biomass, although this constituted only 5% of the total biomass (Godfree et al. 2006). This patchiness is a feature of the white clover abundance and may be related to past disturbances in these areas. Although white clover does not dominate the invaded communities (Godfree et al. 2006; Godfree et al. 2007), it is considered an environmental weed in south eastern Australia, mostly due to high adaptability. Where it is present in semi-natural native plant communities at a relatively high abundance, it competes for growing space with native analogues and may even exclude native species altogether by forming a mat. This is especially the case along creek lines or in mesic grasslands (Godfree et al. 2006).

AMV

195. As discussed in Chapter 1 Section 5, AMV is a plant virus transmitted by aphids. The aphids transmit the virus in a non-persistent manner, meaning that they only carry the virus temporarily after contact with an infected plant (Garrett 1973). AMV is not transmitted in clover through seed so infected plants only pass on the virus to their offspring through vegetative reproduction (Barnett & Gibson 1975).

196. 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).

197. There is little information published on the incidence of AMV outside of a pastoral situation in more marginal areas such as roadsides, native plant habitats and home gardens. In subalpine regions of NSW and the ACT, 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 no AMV was detected in native grasslands in the conserved parts of the national parks, although AMV infection was common in local tourist parks (Godfree et al. 2004).

198. There is one report which suggests that some white clover plants may have natural resistance to AMV (Barnett & Gibson 1975). A number of white clover plants were found to be resistant when mechanically inoculated with AMV in the glasshouse, although it was suggested by the authors that failure to infect all plants of particular clones may have been due to experimental limitations. No other literature indicating natural AMV resistance in white clover has been identified.

Summary

199. The risk of increased weediness as a result of transfer of the *AMV CP* gene to other white clover plants would depend on the importance of AMV in limiting the spread and persistence of white clover (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The risk is assessed against the baseline of the weediness potential in the non-GM parent organism and in the context of the small scale and short duration of the proposed release and the control measures proposed to restrict gene flow.

2.1.1 Consequence assessment

200. As discussed in the introduction of this Chapter, a weed could have a number of negative impacts including adversely affecting the health of people, animals or the environment, restricting movement, or reducing the establishment or yield/amount of desired vegetation.

201. White clover containing the *AMV CP* gene is not expected to adversely impact the health of people or animals compared to non-GM white clover (refer to Event 1). With regards to environmental health effects, white clover containing the *AMV CP* gene is not expected to adversely impact the fire regime of an area, soil salinity or stability, or water table levels. White clover containing the *AMV CP* gene is not expected to restrict the movement of people, animals, vehicles, machinery or water due to the characteristic morphology of white clover.

202. However, transfer of the introduced *AMV CP* gene to non-GM white clover could potentially result in the presence of white clover with resistance to AMV in native plant habitats which could reduce the establishment of native vegetation, giving rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. This could in turn have secondary impacts such as adversely changing animal or microorganism species composition due to altered food/shelter availability.

203. The *AMV CP* provides resistance to AMV infection. In environments where AMV infection limits one or more of the key life stages of white clover plants, this could lead to increased weediness of white clover containing the *AMV CP* gene compared to AMV infected non-GM white clover plants. If this were to occur, AMV-resistant white clover may reduce the establishment or amount of desired native plants/vegetation, or may increase nutrient levels (by increasing nitrogen amounts via fixation) in the soil which may affect native vegetation growth. However, in an environment in which AMV infection is not present, or does not adversely affect the survival or persistence of the white clover plants, the introduced gene will offer no selective advantage to an individual white clover plant. This means that the potential impact of white clover with AMV resistance in the Australian environment is expected to be similar to that of uninfected non-GM white clover plants in areas free of AMV.

204. As discussed in the introduction to this Section (Section 2.1), white clover is present in natural ecosystems of temperate Australia but tends only to exist at low densities (generally up to 5% of total cover/biomass) and is more common in communities with a higher cover of native herbs where it competes for growing space (Godfree et al. 2004). White clover is generally found in areas that have been recently disturbed or have minimal competition from other plants and it does not establish under closed shrub canopies (Garrett & Chu 1997). Its presence is negatively related to tree cover and is not related to the cover of shrubs, perennial grasses, rushes and sedges (Godfree et al. 2004). This indicates that any potential adverse impact of AMV-resistant white clover, like non-GM white clover, will be limited mainly to native herbaceous plants and will only have a marginal effect on the overall plant biodiversity of an area.

205. Importantly, current information indicates that the majority of white clover populations in native plant habitats are not infected with AMV (refer to Section 2.1). This suggests that, if white clover with resistance to AMV is introduced into native plant habitats, these plants will behave similar to non-GM white clover plants and have minimal impact on most native vegetation. However, it may be possible that AMV infection only occurs in intermittent years in some native plant habitats. If this happens and AMV does play a role in controlling white clover abundance, it is possible that AMV-resistant white clover could become more abundant than the non-GM white clover over a long period of time.

206. If white clover containing the *AMV CP* gene was present in the environment, it is not expected to expand beyond the current distribution of non-GM white clover ie temperate and some subtropical parts of Australia. This is because white clover is mainly limited by temperature, water availability, and nutrient availability (see discussion in Section 2.1.2 of this Chapter), and the genetic modification is not expected to increase the plant's ability to withstand these abiotic stressors.

Conclusion

207. White clover plants containing the *AMV CP* gene could have increased weediness compared to AMV-infected white clover plants in environments where AMV infection limits white clover. Uninfected non-GM white clover mainly impacts on herbaceous plant and many native plant habitats do not currently have AMV infected white clover plants. Therefore, white clover resistant to AMV is expected to have a similar impact to non-GM white clover in these areas. Furthermore, the genetic modification is not expected to extend the range of the GM white clover compared to non-GM white clover.

208. However, due to the uncertainty stemming from a lack of information about the role of AMV in limiting white clover populations and the impact that AMV-resistant white clover may have on native vegetation in areas where AMV is present, the consequences of resistance to AMV in white clover due to expression of the *AMV CP* gene is assessed as **minor**.

2.1.2 Likelihood assessment

209. The adverse outcome of weediness resulting from gene transfer from the trial site and an increase in the spread and persistence of white clover plants in native plant habitats is contingent on a number of steps occurring, including:

- A bee needs to carry GM white clover pollen on its body. In this trial, only a maximum of 28/72 of the plots will actually contain GM white clover so if a bee visits the trial site there is less than 40% chance that the flower visited will be GM
- The bee must visit no, or very few, flowers in the inner 1m flowering non-GM white clover pollen trap, the 35 m flowering lucerne pollen trap and the outer 1 m flowering non-GM white clover pollen trap
- The bee must visit no, or very few, flowers in the 500 m isolation zone
- After flying for 500 m and finding very few attractive flowers, the bee must not return to the hive to empty its pollen baskets of the collected GM clover pollen
- The bee needs to locate a flowering white clover plant in the surrounding area (in which white clover is not generally planted) and transfer pollen to the plant
- The plant needs to be fertilised with the GM white clover pollen and set viable seed
- The AMV-resistant white clover seed needs to fall into a suitable habitat to germinate
- The AMV-resistant white clover plant needs to survive the main limiting factors for white clover (ie moisture stress, temperature extremes and soil fertility) to reach flowering
- The pollen for subsequent AMV-resistant white clover plants needs to be collected by a bee and transferred to another white clover plant, or the seed or vegetative plant material needs to be dispersed significant distances
- This cycle of fertilisation, survival and dispersal needs to be repeated until the seed reaches a sensitive native plant habitat
- Both aphids and AMV need to be present in this environment
- The GM white clover plant needs to have a fitness advantage to compete with other white clover plants and other native plants.

