



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

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# Risk Assessment and Risk Management Plan for

## **DIR 176**

Limited and controlled release of white clover  
genetically modified for increased condensed  
tannins

Applicant: PTM Solutions Australia Pty Ltd

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# Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 176

## **Decision**

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

## **The application**

Application Number	DIR 176
Project Title	Limited and controlled release of white clover genetically modified for increased condensed tannins <sup>1</sup>
Parent organism	White clover ( <i>Trifolium repens</i> L.)
Introduced genes	<p>Introduced gene conferring increased condensed tannins in white clover:</p> <ul style="list-style-type: none"> <li>• <i>TaMYB14-1</i> - transcription factor involved in regulation of the pathway controlling condensed tannin production from <i>Trifolium arvense</i> (Hares foot clover)</li> </ul> <p>Introduced marker gene:</p> <ul style="list-style-type: none"> <li>• <i>nptII</i> selectable marker – antibiotic resistance gene from <i>Escherichia coli</i></li> </ul>
Genetic modification method	<i>Agrobacterium</i> -mediated transformation
Number of lines	Two events crossed into up to six lines
Proposed location/s	The trial is proposed to take place on sites selected from 55 LGAs in NSW, 35 in Victoria, 16 in WA and 11 in Qld
Proposed release size	Up to a total of 1 ha per year across a maximum of four sites per year, with a maximum of 0.3 ha on any single site in any year
Proposed period of release	From April 2021 until December 2026 (five and a half years)
Principal purpose	To study the agronomic performance, nutritional analysis, compositional analysis, molecular analysis and genetic stability of the GM white clover under field conditions

<sup>1</sup> The original title for the application was “Limited and controlled release of *Trifolium repens* L. genetically modified for increased condensed tannins.”

***Risk assessment***

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks.

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short and long term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM white clover plants. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed and that the limits and controls will effectively minimise exposure to the GMOs.

***Risk management***

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food and animal feed, to minimise dispersal of the GMOs or GM pollen from the trial site, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs at the end of the trial and to conduct post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

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## Abbreviations

Act	<i>Gene Technology Act 2000</i>
AMV	Alfalfa mosaic virus
APVMA	Australian Pesticides and Veterinary Medicines Authority
ANR	Anthocyanidin reductase ( <i>ANR</i> gene in italics)
ANS	Anthocyanidin synthase ( <i>ANS</i> gene in italics)
CaMV	Cauliflower mosaic virus
CCIA	California Crop Improvement Association
CCoA	Coumaryl CoA
CHI	Chalcone isomerase ( <i>CHI</i> gene in italics)
CHS	Chalcone synthase ( <i>CHS</i> gene in italics)
CSGA	Canadian Seed Growers' Association
CT	Condensed tannins
CYVV	Clover yellow vein virus
DAWE	Department of Agriculture, Water and the Environment
DFR	Dihydroflavonol reductase ( <i>DFR</i> gene in italics)
DIR	Dealings involving Intentional Release
DM	Dry matter
DNA	deoxyribonucleic acid
DSE	Dry sheep equivalent (stocking rates)
DW	Dry weight
F3H	Flavanone hydroxylase ( <i>F3H</i> gene in italics)
F3'5'H	Flavonoid-3'5'-hydroxylase ( <i>F3'5'H</i> gene in italics)
FAOStat	Statistics Division, Food and Agriculture Organization of the United Nations
FLS	Flavonol synthase ( <i>FLS</i> gene in italics)
FSANZ	Food Standards Australia New Zealand
ft	Feet
GM	genetically modified
GMO	genetically modified organism
ha	Hectare
HCN	Hydrogen cyanide
HGT	Horizontal gene transfer
K	Potassium
LAR	Leucoanthocyanidin reductase ( <i>LAR</i> gene in italics)
LGA	Local Government Area
m	Metre
<i>MATE1</i>	Multidrug and toxin extrusion-1 gene
<i>MATE2</i>	Multidrug and toxin extrusion-2 gene
mDP	Mean degree of polymerisation (tannins)
Mo	Molybdenum
N	Nitrogen
NLRD	Notifiable Low Risk Dealing
<i>nptII</i>	Neomycin phosphotransferase II gene

