



Office of the Gene Technology Regulator

APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 047/2003

SUMMARY INFORMATION

Project Title:	Field Evaluation of Genetically Modified White Clover Resistant to Infection by Alfalfa Mosaic Virus
Applicant:	Department of Primary Industries (Victoria) PO Box 500 East Melbourne VIC 3002
Common name of the parent organism:	White Clover
Scientific name of the parent organism:	<i>Trifolium repens</i> L.
Modified trait(s):	Viral Disease Resistance, Antibiotic resistance
Identity of the gene(s) responsible for the modified trait(s):	<i>AMV CP</i> gene from Alfalfa Mosaic Virus (viral disease resistance) <i>nptII</i> gene from the bacterial Tn5 transposon (antibiotic resistance)
Proposed Location(s)	Shire of Southern Grampians, Victoria
Proposed Release Size:	Maximum of 2 hectares at one site
Proposed Time of Release	May 2004 - April 2007 (four planting seasons)

Introduction

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

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

The legislation sets out the requirements for considering applications for licences for dealings with GMOs and the matters that the Regulator must take into account before deciding whether to issue a licence.

The application and the proposed dealings

The OGTR has received an application from the Department of Primary Industries (DPI) (Victoria) for a licence for the intentional release of a genetically modified (GM) virus resistant white clover derived from a single transformation event into the environment, on a limited scale and under controlled conditions

DPI (Victoria) proposes to carry out the field trial of the GM white clover over four planting seasons between May 2004 and April 2007 on one site in Victoria, on a total area of two hectares (consisting of a 26 x 19 metre GM white clover plot in the centre of a 32 metre (minimum) pollen trap of non-GM clovers and other legumes). The GM white clover contains the Alfalfa Mosaic Virus coat protein (*AMV CP*) gene intended to provide resistance to Alfalfa Mosaic Virus (AMV), and an antibiotic resistance gene (*nptII*) which provides resistance to the antibiotic kanamycin.

The main aims of the proposed release are the field evaluation of a GM white clover resistant to 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 clover white clover plants showing superior agronomic performance and AMV resistance.

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

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

- specific containment, transport and storage conditions in accordance with OGTR guidelines;
- surrounding the release site by both a livestock-proof and a rabbit-proof fence to prevent seed dispersal by grazing animals;
- surrounding the GM white clover plot by a pollen trap, consisting of an inner one metre wide band of non-GM white clover, concentric 10 metre wide (minimum) bands of red clover, lucerne and Persian clover and an outer one metre wide band of non-GM white clover;
- removal of flowers from the GM white clover plants in the event of the pollen trap plants not flowering simultaneously;

- removal of mature flower heads during peak flowering time to reduced the build-up of a seed bank;
- monitoring for, and destroying any, white clover plants that may occur within 500 metres of the release site;
- destroying GM material not required for subsequent research;
- placing a footbath and washbasin at gate of release site to clean all implements; and
- monitoring for, and destroying any, volunteer GM white clover that may occur in the release area for five years after completion of the trial.

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

Previous releases of the GMO

Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been four field trials of the GMO proposed for release under the current application. Three of the trials were conducted by La Trobe University (PR-64, PR-64X and PR-64X(2)) and the other was by CSIRO (PR-67). The GM clovers were assessed for plant growth, expression of the introduced gene and resistance to viral infection. Gene flow to non-GM white clover was also analysed. The size of the releases ranged from two to four hectares. These releases 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 these releases.

Parent organism

The parent organism is white clover (*Trifolium repens* L.), which is exotic to Australia and is grown as a component of improved pastures in south-eastern Australia and in the wetter parts of Western Australia. It is the dominant pasture clover for the Australian dairy industry.

Genetic modification and its effect

The GM white clover line contains the coat protein gene from Alfalfa Mosaic Virus (AMV), which is expected to reduce the susceptibility of the clover plants to AMV. Evaluation in contained facilities showed *AMV CP* gene expression and virus resistance in the GM white clover line proposed for release in this field trial. The GM white clover plants also contain a selectable marker gene (*nptII*) which confers resistance to aminoglycoside antibiotics related to kanamycin and neomycin.

Short regulatory sequences that control expression of the gene are also present in the GM white clover. These are derived from Cauliflower Mosaic Virus (CaMV) and pea. Although some of these sequences are derived from a plant pathogen (CaMV), the regulatory sequences comprise only a small part of the pathogen's total genome, and are not in themselves capable of causing disease.

Method of gene transfer

The coat protein gene, *nptII* gene and associated regulatory sequences were originally introduced into the white clover cultivar 'Mink' on a plasmid vector carried by

Agrobacterium tumefaciens (a common soil bacterium). The vector is 'disarmed' since it lacks the genes that encode the tumorigenic functions of *A. tumefaciens*. The subsequent GM plants produced from one transformation event were conventionally bred with the white clover cultivar 'Sustain'. Plants containing the *AMV CP* gene in a homozygous state will be used in this proposed field trial.

Consultation on draft Risk Assessment and Risk Management Plan

The Regulator has made an initial assessment as to whether the proposed release may pose significant risks to human health and safety or the environment, in accordance with section 49 of the Act. Due to the low risk potential of the GMOs, the control measures that will be imposed, and the limited scale and scope of the dealings, **the Regulator has decided that the proposed release does not pose a significant risk to human health and safety or the environment.**

This means that the Regulator is **not required to seek public comment** on the assessment of this proposal until a risk assessment and risk management plan (RARMP) has been prepared. At this stage, the consultation version of the RARMP is expected to be issued for an extended public consultation in **early April 2004**. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 047/2003.

In preparing the RARMP, the Regulator will seek input from a wide range of key stakeholders and expert groups comprising State and Territory Governments, relevant Australian Government agencies, the Minister for Environment and Heritage, the Gene Technology Technical Advisory Committee and appropriate local councils, as required by section 50 of the Act.

In accordance with section 52 of the Act, the Regulator will again consult with these prescribed agencies and authorities and the public in finalising the RARMP. The public will be invited to provide submissions on the RARMP over a consultation period of up to six weeks, via advertisements in the media and direct mail to anyone registered on the OGTR mailing list. Summaries and copies of the RARMP will be available from the OGTR, or on the OGTR website.

If you have any questions about the application or the assessment process, please contact the OGTR at:

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