Gene transfer

210. White clover is widely planted in Australian pastures and is the main legume grown in perennial pastures in cool temperate, summer rainfall zones (Archer & Robinson 1989). The trial will be planted in Howlong in Southern NSW, under irrigation as this area does not generally receive enough rainfall for white clover production (see Table 4). No white clover

trials are currently being conducted on the research farm where the GM trial is proposed and the area is principally a lucerne-growing district rather than a major white clover grazing area (information supplied by applicant). However, some white clover plants may potentially be growing in nearby areas.

211. White clover is a predominately outcrossing species with a well-developed genetic gametophytic self-incompatibility mechanism in place which limits self-pollination (Thomas 1987). 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 stimulates pollen germination (Harris 1987). Honey bees are the most frequent visitors to this crop and are thought to be the major pollinators, although native bees (*Lasioglossum sp.*) have also been observed collecting pollen (Goodman & Williams 1994). The bees are attracted to the nectar produced by the flower (Anon. 2005). Pollen viability is thought to be high. In pollen germination tests of mature florets, germination *in vitro* was found to be 98.4%. However, as florets mature from the base of the flowers upwards, old florets may contain no viable pollen. (information supplied by applicant). Pollen has been shown to remain viable for at least 60 mins in a sucrose solution (Erith 1924 as cited in Thomas 1987). Long term viability of white clover pollen when carried on bees is unknown but its viability is expected to rapidly decrease upon desiccation.

212. Although honey bees can travel up to 10 km and distances of 2.5 km are regularly recorded (Beekman & Ratnieks 2000), when there is abundant nectar source the forage area is much 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). Observations on two individual bees indicated that they collected a full load of pollen in their corbiculae in an area 3.6 x 4.6 m and 1.5 x 9.1 m, respectively (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 50th inflorescence (Marshall et al. 1999). Bees have been reported to visit an average of 2.5-3.5 white clover florets per inflorescence (Weaver 1965; Michaelson-Yeates et al. 1997) and generally the pollen most effective in fertilisation comes from the last pollen donor visited by the bee (Michaelson-Yeates et al. 1997; Marshall et al. 1999). Honey bees will be present at the trial site as beehives will be set up in the centre of the seed production plot in the second season to allow poly-crosses to be made between the *Syn2* plants (information supplied by applicant).

213. Gene flow rates have been studied on an experimental scale and for commercial seed standards (reviewed in OGTR 2008). Gene flow rates are seen to be high over distances of less than 1 m (Marshall et al. 1999). Gene flow in white clover from a 50 m² site containing a bee hive was measured over two years. Gene flow was seen to be 3% at 1 m, but reduced to less than 0.04% at 10 m, with less than 0.01% beyond 24 m from the pollen source indicating that 95% of successful pollination events occurred within 10 m of the pollen source. Rare instances of gene flow were detected up to 250 m from the pollen source (Clifford et al. 1996).

214. No information has been found on GM white clover releases internationally. However, data is available on separation distances for commercial seed production. These seed production standards are imposed for white clover to keep cultivars distinct. In the USA, standards for fields of less than two hectares, require an isolation distance of 302 m for foundation seed (0.1% contamination with other varieties), 151 m for registered seed (0.25% contamination with other varieties) and 50 m for certified seed (1.0% contamination with other varieties) (Association of Official Seed Certifying Agencies 2001). In Europe, 200 m isolation distance is required (for fields of two hectares or less) for multiplication seed (OECD 2008). In South Australia, basic seed requires an isolation distance of 200 m for areas

of two hectares or less, and 100 m for certified seed (Smith & Baxter 2002). These distances are also used for the New Zealand seed standards for legumes (MAF Biosecurity 2007). In New Zealand, it has been concluded that isolation distances of 100 m are adequate for both field releases of GM plants and normal cultivar isolation (Woodfield et al. 1995).

215. Furthermore, seed collected from a previous field trial with AMV-resistant GM white clover showed no gene flow at 39 m or beyond (Emmerling et al. 2004). Analysis of non-GM white clover plants, which were located immediately adjacent to the GM white clover, indicated that only 0.5% of the seed were the product of gene flow from the GM white clover (information supplied by applicant).

216. Thus, pollen transfer to plants close to the GM white clover plot could occur. However, the applicant has proposed control measures to limit this pollen transfer to plants outside the trial site.

217. The applicant has proposed to surround the GM white clover plot by a pollen trap at least 37 m wide, consisting of non-GM white clover and lucerne plants (refer to figure 2 for details) and to ensure that the GM white clover does not flower when there are insufficient lucerne flowers present to attract bees.

218. Lucerne has been intentionally chosen as the major component of the pollen trap. Lucerne flowers produce a large amount of nectar, which is highly attractive to honey bees (McGregor 1976). The volume and concentration of sucrose in the nectar varies between different cultivars, with higher nectar volume and higher sucrose concentration correlated with seed set and thus bee visitation (Holtkamp et al. 1992).

219. Lucerne pollen has a protein content of between 20-24%, similar to white clover pollen, but with low levels of the amino acid iso-leucine (Stace 1996). Bees do not generally collect lucerne pollen and can obtain nectar from flowers without triggering pollination (Manning 2008).

220. The first flowers of lucerne generally appear in October (De Barro 2001), although the crop is generally managed by grazing or mowing which alters the time of flowering. This is similar to the observed peak flowering of clover which begins in October (Caradus et al. 1997). A lucerne flower may open at any time of the day and will remain open for about one week if it is not pollinated but starts to wilt a few hours after pollination (McGregor 1976; Somerville 2002).

221. The applicant also proposes to provide a 500 m isolation distance between the GM white clover and any non-GM white clover plants (other than those in the pollen trap). This will remove any potentially sexually compatible recipients of the GM white clover pollen from a 500 m radius.

222. Thus, there will be an attractive food source close to the non-GM white clover plants to limit the foraging area for bees, and an absence of sexually compatible non-GM white clover plants (not including those that are part of the trial) that could set seed for 500 m.

223. As summarised previously, for vertical gene transfer to occur, the GM white clover (either planted or volunteers) would need to be within pollination distance of other white clover plants. The GM white clover line would also need to be flowering simultaneously with the non-GM white clover plants. Therefore, the introduced gene could be transferred to white clover plants close to the trial site; however, long distance gene transfer is highly unlikely.