NSW	New South Wales
NSW DPI	NSW Department of Primary Industries
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
P	Phosphorous
PA	Proanthocyanidin
PC2	Physical Containment level 2
PIRSA	Primary Industries & Resources South Australia
PPE	Personal protective equipment
PTM	PTM Solutions Australia Pty Ltd
Qld	Queensland
RAF	Risk Analysis Framework
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
S	Sulfur
SA	South Australia
SCRLV	Subterranean clover red leaf virus
t	Tonnes
T2	Second generation transgenic plant
Tas.	Tasmania
<i>TaMYB14-1</i>	<i>MYB14-1</i> gene from <i>Trifolium arvense</i> L.
TGA	Therapeutic Goods Administration
UN	United Nations
USDA	United States Department of Agriculture
USDA-APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
Vic.	Victoria
WA	Western Australia
WCMV	White clover mosaic virus
WRA	Weed risk assessment
WT	Wild type

# Chapter 1 Risk assessment context

## Section 1 Background

1. An application has been made under *the Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) [website](#).
5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

RISK ASSESSMENT CONTEXT	
<b>The GMO</b>	<b>Proposed GMO dealings</b>
Modified genes	Activities
Novel traits	Limits
	Controls
<b>Parent organism (comparator)</b>	<b>Previous releases</b>
Origin and taxonomy	Australian approvals
Cultivation and use	International approvals
Biology	
<b>Receiving environment</b>	
Environmental conditions: abiotic and biotic factors	
Production practices	
Related organisms	
Similar genes and proteins	

**Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF ((Risk Analysis Framework).**

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Four public submissions were received and their consideration is summarised in Appendix B.

7. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA) and the Department of Agriculture and Water Resources (DAWE). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

## Section 2 The proposed dealings

8. PTM Solutions Australia Pty Ltd (PTM) proposes to release white clover genetically modified for increased condensed tannin concentrations. They are proposing to release white clover containing one of two events and potentially incorporating either of these events into up to six lines of white clover by conventional crossing and backcrossing.

9. The purpose of the release is to evaluate characteristics of GM white clover lines under field conditions.

10. The dealings involved in the proposed intentional release are:

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- growing the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

and the possession, supply or use of the GMOs in the course of any of these dealings.

### 2.1 The proposed limits of the dealings (duration, size, location and people)

11. The release is proposed to take place for up to five and a half years from the issue of the licence until December 2026. The applicant has proposed that the GM white clover would be grown on up to four sites per year, with a maximum total growing area across all sites of one ha per year. Most sites would have a single planting in a year, however, some sites may have an autumn and spring planting. Some sites may have planting areas that would be managed as perennial crops and would last over more than one year. Sites would be chosen from the list in Table 1, which includes Local Government Areas (LGAs) in New South Wales, Victoria, Western Australia and Queensland.

**Table 1: List of LGAs from which trial sites are proposed to be selected**

New South Wales	Victoria	Western Australia	Queensland
Armidale <sup>1</sup>	Ballarat <sup>4</sup>	Albany <sup>7</sup>	Gympie Regional <sup>1</sup>
Bathurst <sup>1</sup>	Bass Coast <sup>2</sup>	Augusta-Margaret River <sup>6</sup>	Ipswich <sup>4</sup>
Bega Valley <sup>2</sup>	Baw Baw <sup>2</sup>	Bridgetown-Greenbushes <sup>6</sup>	Lockyer Valley <sup>1</sup>
Bellingen <sup>2</sup>	Benalla <sup>5</sup>	Busselton <sup>6</sup>	Logan <sup>4</sup>
Berrigan <sup>2</sup>	Campaspe <sup>2</sup>	Capel <sup>6</sup>	Moreton Bay <sup>1</sup>
Blayney <sup>2</sup>	Cardinia <sup>2</sup>	Dardanup <sup>6</sup>	Scenic Rim <sup>1</sup>
Byron <sup>2</sup>	Casey <sup>4</sup>	Denmark <sup>6</sup>	Somerset <sup>1</sup>
Cabonne <sup>2</sup>	Colac-Otway <sup>2</sup>	Harvey <sup>6</sup>	South Burnett <sup>1</sup>
Central Coast <sup>3</sup>	Corangamite <sup>2</sup>	Manjimup <sup>6</sup>	Southern Downs <sup>1</sup>
Cessnock <sup>4</sup>	East Gippsland <sup>2</sup>	Murray <sup>6</sup>	Tablelands <sup>1</sup>
Lake Macquarie <sup>4</sup>	French Island <sup>8</sup>	Nannup <sup>6</sup>	Toowoomba <sup>1</sup>
City of Lithgow <sup>3</sup>	Gannawarra <sup>2</sup>	Nedlands <sup>7</sup>	

