



EXECUTIVE SUMMARY
of
THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN
for
APPLICATION NO. DIR 047/2003
from
DEPARTMENT OF PRIMARY INDUSTRIES (VICTORIA)

INTRODUCTION

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

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

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

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

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

THE APPLICATION

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

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

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

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

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

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

THE EVALUATION PROCESS

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

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

The risk assessment considered information relevant to the evaluation of potential impacts on human health and safety and the environment contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, the proposed dealings

and containment measures), submissions received during consultation with expert groups and authorities, and current scientific knowledge.

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of the GM white clover were identified. These have been evaluated to determine whether risks might arise, based on the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised.

The identified potential hazards relate to:

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

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

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

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

CONCLUSIONS OF THE RISK ASSESSMENT

It is concluded that the proposed release of the GM virus resistant white clover does not pose significant risks to human health and safety or to the environment as a result of the genetic modification. The Regulator has imposed stringent licence conditions that differ from those

proposed by the applicant to minimise potential exposure of humans and other organisms, and to limit the spread and persistence of the GMO and the introduced genes while more data is gathered on the behaviour and interactions of the GMO in the Australian environment. The risk assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

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

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

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

Toxicity to other organisms

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

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

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

Weediness

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

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

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

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

Transfer of introduced genes to other organisms

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

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

The applicant had proposed to surround the trial site by a pollen trap consisting of non-GM white clover and other legumes, and to isolate the trial site from all other white clover populations to minimise gene flow and persistence in the first two planting seasons. However, because there is uncertainty about the potential for enhanced weediness of GM white clover and the ability of AMV to limit white clover growth and persistence in the area surrounding the trial site, the Regulator has taken a cautious approach to manage potential enhanced weediness through gene flow to non-GM white clover plants in the proposed field trial.

Therefore, stringent licence conditions have been imposed to minimise the risk through cross-pollination to non-GM white clover plants outside the release site by enclosing the GM plot in a bee-proof cage during the flowering period of the GM white clover and ensuring that the integrity

of the cage is maintained (refer to key licence conditions below). Additionally, any bees used in the pollination of the GM white clover are required to be killed at the end of the period to ensure that no GM pollen is transferred outside the bee cage.

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

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

Interactions between the introduced viral gene and viruses

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

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

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

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

Anti-viral resistance

New AMV variants could arise as a result of the trial that overcome AMV coat protein (CP)-mediated resistance (anti-viral action) of the GM white clover and could potentially cause increased disease burden in white clover plants and other plant species. However, given the limited scope of the release in both scale and time, the likelihood of this risk resulting from the release is negligible. This issue is discussed in Appendix 6 of the RARMP.

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

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

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

Toxicity or allergenicity to humans

Licence conditions have been imposed which require the applicant to:

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

Toxicity to other organisms

Licence conditions have been imposed which require the applicant to:

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

Weediness

Licence conditions have been imposed which require the applicant to:

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

Transfer of introduced genes

Licence conditions have been imposed which require the applicant to:

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

- clean equipment used at the release site; and
- monitor release area during and after trial and destroy volunteers before flowering.

Interactions between the introduced viral gene and viruses

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release.

Anti-viral resistance

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

General conditions

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

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

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

Research requirements

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

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

Additional data

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

- molecular characterisation of the introduced genetic materials;
- the levels of the natural toxicants e.g. cyanogenic glucosides, phytoestrogens or saponins in the GM white clover;
- toxicity of the GM white clover expressing the AMV CP, via livestock feeding studies;

- weediness of the GM white clover under Australian field conditions, including invasiveness, enhanced reproductive capacities, and limitation of white clover persistence by AMV outside of pastoral situations, such as roadsides, home gardens and natural environments; and
- gene transfer to non-GM white clover.

Monitoring and enforcement of compliance by the OGTR

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