Dispersal, establishment and persistence

224. If a low amount of gene transfer was to occur from the trial site, the GM white clover could cross with non-GM white clover plants growing in the vicinity. Therefore, dispersal of

any white clover seed containing the *AMV CP* gene (as a result of gene transfer) as well as germination, survival and persistence needs to be considered.

225. In pastures, 89-97% of white clover flowerheads are removed by sheep before they mature and thus no seeds are set (Chapman & Anderson 1987). In the event of seed set, most white clover seed is not harvested but would fall to the ground close to the parent plant. Some clover seed is grown commercially for re-planting. As discussed in Event 3, Chapter 2, white clover seed is passively dispersed locally and may also be dispersed over short distances by ants, worms or grazing animals, although Chapman and Anderson concluded that distribution of ripe seeds in sheep dung is unlikely to be significant (Chapman & Anderson 1987). It is also possible that people may disperse clover seed, either unintentionally eg. in hay, or intentionally as planting seed.

226. In the unlikely event of white clover seed entering a waterway the only information available on the survival of white clover seed for extended periods in water is that germination of white clover seeds can occur after 6 days in water (Morinaga 1926).

227. Following outcrossing, the white clover seed containing the GM trait has to germinate or survive until conditions are suitable for germination. Studies on germination of seed on the soil surface indicated that only a small proportion of seed actually germinates. Only 2% of seed sown on undisturbed ground in grass land survived to produce a true leaf, and none of these plants survived the summer. Seed that was sown into bare (herbicide treated) ground established better (16% establishment) and 1% of these initial seed sown survived over the summer (Dowling et al. 1971). Thus, the clover establishes better without competition from other plants. White clover seedlings failed to recruit into permanent pasture except on specific types of disturbed sites such as molehills (Turkington & Harper 1979) or cow pats (Barrett & Silander, Jr. 1992). Although the disturbed sites were found to be better for seedling recruitment, seedling establishment rates were higher in undisturbed areas, presumably due to shading providing favourable temperature and humidity (Barrett & Silander, Jr. 1992). Seedlings face competition from surrounding vegetation, or may be lost due to predators, treading damage and burial during grazing. A New Zealand study over three years found seedling death or disappearance to be the major cause of seedling loss (83%), with the other factors comprising the final 17% (Chapman 1987). They concluded that the slow-growing seedlings of white clover are less suited to establishment in undisturbed sites than annuals which have faster initial growth. Thus white clover seeds are only important in the colonisation of disturbed areas and vegetative growth is more important in established populations (Chapman 1987).

228. If the seed does not germinate immediately then the seed may be buried. Buried seed populations in pastures may not be as high as anticipated from the seed yields. A half-life of one year is indicated by a field study in New Zealand, with few seeds surviving more than two years (Chapman & Anderson 1987). Persistence, as well as productivity and seed production, of white clover is limited by a number of factors in Australia as outlined by Ayres and Reed (1993). These factors include:

- limited growth in cold winters
- low tolerance to summer moisture stress
- local ecotypes being more persistent than introduced ecotypes
- poor performance in soils with low fertility, high acidity or poor drainage
- competition with companion grasses and close grazing
- pests and diseases.

229. Summer moisture stress is the primary environmental constraint limiting the persistence and agronomic performance of white clover in Australia (Jahufer et al. 2002). In a study carried out over 30 years, moisture stress caused by low rainfall and/or high temperature, and high stocking rate were determined to have significant detrimental effects on white clover persistence in pastures located in the Northern Tablelands of New South Wales, Australia (Hutchinson et al. 1995). A study of white clover in lowland grasslands identified three main factors limiting the growth and survival of white clover in these areas (Garrett & Chu 1997). These were low soil fertility, especially phosphorus and trace elements; presence of very wet, acid land and lack of summer moisture in other areas.

230. In summary, a complex interaction between the genotype/phenotype of the white clover, climatic conditions and the physical environment will determine if the white clover can be successful in establishing itself and persisting in a given area. As discussed in *The Biology of Trifolium repens L. (White clover)* (OGTR 2008), white clover populations are highly variable. An investigation in Wales in a permanent pasture identified 50 different clones of white clover in an apparently homogenous area (Burdon 1980). Clones appear adapted to specific ecological niches so the introduction of a new genotype/phenotype may not always be advantageous.

231. The cycle of fertilisation, survival and dispersal would need to be repeated multiple times before the white clover with resistance to AMV could reach sensitive native plant habitat such as the montane and subalpine woodlands and grasslands. As discussed earlier, gene flow to white clover plants outside of the GM trial site, its pollen trap and isolation zone is considered unlikely based on the available information on gene flow. In addition, since no white clover trials are being conducted on the farm and the area is principally a lucerne growing area, only isolated white clover populations are expected to be present further reducing the likelihood of successful gene flow. As discussed immediately above, the area around the trial site is not conducive for white clover seedling survival, establishment and persistence due in large part to insufficient rainfall. This further reduces the likelihood of subsequent gene transfer to other white clover plants in the area. The likelihood of subsequent dispersal (either via gene flow or seed or reproductive plant material) and the establishment of white clover with resistance to AMV into sensitive native plant habitats is also expected to be low due to limited dispersal opportunities, the large dispersal distances required to reach these areas and environmental factors limiting white clover persistence.

Selection pressure and weediness

232. For a white clover plant containing AMV resistance to persist in the clover population and compete with other white clover plants, AMV needs to be present and needs to be an important factor limiting white clover growth.

233. Experiments with burr medic (*Medicago polymorpha*) plants in pastures with capeweed (*Arctotheca calendula*) have compared the competitive ability of AMV-infected and uninfected burr medic in grazed self-regenerating mixed sward pasture (Jones & Nicholas 1998). In pastures in which AMV infection was present the capeweed became dominant as the AMV infection decreased the competitive ability of the burr medic plants. Similar effects of viral infection altering species composition have been seen for other plant-viral combination reviewed in (Cooper & Jones 2006).

234. In glasshouse and controlled field experiments, infection of white clover with AMV decreases growth, flowering and seed number (Barnett & Gibson 1975; 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; Garrett 1991). 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). No literature has been found on the effects of AMV on white clover in native plant habitats.

235. AMV is transmitted in a non-persistent manner by aphids (Garrett & Chu 1997). 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 m 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). AMV has been seen at high levels in 20 year old white clover pastures, but at very low levels in pastures that were sown in the past five years, even in those adjacent to AMV infected areas (Mckirdy & Jones 1995). Similarly, virus spread by aphids from plots containing AMV infected burr medic plants did not occur over a 5 m grass buffer to uninfected plants over six growing seasons (Jones & Nicholas 1998). 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). Thus the incidence of viruses in pastures with perennial growth is greater than those in which clover persists primarily by annual reseeding (Barnett & Gibson 1975).