New South Wales	Victoria	Western Australia	Queensland
Clarence Valley <sup>3</sup>	Glenelg <sup>2</sup>	Serpentine-Jarrahdale <sup>6</sup>	
Coffs Harbour <sup>4</sup>	Golden Plains <sup>2</sup>	Subiaco <sup>7</sup>	
Cootamundra-Gundagai <sup>1</sup>	Greater Shepparton <sup>4</sup>	Swan <sup>7</sup>	
Cowra <sup>2</sup>	Hepburn <sup>2</sup>	Waroona <sup>6</sup>	
Dubbo <sup>1</sup>	Indigo <sup>2</sup>		
Dungog <sup>2</sup>	Latrobe <sup>4</sup>		
Glenn Innes Severn <sup>3</sup>	Loddon <sup>2</sup>		
Goulburn Mulwaree <sup>3</sup>	Macedon Ranges <sup>2</sup>		
Gwydir <sup>2</sup>	Mitchell <sup>2</sup>		
Hawkesbury <sup>4</sup>	Moira <sup>2</sup>		
Hilltops <sup>3</sup>	Moorabool <sup>2</sup>		
Inverell <sup>2</sup>	Mornington Peninsula <sup>2</sup>		
Kempsey <sup>2</sup>	Moyne <sup>2</sup>		
Kyogle <sup>3</sup>	Pyrenees <sup>2</sup>		
Lismore <sup>4</sup>	South Gippsland <sup>2</sup>		
Liverpool Plains <sup>2</sup>	Southern Grampians <sup>2</sup>		
Maitland <sup>4</sup>	Surf Coast <sup>2</sup>		
MidCoast <sup>3</sup>	Towong <sup>2</sup>		
Mid-Western <sup>1</sup>	Wangaratta <sup>5</sup>		
Muswellbrook <sup>2</sup>	Warnambool <sup>4</sup>		
Nambucca <sup>2</sup>	Wellington <sup>2</sup>		
Narrabri <sup>2</sup>	Wodonga <sup>4</sup>		
Oberon <sup>3</sup>	Yarra Ranges <sup>2</sup>		
Orange <sup>4</sup>			
Port Macquarie-Hastings <sup>3</sup>			
Port Stephens <sup>3</sup>			
Queanbeyan-Palerang <sup>1</sup>			
Richmond Valley <sup>3</sup>			
Shoalhaven <sup>4</sup>			
Singleton <sup>2</sup>			
Snowy Monaro <sup>1</sup>			
Snowy Valleys <sup>3</sup>			
Tamworth <sup>1</sup>			
Tenterfield <sup>2</sup>			
Tweed <sup>2</sup>			
Upper Hunter <sup>2</sup>			
Upper Lachlan <sup>2</sup>			
Uralla <sup>2</sup>			
Wagga Wagga <sup>4</sup>			
Walcha <sup>3</sup>			
Walgett <sup>2</sup>			
Warrumbungle <sup>2</sup>			
Wingecarribee <sup>2</sup>			

As different states have different titles for LGAs, these are identified in the table as: <sup>1</sup> Regional Council; <sup>2</sup> Shire Council; <sup>3</sup> Council; <sup>4</sup> City Council; <sup>5</sup> Rural City Council; <sup>6</sup> Shire of; <sup>7</sup> City of; <sup>8</sup> Unincorporated area