236. 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). 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).

237. Even if white clover populations, aphid populations and AMV are all present in a particular location, the likelihood of GM white clover having a greater negative impact than non-GM white clover at that location can only be more accurately estimated when it is established whether AMV is limiting the spread and persistence of non-GM white clover and to what degree.

238. If the AMV CP confers a selective advantage then its prevalence in wild populations would be expected to increase, but only if the AMV CP confers an advantage relative to the factors that limits population size (Tepfer 2002). However, it is likely that the survival of the white clover plants resistant to AMV would still be limited by high temperatures, low water availability, nutrient availability and other environmental factors that normally limit the spread and persistence of white clover plants in Australia (OGTR 2008).

239. Therefore, the expression of the introduced gene for AMV resistance is only expected to provide the GM white clover plants with a significant selective advantage over non-GM white clover plants in areas where AMV is the main limiting factor for the spread and persistence of white clover.

Conclusion

240. Although a causal pathway from gene flow to increased weediness can be identified, the number of seeds that could actually result from gene flow out of this field trial (which is of small size, short duration and has control measures to prevent gene flow) will be very limited and the chance of these seeds being spread to a native plant habitat where they are able to establish and persist is also very low. Furthermore, any native plant habitat where the GM white clover plants might be able to survive must have both aphids and AMV present and the

white clover population must be limited by AMV. Therefore, the likelihood of increased weediness resulting from Identified Risk 1 is assessed as **highly unlikely**.

Section 3 Risk estimates

241. The risk estimate (which can range from negligible to high), is based on a combination of the consequences and likelihood assessments, using the Risk Estimate Matrix (see Chapter 2).

242. The risk estimates for the adverse outcome of weediness of white clover resistant to AMV in native plant habitats as a result of the transfer of the introduced *AMV CP* gene via gene flow to non-GM white clover plants have been made relative to the baseline of the weediness potential of the non-GM parent organism and in the context of the small scale, short duration and the containment measures proposed by the applicant for this proposed release.

243. The consequences of gene transfer from the GM white clover line to non-GM white clover plants resulting in weediness (Identified Risk 1) have been assessed as **minor**, and the likelihood of this resulting in increased weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

244. The risk of the event (above) that may lead to increased weediness is estimated to be **negligible** and therefore, no risk treatment measures for weediness are proposed.

245. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM white clover line into the environment are considered to be negligible. Hence, the Acting Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Table 6. Summary of risk assessment

Event that may give rise to weediness	Consequence assessment	Likelihood	Risk estimate	Does risk require treatment?
<p>Identified Risk 1</p> <p>Expression of the introduced <i>AMV CP</i> gene in other white clover plants as a result of gene transfer leading to increased spread and persistence in native plant habitats.</p>	<p>Minor</p> <ul style="list-style-type: none"> • Non-GM white clover mainly impacts on herbaceous plants in native plant habitats and AMV-resistant white clover is expected to do the same. • Many native plant habitats have no AMV infection and therefore white clover with resistance to AMV will have no selective advantage. • The genetic modification will not extend the range of white clover with resistance to AMV compared to non-GM white clover. • In native plant habitats where AMV is present, the degree to which AMV-resistant white clover may adversely impact native vegetation is uncertain. 	<p>Highly unlikely</p> <ul style="list-style-type: none"> • Outcrossing to other white clover plants would be rare due to containment measures proposed by the applicant, and the small size and short duration of the trial. • The chance of volunteer GM plants arising from seed dispersal finding suitable conditions to establish as weeds would be no greater than for non-GM white clover. • Aphids and AMV must both be present in a native plant habitat and a white clover population must be limited by AMV before a selective advantage could potentially be conferred. • Although resistance to AMV infection may offer a small competitive advantage, abiotic and biotic factors, such as temperature, soil type, water and nutrient availability, are likely to be more important in limiting the spread and persistence of white clover. 	Negligible	No

Section 4 Uncertainty

246. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

247. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁴. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

¹⁴ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

248. For DIR 089, which involves early stage research, uncertainty exists in relation to the characterisation of:

Potential increases in toxicity

249. Some data has been provided on the levels of toxins and anti-nutrients in the GM white clover line but further verification may be required to inform future assessments.

Potential for gene flow to non-GM white clover

250. CCI data has been provided from an experiment designed to maximise gene flow in white clover. However, there is a lack of information available on gene flow under more realistic field conditions.

Potential for increased survival and persistence of white clover resistant to AMV in native plant habitats

251. Although factors limiting the establishment of the GM white clover are expected to be the same as those limiting the non-GM white clover, there remains some uncertainty about whether AMV infection is a significant factor limiting white clover in native plant habitats. Data comparing the fitness of AMV infected and uninfected white clover plants would assist in determining the potential impact of the *AMV CP* gene on the weediness potential of white clover resistant to AMV.

252. Chapter 4, Section 5 also discusses information that may be required for future releases.

Chapter 4 Risk management

253. Risk management includes evaluation of risks identified in Chapter 2 and 3 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

254. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment. All licences are required to be subject to three conditions prescribed in the Act.

255. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

256. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

257. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁵.

258. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. *The Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

¹⁵ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

As the trial involves early stage research, the applicant does not intend any material from the GM white clover line proposed for release to be used in human food. All genetically modified foods intended for sale in Australia must undergo a safety evaluation by FSANZ).

Accordingly, the applicant has not applied to FSANZ to evaluate the GM white clover line. However, in the event of a commercial release, FSANZ approval may need to be obtained before materials or products derived from the GM white clover line could be sold for human consumption.

259. The APVMA, which has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia, has previously issued a research permit for the small scale use of the Alfalfa mosaic virus coat protein in GM white clover plants released under DIR 047/2003. DPI Victoria would require a research permit for this proposed release of GM white clover containing the *AMV CP* gene.

260. No other approvals are required.

Section 3 Risk treatment measures for identified risks

261. The risk assessment of events listed in Chapter 2 and the identified risk in Chapter 3 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM white clover. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

262. These events were considered in the context of the scale of the proposed release (a maximum area of 633 m² per year between March 2009 and August 2011, on one site in the local government area of Corowa, NSW), the containment measures (Chapter 1, Section 3), and the receiving environment (52).

Section 4 General risk management

263. Licence conditions have been imposed to control the dissemination and persistence of the GMO and its genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence¹⁶ and summarised in this Chapter, Section 4.1.2.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by DPI Victoria

264. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by DPI Victoria in their application, and discussed in the events characterised for the release in Chapter 2. The appropriateness of these limits and controls are considered further below.

265. The release would be confined to one site, which occurs within the Heritage Seeds Research Farm, currently encompassing the trial site for a GMAC authorised trial (PR64X, currently being monitored under DIR 047/2003). Only staff with appropriate training will be allowed access to the site. Additionally, the applicant does not intend to use any of the GM

¹⁶ The licence for DIR 089 is available on the OGTR website via the link to DIR 089 (<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir089>)

plant material as human food or animal feed. Furthermore, the duration of the proposed release will be limited to two years and a half years. These measures will limit the potential exposure of humans and vertebrates to the GMO (Event 1) and the potential for the GM white clover line to persist or to establish outside the proposed release site (Event 3).

266. Beehives will be located on the trial site for cross-pollination. The applicant proposed to euthanize the bees and destroy the honey by burning after pollination is completed. The destruction of bees, honey and pollen from these hives will limit potential exposure of humans to the pollen from the GM white clover (Event 1).

267. The applicant's proposal to limit gene flow from the GM white clover (Event 4; Identified Risk 1) includes surrounding the proposed release site with a pollen trap, consisting of an inner one metre wide band of non-GM white clover, surrounded by a band of lucerne at least 35 m wide and an outer one metre wide band of non-GM white clover. This will be surrounded by a 500 m isolation zone which will be monitored for white clover plants during the trial and any plants detected will be destroyed.

268. Differences in pollen flow have been observed between different pollen flow studies in white clover. A number of variables, particularly pollen source size, climatic conditions and the difficulty of detecting rare events, could influence the accuracy and reproducibility of these measurements. Low rates of outcrossing occur up to 10 m with even lower rates up to 24 m from the pollen source (Woodfield et al. 1995; Clifford et al. 1996).

269. As discussed in Chapter 3, on the basis of the scientific literature on gene flow and the rules for producing seed, gene flow over long distances under Australian conditions is highly unlikely. The data suggests that pollen trap rows consisting mainly of lucerne and the absence of white clover plants for 500 metres surrounding the trial provides adequate containment to limit gene flow to non-GM white clover. Therefore, a pollen trap of at least 37 metres (35 metres of lucerne and 2 x 1 metre of white clover) and 500 metre isolation zone are included as licence conditions (Event 4; Identified Risk 1).

270. The applicant has proposed removal of the flower buds of the GM white clover if less than 25% of the lucerne plants in the surrounding pollen trap are flowering. The applicant has also proposed fortnightly monitoring of the GM white clover to look for indications of flowering. Generally it takes nine weeks from flower head initiation to the appearance of the first flower (Anon. 2005), although this does depend on conditions. Therefore, fortnightly monitoring would be sufficient to detect flowering before it occurs and to effect GM white clover flower bud removal. This will limit the potential for gene flow from the GM white clover in the event of inadequate flowering of the lucerne pollen trap (Event 4; Identified Risk 1).

271. The applicant has proposed monthly monitoring of the lucerne component of the pollen trap for the presence of white clover plants and remove these prior to flowering. This will limit the potential for gene flow from the GM white clover (Event 4; Identified Risk 1).

272. The applicant proposes to surround the trial with two fences. The trial site will be surrounded by a rabbit-proof fence and the pollen trap will be surrounded by a stock-proof fence with a locked gate. These measures will aid in excluding grazing animals which may have access to the GMOs and preventing inadvertent access by people. This will limit the potential exposure of vertebrates to the GMOs (Event 1) and the potential dispersal of the GMOs (Event 3).

273. The applicant has also stated that the trial site will be at least 280 metres from the river. A standard DIR licence condition has been imposed requiring the trial site to be located at least 50 metres from a natural waterway which will limit the dispersal of viable GM plant material in the event of flooding (Event 3).

274. Non-GM white clover and non- GM perennial ryegrass will be planted amongst the GM white clover at the site (refer to Chapter 1, Section 3.1 for details). As there is a possibility of viable GM material being present in the non-GM plants, a licence condition has been imposed requiring these non-GM plants to be treated as the GMO and therefore subject to all the same licence conditions. This will limit the dispersal of viable GM plant material (Event 3).

275. The applicant has proposed a number of conditions to minimise the persistence of any GM white clover plants and seeds in the seed bank at the proposed release site after harvest of the proposed trial (Event 2). These conditions include removing mature flower heads during peak flowering time to reduce the build-up of a seed bank, seed head removal and herbicide application at the end of the trial. The applicant has also proposed to monitor the proposed release site for five years after harvest with cultivation in spring and autumn under conditions of adequate moisture to promote seed germination. All volunteers will be destroyed by hand pulling or by herbicide application.

276. White clover forms a persistent seedbank (Lewis 1973). Shallow cultivation of the soil following a white clover seed crop reduced the seed load in the germinating zone (top 50 mm of soil), but even with annual shallow cultivation plus herbicide removal of volunteers it took five years to meet the first generation certification standard of one off-type or less per 10 m² (Clifford et al. 1990). There is no data presented to suggest how long seeds would persist without cultivation. However, data from other studies has found viable clover seeds after 20 years (Lewis 1973). White clover seeds require moisture for germination. Germination of seed occurs in winter and spring and timing is dependent on environmental conditions (Archer & Robinson 1989).

277. It is considered that irrigations, combined with tillage, and monitoring for and destruction of volunteers would effectively reduce survival and persistence of viable white clover seeds in the soil at the completion of the proposed release. Tilling the soil to the depth of the original cultivation each spring and autumn, with irrigation if there has not been sufficient rainfall to encourage germination, will bring buried seed to the surface and encourage germination. Post harvest monitoring of the proposed trial site and pollen traps for at least five years after harvest with no volunteers observed in the most recent twelve months, needs to be completed before an application that inspection conditions no longer apply can be made to the Regulator. These measures will minimise the persistence of the GMO at the trial site (Event 2). Although the removal of seed heads during and at the completion of the trial would reduce the number of seeds in the seedbank, it is considered that a combination of irrigation/cultivation and monitoring will adequately limit persistence of seed at the trial site. Additionally, there is no proposed licence condition to monitor the 500 metre isolation zone post-harvest. Other proposed licence conditions require that this area is to be kept free of white clover plants during the trial and will limit the presence of seed or vegetative material of GM white clover in this area. Thus it is unlikely that there is any GM white clover seed in this area to necessitate monitoring.

278. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs*, <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>> and will be destroyed by autoclaving immediately after analysis. These are standard protocols for the handling of GMO to minimize exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer [Events 4 (Identified Risk 1) & 5].

4.1.2 Summary of measures imposed by the Regulator to limit and control the release

279. A number of licence conditions have been imposed by the Acting Regulator to limit and control the release, including requirements to:

- conduct the release on a total area of up to 633 m² per year at one site in the local government area of Corowa, NSW, between March 2009 and August 2011
- locate the trial site more than 50 m away from natural waterways
- surround the release site by both a livestock-proof and a rabbit-proof fence to reduce seed dispersal by grazing animals
- surround the GM white clover plot by a pollen trap, consisting of an inner one metre wide band of non-GM white clover, surrounded by a band of lucerne at least 35 metres wide, and an outer one metre wide band of non-GM white clover
- monitor the GM white clover fortnightly during flowering, and remove flowers from the GM white clover plants in the event of less than 25% of the lucerne plants in the pollen trap flowering at the same time as the GM white clover plants
- monitor the lucerne band monthly and destroy any white clover plants prior to flowering
- monitor monthly for, and destroy any, white clover plants that may occur within 500 metres of the release site
- specific containment, transport and storage conditions in accordance with OGTR guidelines
- destroy all GM plant material not required for testing or future trials
- destroy bees, honey and pollen from beehives used on the trial site
- monitor for, and destroy any, volunteer GM white clover that may occur in the release area for at least five years after completion of the trial, until no volunteers have been detected for a continuous twelve month period.