12. Only trained and authorised staff would be permitted to deal with the GM white clover.

## 2.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment

13. The applicant has proposed a number of controls to restrict the spread and persistence of the GM white clover and the introduced genetic material in the environment. These include:

- locating trial sites at least 50 m from waterways
- containing planting areas by:
  - covering with an insect-proof tent, surrounded by a monitoring zone and an isolation zone, such that there is a distance of 100 m between planting areas and any other intentionally planted white clover; or
  - surrounding planting areas with an inner pollen trap (non-GM white clover), a pollen buffer (lucerne), an outer pollen trap (non-GM white clover) and an isolation zone such that there is a distance of 200 m between planting areas and any other intentionally planted white clover
- where pollen traps are used, surrounding the outer pollen trap with a stock-proof fence with lockable gate
- where beehives are used in the planting areas, all bees, honey and pollen in the beehives would be destroyed following pollination
- only permitting trained and authorised staff to access the trial site
- all sites would be on private land with controlled access, for example within fenced paddocks
- treating non-GM plants used in the trial as if they were GM
- inspecting all equipment after use for GM seeds and asexual propagules and cleaning as required
- transporting and storing GM plant material in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all plant material from the trial not required for testing or future trials
- post-harvest monitoring of the trial site at least monthly and destroying any white clover volunteers prior to flowering, for either 24 months or 36 months with a period of 12 months in which no volunteers are detected prior to sit sign-off
- shallow tillage postharvest when conditions are conducive to germination of volunteers
- postharvest irrigation of the site, if required, to promote germination of volunteers
- not allowing the GM plant materials or products to be used in commercial human food or animal feed.

## Section 3 The parent organism

14. The parent organism is *Trifolium repens* L. (white clover). Detailed information about white clover is available in the reference document *The Biology of Trifolium repens L. (white clover)* (OGTR, 2020), which was produced to inform the risk analysis for licence applications involving GM white clover. Baseline information from this document, which includes information specific to Australian production and management of white clover as well as information on white clover production and characteristics from a global perspective, will be used and referred to throughout the RARMP.

15. White clover is grown mainly in temperate higher rainfall (over 750 mm per year) areas of Australia and is tolerant of wide range of soil types provided phosphorus (P) and (S) levels are sufficient (NSW DPI, 2020). It is usually grown as part of a mixed pasture sward with perennial grasses and other legumes. It is most frequently grown with perennial grasses such as perennial ryegrass (*Lolium perenne*) and in Tasmania, cocksfoot (*Dactylis glomerata*) (Donald, 2012).

16. In 2011, white clover was cultivated on 296,968 ha (0.6% of total pastures) in New South Wales (NSW), 261,957 ha (3.5%) in Victoria (Vic.), 37839 ha (3.2%) in Tasmania (Tas.) and 6308 ha (0.1%) in Western Australia (WA) (Donald, 2012). Export of seed of *Trifolium* species for planting (any *Trifolium*

species, not only *T. repens*) from Australia ranged from 1,690 t to 2,803 t over the period from 2015 to 2018, with a value of between \$US 6.1 and \$US 8.6 million (United Nations (UN) Comtrade database, accessed 20 August 2020). However, much of the value attributed to white clover is through its use in the meat and dairy industries as stock feed, through its ability to increase productivity and stocking rates, thereby increasing overall outputs from these industries (Ayres et al., 2000).

17. White clover seeds are very small (1.7 million seeds per kg – or approximately 0.6 mg per seed) (Jahufer et al., 2001), so planting depth is important to ensure good establishment of pasture seed planting. Seeds can be planted up to 15 mm deep, with 10-12 mm recommended (Jahufer et al., 2001). Seed may be surface sown into freshly-prepared seed beds with companion grasses, or may be sown into existing grass or mixed pastures to increase the clover content of the pasture mix if clover production in established pastures have declined. Clover is best planted into moisture and in Australian white clover growing areas, autumn and spring planting are most common. Although recommendations for planting rates vary markedly, in general, recommended planting rates range from 0.5 – 5.0 kg/ha, with rates at the lower end of this range when planting as part of pastures mixes with perennial grasses (Jahufer et al., 2001; NSW DPI, 2012).

18. White clover grows best in areas with minimum annual rainfall of 700 mm, preferably 750 mm or more. It has a relatively shallow root system, so is largely intolerant of drought and needs summer rainfall or some irrigation for optimal growth and production. In most areas growth declines over summer, with best pasture growth of white clover in winter and spring (Jahufer et al., 2001; NSW DPI, 2012).

19. Although tolerant of a wide range of soils and able to grow in relatively infertile soils, white clover does require adequate levels of P and S in order to thrive. Main nutrient deficiencies that affect white clover are P, S, molybdenum (Mo) and potassium (K) (NSW DPI, 2012). White clover is able to fix atmospheric (N) as a result of its symbiotic relationship with rhizobia. Most perennial clover seeds are pre-inoculated with *Rhizobium leguminosarum* bv. *trifolii* (Group B inoculant) and should be planted soon after seed inoculation. A soil pH of 5.5 or higher is best for good rhizobial survival and root nodulation (Drew et al., 2014). White clover will however tolerate slightly acidic soils (Smoliak et al., 2008; NSW DPI, 2012), although it will not tolerate highly acidic, highly alkaline or saline soils (Jahufer et al., 2001).

20. White clover is used for grazing and pasture hay, particularly in dairy, meat and wool production, where it has the potential to increase yields, and as ground cover in horticultural situations. It is highly important in the dairy, meat and wool industries (Ayres et al., 2000). In extensive grazing systems common in Australian livestock production, legume-based pastures are a major feed source; as part of such systems, white clover increases pasture productivity, enables N fixation to improve soil fertility and provides high quality forage enabling increased livestock production (Ayres and Lane, 2008). Average stocking rates for mixed pastures containing white clover are approximately 8 dry sheep equivalents per hectare (DSE ha<sup>-1</sup>) in NSW, 18 DSE ha<sup>-1</sup> in Vic. and 17.5 DSE ha<sup>-1</sup> in Tas., compared to unimproved pasture at 3 DSE ha<sup>-1</sup> (Donald, 2012).

21. White clover can be associated with bloat in grazing animals. Ingestion of foliage containing high levels of starch and carbohydrates may promote bloat, and saponins, colloidal particles and soluble proteins present in white clover may all play a role in bloat, which can lead to respiratory and circulatory malfunction (Hart, 1987; Lane et al., 2000). Risks of bloat can be managed or prophylactics may be used, but these are not always effective and may be expensive (Berg et al., 2000).

22. White clover produces cyanogenic glycosides which are released when plants are damaged - such as during grazing - and hydrolysed by an enzyme that is produced and stored in separate plant tissue to form hydrogen cyanide (HCN). The pathways for the formation of cyanoglucosides in white clover - linamarin and lotaustrin - have been elucidated. The alleles that control of presence or absence of these compounds is well characterised (Hughes, 1991; Olsen et al., 2007; Olsen et al., 2008; Olsen et al., 2013). White clover phenotypes for cyanogenesis vary across populations based on genetic variation (Olsen et al., 2007; Olsen et al., 2008) and also environmental factors (Vickery et al., 1987; Ballhorn and Elias, 2014), although relationships between environmental conditions and cyanogenic potential of white clover populations are not always simple (Richards and Fletcher, 2002; Kooyers et al., 2018).