4.1.3 Measures to control other activities associated with the trial

280. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs*¹⁷; *Policy on transport and supply of GMOs*¹⁸). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

281. Conditions applying to the conduct of experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

282. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

¹⁷ Guidelines for the transport of GMOs
<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>>

¹⁸ Policy on transport and supply of GMOs
<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/policies-1>>

- applicant suitability
- contingency and compliance plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

283. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

284. On the basis of information supplied by the applicant and records held by the OGTR, the Acting Regulator considers DPI Victoria suitable to hold a licence.

285. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

286. DPI Victoria must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

287. Prior to planting the GM white clover line, DPI Victoria is required to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of the GM white clover line occurs.

288. DPI Victoria is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM white clover line outside of the permitted areas.

289. DPI Victoria is also required to provide a method to the Regulator for the reliable detection of the presence of the GMO and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.2.3 Identification of the persons or classes of persons covered by the licence

290. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting structures

291. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

292. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

293. A number of written notices are required under the licence that will assist the OGTR in designing and implementing a monitoring program for all licensed dealings. The notices include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of destruction and cleaning after destruction.

4.2.5 Monitoring for Compliance

294. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

295. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

296. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the provision for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Issues to be addressed for future releases

297. Additional information has been identified that may be required to assess an application for a large scale or commercial release of this GM white clover line, or to justify a reduction in containment conditions. As detailed in Chapter 3, Section 4, this would include:

- additional data on the potential toxicity of plant materials from the GM white clover line
- weediness of the GM white clover under Australian field conditions, including invasiveness and enhanced reproductive capacities
- the degree to which AMV limits white clover spread and persistence outside of pastoral situations, in particular in native plant habitats
- additional data on gene transfer to non-GM white clover.

Section 6 Conclusions of the RARMP

298. The risk assessment concluded that this limited and controlled release of one GM white clover line on a maximum total area of 633 m² per year over two and a half years in the NSW local government area of Corowa, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

299. The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

the process of analysing hazards and the events that may give rise to harm

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk

the chance of something happening that will have an undesired impact

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices

Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective

High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

the overall process of risk assessment, risk management and risk communication

Risk analysis framework

systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context

parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate

a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation

the process of determining risks that require treatment

Risk management

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁹ on the consultation RARMP for DIR 089

The Acting Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence that was used in finalising the RARMP that formed the basis of the Acting Regulator's decision to issue the licence. A number of submissions received raised issues relating to risks to the health and safety of people and the environment as summarised below.

Summary of issues raised	Comment
White clover is pollinated by bees which may travel up to 2 km from the hive. Notes that an earlier GM white clover trial will soon be signed off which provides assurance that the proposed containment measures do effectively minimise risk of gene flow.	The risks that may be associated with gene flow via pollen dispersal by bees are discussed in Chapter 3 of the final RARMP. Previous GM white clover trials [DIR 047/2003 and those conducted under the Genetic Manipulation Advisory Committee (GMAC)] cannot be directly compared with the current application which proposes a range of different containment measures.
The trial is an excellent opportunity for the applicant to carry out a thorough study of gene flow from white clover before more commercial development of the GM white clover.	Chapter 4, Section 5 of the final RARMP outlines issues, including gene flow in white clover, that the applicant may need to address if a future licence application was made to release this GMO on a larger scale or if reduced containment measures were proposed.
Information about the results of post trial monitoring of the earlier GM white clover trial would provide assurance that at least five years of post-trial monitoring is adequate to remove the seed bank.	Information from previous GM white clover trials and information presented in the biology document for white clover supports the licence condition requiring at least five years of inspection with the last 12 months being volunteer free.
The size of the bee hive to be used in the last year of the trial will be an important factor affecting potential for gene flow.	There are a number of factors that affect the likelihood of gene flow from the trial site. These have been discussed in detail in Chapter 3 of the final RARMP.
Raised the issue of transfer of pollen from GM plants to non-GM plants and the proposed pollen trap attracting insects. A more stringent containment option should be considered eg enclose site with fine mesh nets.	The likelihood and consequences of gene flow from the trial site is now considered in detail in Chapter 3 of the final RARMP and risks that may be associated with gene flow were considered to be negligible. Containment measures for this trial were considered in Chapter 4 and are considered adequate to restrict gene flow.
Feels that the potential for increased weediness due to the expression of the AMV gene improving survival should be an identified risk. An analysis of the consequences of a breach of the containment measures warrants further examination.	Chapter 2, Event 4 (gene flow and a potential for increased weediness) now identifies a risk, and the likelihood and consequence of this identified risk are considered in detail in Chapter 3 of the final RARMP. The risk for this event was estimated as negligible.

¹⁹ GTTAC, State and Territory Governments, Australian Government agencies, the Minister for the Environment, Heritage & the Arts and the Local Council(s) where the release may occur

Summary of issues raised	Comment
White clover is a serious weed of many agricultural and native habitats. The incorporation of a trait that confers resistance to AMV could increase the weediness of white clover in regions where infection with AMV is prevalent.	Chapters 2 and 3 of the final RARMP discuss in detail the potential for increased weediness of AMV-resistant white clover in areas where AMV is present.
The proposed containment measures reduce but do not eliminate the risk of pollen spread from the GM white clover to wild type white clover by insect pollinators. With increasing levels of bee visitation there is the cumulative risk that a bee would fly directly from the central plot, past the lucerne pollen trap and to a clover plant outside the relatively small exclusion zone. Without additional environmental safety information the consequences cannot be predicted with certainty and therefore a degree of caution is prudent.	The likelihood of gene flow from the trial site resulting in increased weediness is now considered in detail in Chapter 3 of the final RARMP. Containment measures for this trial are considered in Chapter 4 and are assessed as adequate to restrict gene flow from this trial. Taking into consideration the range of containment measures proposed, gene flow was considered to be highly unlikely.
Recommends that the statement in paragraph 119 about the ecological equivalence of GM and non-GM white clover in environments in which AMV infection is not prevalent should be appropriately modified. This conclusion is not substantiated by any supporting evidence and does not appear to take into account possible pleiotropic and unintended effects of the transgene in the receiving genome.	The applicant has previously trialled this GMO, and the available data shows no evidence of unintended effects. Event 7 evaluates the potential for harm as a result of unintended effects and this event was not considered to be an identified risk that warranted further detailed assessment. Paragraph 119 has been amended in the final RARMP to provide more clarity.
Recombination between viral transgenes and viral genomes could result in the emergence of novel viruses with altered virulence and/or altered host range and vector specificity. It is widely accepted that virus-resistant transgenic plants for commercial release should not include the 3' untranslated region (UTR) sequence to reduce the appearance of recombinants. There are alternative approaches to construct design that are less likely to result in undesirable recombination events.	Chapter 2, Event 5 evaluates the potential for harm as a result of viral recombination, including possible effects of the viral 3' UTR. It is unlikely that recombinant viruses that might occur in the AMV CP-expressing GM white clover would differ significantly from those arising from recombination between infecting viruses and AMV occurring naturally in white clover.
Concerned about the efficacy of the measures proposed to manage the risk of pollen spread and suggests it should be managed by the installation of a bee-proof cage during flowering of the GM white clover and maintaining the cage's integrity (as was imposed under DIR 047). In the absence of new environmental safety data and to ensure consistency with previous assessments, it is recommended in the strongest possible terms that the same licence conditions should be imposed upon the present application. If these conditions are incorporated in the conduct of the trial, the risk posed by this trial would be comparable to the previous trial.	The containment measures and location for DIR 089 differs from DIR 047/2003. Additional information has been considered in the RARMP for DIR 089 including new data on gene flow (collected from a previous GM white clover trial, which was conducted without a bee-proof cage), and surveys of white clover and AMV prevalence in native plant habitats. Chapter 3 of the final RARMP discusses the identified risk of increased weediness as a result of gene flow and estimates the risk to be negligible.