23. A number of phytoestrogens, including isoflavones and coumesterol are produced in white clover, but generally not at levels that impact stock feeding on white clover forage. Although these compounds are produced as part of the broader PA biosynthesis pathway (see Figure 2, section 4.4.1), they are produced by a pathway that branches from the biosynthesis of CTs.
24. Only one report of allergy in humans to white clover was found (Jovanovic et al., 2003) and while one online source does list white clover as a mild allergen, no further information is supplied in this site ([Pollen Library website](#), accessed 27 July 2020). Horses may have adverse food reactions to white clover (Reed et al 2010, as cited by Pali-Schöll et al., 2017).
25. White clover is mainly self-incompatible and as such is primarily a species that depends on outcrossing between individuals in the population. Following on from earlier work (Ellison et al., 2006) examining the taxonomy of *Trifolium*, *T. repens* has been noted as having a primary gene pool that consists only of different individuals and lines of the same species and does not cross naturally with other *Trifolium* species (Williams, 2014).
26. Although natural interspecies crossing does not occur in *T. repens*, there are a number of species with which it may cross to form partially fertile offspring when hybridised, but production of fertile offspring requires extensive backcrossing programs or artificial crossing techniques such as chromosome doubling or embryo rescue in order to produce fertile, viable offspring (Williams, 2014).
27. White clover is principally insect pollinated, with bees (*Apis mellifera*) as the main pollinator. White clover pollen is not easily spread by wind. The distance pollen travels and successfully pollinates other white clover plants is dependent on a number of factors, including the size of donor and recipient crops, competition for pollination and environmental conditions. Different studies have concluded that outcrossing rates are less than 1 % within a distance of 10 m (Woodfield et al., 1995), while other show outcrossing rates higher than this even at distances of 200 m (De Lucas et al., 2012).
28. The rate of flower emergence is dependent on the rate of leaf emergence from the apical bud, which is related to temperature (Thomas, 1987). Under conditions where one leaf emerges per week, the time of full flower emergence from the appearance of the first leaf is about nine weeks (FAR, 2005), although it can be as short as four weeks (FAR, 2009). Seed development takes  $26 \pm 5$  days from pollination to full development (Harris, 1987).
29. White clover seeds may be soft – permeable to water and readily germinable – or hard – impermeable to water with delayed germination – depending on the conditions under which they ripen, with higher proportions of hard seed in dry conditions than in higher humidity (Hyde, 1954; Harris, 1987; D'Hondt et al., 2010). One study also indicated that higher seed number per seed head was correlated with a higher proportion of hard seed (D'Hondt et al., 2010). White clover is noted as having high seed output, forming persistent seedbanks (the longest reported is 25+ years, although no information about seed viability is given). A study in the USA found that of a small percentage (2%) of recovered seed was viable after burial at a depth of 42 inches (approximately 107 cm), for up to 16 years (or possibly 21 years with scarification of hard seeds). However, seeds were buried in pots and germination of retrieved seeds was tested in sterilised soils in a greenhouse (Toole and Brown, 1946), rather than under field conditions. White clover is primarily spread by seed, but also by movement of stem fragments and stolons, and can be dispersed long distances by humans and animals; it may also be spread by wind, water, birds, and ants. However, under unfavourable environmental conditions it has low germination rates ([FloraBase, The Western Australian Flora database](#); accessed September 2020). In one pasture grazing study in south east Queensland, soil seed reserves in the top 5 cm of soil (measured in three years) were higher in the wetter site than in the drier site (5,800 seeds m<sup>-2</sup> compared to 2,800 seeds m<sup>-2</sup>). However, seedling emergence from the same study, reported across seven years, ranged from 1 to over 700 seedlings m<sup>-2</sup> (Jones, 1982).
30. White clover is naturalised in many areas of Australia, regarded as established throughout much of Victoria and is present and naturalised in all biodiversity regions of Victoria. It is also regarded as naturalised in the ACT, eastern New South Wales, Tasmania, south-eastern South Australia and south-eastern Queensland and is present but less common in south-western Western Australia and other parts of Queensland ([VICFLORA database](#), [Weeds of Australia](#), [Queensland Biosecurity Edition database](#); accessed

September 2020). It is regarded as a very common and widespread weed of lawns, parks, gardens, playing fields, roadsides, waste areas, disturbed sites, riparian vegetation, grasslands, open woodlands and alpine vegetation, occasional weed of crops ([VICFLORA database](#), [Weeds of Australia, Queensland Biosecurity Edition database](#); accessed September 2020). White clover has been recorded as an environmental weed in Vic., WA and NSW, and in sub-alpine areas of south eastern Australia it may pose a threat to endangered species; however, it is not declared or noxious in any state or territory ([FloraBase](#), [The Western Australian Flora database](#); accessed September 2020). Randall (2017) rates white clover as an ‘Extreme’ weed risk rating, with most Australian reports categorising it as a ‘naturalised species’, some as an ‘environmental weed’ or ‘weed’. In Victoria, a report on environmental weeds classified white clover as a high risk weed based on scores for impact, potential spread to other areas, invasiveness, rate of dispersal and the range of susceptible habitat(s) it could impact (White et al., 2018).

31. The weed risk assessment (WRA) in ‘*The Biology of Trifolium repens L. (white clover)*’ (OGTR, 2020) indicates that white clover, as a volunteer, has some weedy characteristics such as high seed production and short time to seeding, as well as a high ability to establish in many land uses. It may be spread long distances by human activity, and has medium ability to establish among existing plants, but limited ability to reduce the establishment or yield of desirable plants.

## Section 4 The GMOs, nature and effect of the genetic modification

### 4.1 Introduction to the GMOs

32. The GM white clover proposed for release contains a gene for increased condensed tannins (CT) in the leaves. Two events with the same gene are included in this application, with each line containing only a single event. The applicant proposes to cross white clover lines containing each of these events with elite non-GM white clover lines to produce a number of lines, each containing one of the events.

33. Legumes such as white clover have low levels of condensed tannins in leaves and high levels of protein. Increased CTs in white clover leaf tissues are of interest due to their potential to mitigate bloat in ruminants (which may occur when higher levels of legumes are a significant part of animal feed) and improve protein utilisation, with the aim of improving animal production. The presence of leaf CTs with higher mDP (5-10) is valuable in mitigating bloat, and may also potentially reduce greenhouse gas emissions from animal production systems (Hancock et al., 2012; Hancock et al., 2014 and references therein).

34. The introduced gene is sourced from *Trifolium arvense* L. (hare’s foot clover), which is native to most of Europe and grows well in sandy soils. It is commonly found in Australia ([Atlas of Living Australia](#), accessed July 2020), but is not sexually compatible with *T. repens*.

35. The GM white clover plants also contain the *nptII* (neomycin phosphotransferase II) selectable marker gene. Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. This gene is derived from *Escherichia coli* (*E. coli*) strain K12 and encodes an aminoglycoside 3’-phosphotransferase II enzyme that is also known as neomycin phosphotransferase II (NPTII). It provides resistance to kanamycin and related antibiotics. More information on marker genes is available in the document [Marker Genes in GM Plants](#).

36. Short regulatory sequences that control expression of the genes are also present in the GM white clover lines. The regulatory sequences are derived from microorganisms (Cauliflower mosaic virus (CaMV) or *Agrobacterium tumefaciens*).

37. The genes and regulatory elements introduced to GM white clover lines are shown in Table 2.

**Table 2: Genes and regulatory elements introduced to GM white clover lines**

Genetic element	Source	Description	Function
<i>TaMYB14-1</i>	<i>Trifolium arvense</i> L.	Allelic variant of <i>TaMYB14</i>	R2R3-MYB Transcription factor, regulation of proanthocyanidin (PA) biosynthesis in legumes
35S	Cauliflower mosaic virus	Promoter from CaMV <sup>a</sup>	Promoter for <i>TaMyb14-1</i> gene

Genetic element	Source	Description	Function
<i>pNos</i>	<i>Agrobacterium tumefaciens</i>	Promoter	Promoter for <i>nptII</i> gene
<i>nptII</i>	<i>Escherichia coli</i>	Plasmid selectable marker - kanamycin resistance	Selectable marker gene
<i>OCS</i>	<i>Agrobacterium tumefaciens</i>	3'-untranslated sequence of the octopine synthase gene	Terminator sequence for <i>TaMYB14-1</i> gene
<i>nos</i>	<i>Agrobacterium tumefaciens</i>	Nopaline synthase gene from <i>A. tumefaciens</i> Ti plasmid	Terminator sequence for <i>nptII</i> gene