Summary of issues raised	Comment
<p>If a larger scale trial is planned for the future, where containment is not possible, data should be provided on:</p> <ul style="list-style-type: none"> • whether the AMV CP gene confers a competitive advantage to the GM white clover in native habitats • the role of AMV in limiting the spread of white clover populations • the effects of increased weediness of the GM white clover on insects and animals. 	<p>Further information on the potential for the GM white clover to have a competitive advantage in native plant habitats and the role of AMV in limiting the spread of white clover populations are identified in Chapter 4 of the RARMP as requirements for assessing any future applications for a larger scale, or commercial release of the GM white clover or for a reduction in containment measures. The possible impact on animals (including insects) if there was increased weediness of the GM white clover is discussed in Chapter 3 of the final RARMP. The additional information outlined above would determine whether further information may be required on possible secondary effects on animals.</p>
<p>Recommends that the impact of recombination events between the transgene sequence and other virus genomes should be explored. Data should be collected to:</p> <ul style="list-style-type: none"> • determine the rate of recombination between the transgene and challenging viruses • ascertain whether recombination events are resulting in novel viruses with altered virulence and/or host specificity. 	<p>Chapter 2, Event 5 considers it unlikely that recombinant viruses that might occur in the AMV CP-expressing GM white clover would differ significantly from those arising from recombination between infecting viruses and AMV occurring naturally in white clover. Further, the emergence of recombinant viruses as a result of the GM white clover is considered no more likely than the baseline situation with non-GM white clover, given that there is no increase in selection for recombinant viruses. This conclusion is supported by a range of field studies which have not detected any recombinant viruses in commercial releases of a variety of virus CP-expressing GM plants (reviewed by Fuchs & Gonsalves 2007).</p>
<p>Unintended effects of the proposed trial could include changes to growth characteristics that would indirectly influence the potential for invasiveness of the GM clover. Data on growth rates as agronomic information should be collected as part of this trial.</p>	<p>The aim of the current trial is to assess agronomic performance of the GM white clover. The potential for increased weediness is discussed in Chapters 2 and 3 of the final RARMP. Chapter 4 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release of the GMO on a larger scale or if reduced containment measures were proposed including information on the competitive advantage to the GM white clover in native plant habitats.</p>
<p>White clover is a weed of natural areas and can raise soil nitrate levels.</p>	<p>The Identified Risk Chapter (Chapter 3) in the final RARMP acknowledges that an increase in weediness of white clover with resistance to AMV could adversely impact native plant species by increasing soil nitrogen levels.</p>
<p>There may be potential for white clover to hybridise with <i>Trifolium ambiguum</i>.</p>	<p>Section 6.4 in Chapter 1 of the RARMP addresses the likelihood of white clover hybridising with <i>Trifolium ambiguum</i> and concludes that this is highly unlikely to occur under natural conditions. Hybrids between the two species have only been produced using ovule culture and embryo rescue under highly controlled experimental conditions, and many of the resulting hybrids were male sterile.</p>
<p>While gene flow beyond the trial site is unlikely, the consequences could not be properly assessed owing to a lack of information about the potential weediness of the GM white clover.</p>	<p>The final RARMP has been modified so that the potential for increased weediness of white clover in native plant habitats as a result gene flow is now an identified risk (Event 4), and the consequences and likelihoods of adverse impacts are assessed in detail in Chapter 3 (Identified Risk 1). The risk of increased weediness in white clover in native plant habitats as a result of gene flow from the GM trial site is considered to be negligible.</p>

Summary of issues raised	Comment
There is a need for further information on the extent that Alfalfa mosaic virus limits growth of white clover in non-agricultural environments	Chapter 4 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release the GMO on a larger scale or if reduced containment measures were proposed. This includes information on the weediness of GM white clover and the degree to which AMV limits white clover persistence, particularly in native plant habitats.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 089

The Acting Regulator received four submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Acting Regulator's decision to issue the licence.

Position (general tone): n = neutral; x = do not support; y = support.

Issues raised: A: Allergenicity; C: consultation process; FI: further information; DR: data requirements; E: Ethics, EN: Environmental risks, H: human health; HGT: Horizontal Gene Transfer, L: Labelling; LI: Liability issues; OSA: Outside scope of assessment, T: Toxicity.

Other abbreviations: Ch: Chapter; FSANZ: Food Standards Australia New Zealand; GM: Genetically Modified; GMO: genetically modified organism; RARMP: Risk Assessment and Risk Management Plan.

Type: I: individual.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	T	GM white clover would contaminate honey and confound bees. In Ireland, bees will not pollinate red clover until all the white clover has been pollinated.	Licence conditions require the honey from the site beehive to be destroyed. There is no evidence that the GM white clover is toxic to animals, including bees (Chapter 2, Event 1).
			None	Various issues raised regarding the emissions trading scheme, forms of power production, and isolation plant breeding being superior to conventional breeding and genetic modification.	These issues are outside the scope of assessment required by the <i>Gene Technology Act 2000</i> .
2	I	x	H, EN, DR	Premature to test agronomic performance before field trials on ecological ramifications. Human health and safety depend on natural systems within the soil, water and air.	This GM white clover has been trialled previously (under DIR 047/2003 and the previous voluntary system) and no adverse effects have been reported. The risks to people and the environment associated with this trial have been assessed as negligible. Future data requirements have been identified (Chapter 4)
			-	No human communities (except those with vested interests in research and development) have asked for genetically modified organisms.	These issues are outside the scope of assessment required by the <i>Gene Technology Act 2000</i> .

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
3	I	n	T	Raises concerns about the health impacts of cyanogenic varieties of white clover, including sub-lethal quantities of hydrogen cyanide in the meat of sheep and cattle and its effect on human health. Also comments on how the cyanogenic potential of plants can impact on productivity, and that cyanogenic and acyanogenic alleles have been identified and therefore cyanogenic potential is heritable. Considers that it would be appropriate to ensure any GM trial should avoid the use of cyanogenic clovers.	The applicant has demonstrated that levels of cyanogenic glucosides in the GM white clover do not differ significantly from non-GM white clover cultivars (Figure 6, Chap 1). Licence conditions prohibit the use of GM material in food and feed and prevent access by grazing animals.
4	I	x	None	Totally objects to this proposal for GM contamination of white clover	Noted.
			C	Lack of Public Consultation: there has been no referendum available to the Australian public to determine whether we want aberrant GM species given free rein to dominate the land over natural species. The OGTR has not been given a mandate to legislate in favour of the applicant.	Under the <i>Gene Technology Act 2000</i> , the Regulator is required consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any identified risks.
			C	Low Profile Public Objection Process: poorly publicised for public input and objection. The process is not widely publicised and is, in effect, invisible to the public. The process should be widely disseminated in all forms of media and highly visible in the public forum. The consultation process is undemocratic especially as many people don't have access to computer technology and research and the discussion papers present the evidence in scientific language and terms.	As required by the Act, public notification and an invitation for written submissions on the consultation RARMP was undertaken. The consultation was more extensive than prescribed in the Act, and included advertising in <i>The Land</i> in addition to the required general circulation newspaper (<i>The Australian</i>), the <i>Australian Government Gazette</i> and the OGTR website. In addition, an invitation to comment was also sent to interested parties who have registered on the OGTR mailing list, either by email or in writing. An executive summary of the RARMP was prepared to assist those with limited understanding of scientific terminology. All relevant submissions were considered prior to making a decision on whether or not to issue a licence.
			T	GM is having an effect upon the resistance of bees to disease and the lack of a robust immune response is causing devastation in bee colonies. Agricultural disaster could occur due to GM white clover since bees are responsible for pollination and are vital to agricultural production of food crops. This could lead to food shortages and increased food costs.	There is no scientific evidence that supports a link between the decline in bee populations around the world and GM crops. Bees at the trial site will collect honey from the GM white clover but there is no evidence that they will be adversely affected (Chapter 2, Event 1).

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			A, T	Food safety: honey, propolis and royal jelly as food and beeswax in cosmetics pose a risk to consumers. Products sourced from GM nectar exposes humans to a host of GM allergens.	This is a limited and controlled release of GM white clover and the licence stipulates that products from the trial cannot be used in human food or animal feed.
			L	GM honey is unidentifiable, due to the nature of bee gathering activity. Even when labelling laws come into force, it will not be possible to track the source of nectar. GM food and cosmetic sources, such as apiarists products sourced by bees from GM white clover, cannot be controlled.	This is a limited and controlled release of GM white clover and the licence stipulates that the bees, honey and pollen from the on-site bee hive must be destroyed. Labelling of GM foods is the responsibility of FSANZ.
			E	Ethics: It is grossly unethical to impose GM 'foods' upon consumers. Forcing GM food upon an unwilling public and concealing its origins is unethical and a travesty of justice.	The regulation of GM foods is the responsibility of FSANZ.
			T, A	Immunity and GM Substances: GM substances including honey, propolis, royal jelly and products made from beeswax sourced from GM white clover, present a challenge to the defence and recovery of the immune system. GM peas expressing a bean gene show mice develop an immune response. Danger to human health from the potential allergenicity of GM white clover pollen. Must acknowledge the major documented health risks of GM 'food' substances. This interminable list includes: organ deformity, allergic immune response, nerve damage, skin hypersensitivity, eczema, asthma, airway inflammation, lung damage, teratogenic effects of GM substances on newborns, stunted growth and fatalities caused by ingesting GM substances (eg Ermakova 1991-2003, et al.)	There is no evidence that organisms including humans will be adversely affected by this limited and controlled release of GM white clover (Event 1). The licence stipulates that products from the trial cannot be used in human food or animal feed.
			E	The prognosis for Australia's GDP and consequent impact of GM crops upon the financial sector indicates an untenable burden to the public health system, reduced productivity, and an unstable economy.	Economic issues are outside the scope of assessments conducted under the Act.
			LI	Legal Accountability and the effects of GM substances: Who will fund the cost in human health - OGTR? Who will service these ongoing social and financial costs to health?	The risks to human health and safety associated with this trial have been assessed as negligible.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			T	GM white clover poses a threat to the dairy, beef, pork and poultry industries. Contaminated feed will affect dairy and meat products; the general public will be subjected to GM through these products.	This is a limited and controlled release of GM white clover and the licence stipulates that products from the trial cannot be used in human food or animal feed.
			EN	GM plant species is without merit; it is not in the public interest and has no social, environmental, or agricultural benefit.	The Gene Technology Regulator is required to consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any risk. Benefits of gene technology are outside the scope of assessments required by the Act.
			T, EN	Flora and Fauna issues: wildlife, including native birds and animals, will become unhealthy and endangered due to the food chain effect. Australian flora and fauna will be contaminated by GM through the food chain.	The GM white clover contains similar levels of natural toxicants as non-GM white clover. The same or similar proteins encoded by the introduced genes are widespread in the environment and there is a lack of known toxicity or evidence of harm from them (Chapter 2, Event 1).
			W	Environmental impacts of GM white clover are the same as any other GM crop. Contamination and pollution of the natural environment by GM white clover species creates an impact on the ecology and an unnecessary burden upon the health of native plant species.	Chapter 2 and 3 considers the risk of increased weediness and it is considered to be negligible. Chapter 4 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release the GMO on a larger scale or if reduced containment measures were proposed including information on the competitive advantage to the GM white clover in native plant habitats.
			HGT	Transmuting Alfalfa mosaic virus from resistant GM species poses a danger: a 'wild' virus poses potential risks in carrying infection to wildlife and humans, similarly to the MRSA Staph superbug.	GM white clover is resistant to AMV and therefore the virus is not present in the GM plants. Horizontal gene transfer is considered in Chapter 2, Events 5 and 6, and is not identified as a risk that warrants further detailed assessment. Vertical gene transfer to other white clover plants is considered as an identified risk in Chapter 3 of the final RARMP. The risk is assessed as negligible.
			H	Rashly unleashing foreign DNA in GM species without knowledge of the effects, or any guaranteed safety for human health and wellbeing is a dangerous scientific experiment to put upon our country. Aberrant GM species are not biologically determined. We do not know the long-term effects.	The Regulator considers both short term and long term impacts when assessing the release of a GMO. Current evidence indicates that this limited and controlled release of GM white clover poses negligible risks.