<sup>a</sup> CaMV: Cauliflower mosaic virus

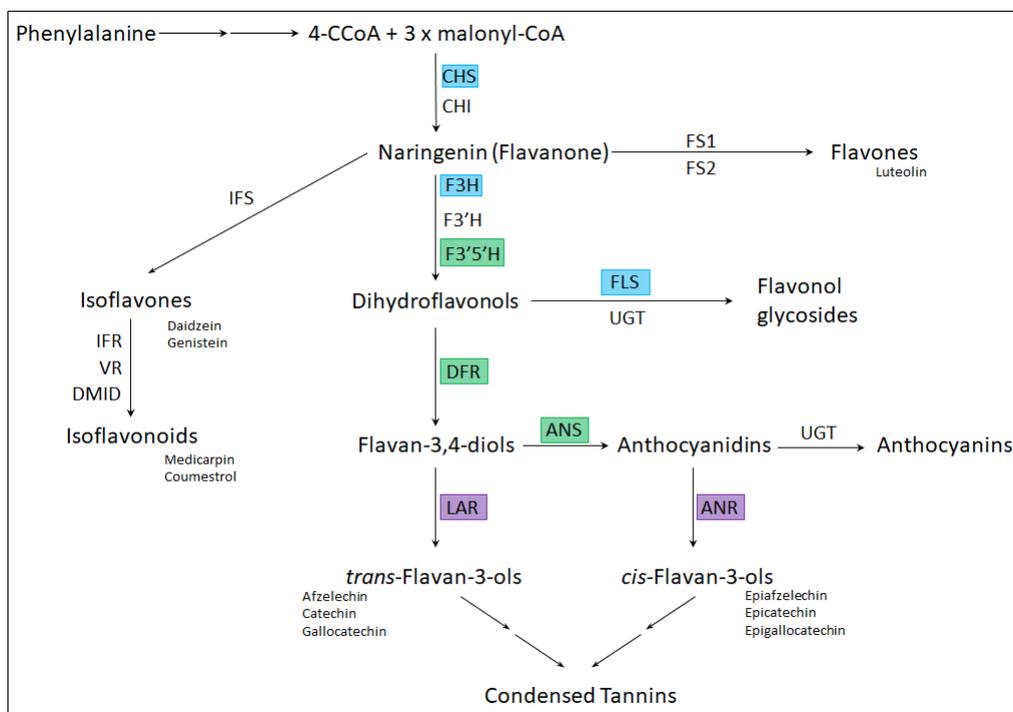
38. The GM white clover lines were produced using *Agrobacterium*-mediated transformation, using a protocol similar to a previously-published method (Voisey et al., 1994). Information about the *Agrobacterium*-mediated transformation method can be found in the document *Methods of plant genetic modification* available from the [OGTR Risk Assessment References page](#). Additionally, the applicant has stated that PCR analysis was undertaken to confirm the presence of t-DNA and the absence of vector backbone in transformed plants. They have stated that no vector backbone was detected in the two events proposed for release in this trial.

## 4.2 The introduced *TaMYB14-1* gene and its products

### 4.2.1 Introduction to the flavonoid pathway

39. Proanthocyanins (PAs) are implicated in a number of functions in plants, including protection from biotic and abiotic stresses (see (for example) reviews by Agati et al., 2009; Agati et al., 2011; Barbehenn and Constabel, 2011) and formation of coloured compounds such as anthocyanins (Winkel-Shirley, 2001). The flavonoid pathway is part of the large and complex PA biosynthetic pathway.

40. In white clover, a diverse set of PA compounds are produced with a range of putative or confirmed functions. A section of this pathway showing a number of the branches in the flavonoid biosynthesis pathway, including the branches relevant to this application is shown in Figure 2, below. It also shows the branches leading to other compounds relevant for white clover.



**Figure 2 Schematic diagram of elements of the PA biosynthetic pathway relevant to this application.** Adapted from Winkel-Shirley (2001); Hancock et al. (2012); Weston and Mathesius (2013). In GM white clover, enzymes highlighted in blue showed no consistent change in expression levels, those in

green were upregulated and those in purple were only expressed in GM white clover. Enzymes shown with no highlighting were not examined in this GM white clover study (Hancock et al., 2012). Abbreviations: CCoA – coumaryl CoA; CHS - chalcone synthase; CHI - chalcone isomerase; F3H - flavanone hydroxylase; F3'H - flavonoid-3'-hydroxylase; F3'5'H - flavonoid-3'5'-hydroxylase; FLS - flavonol synthase; UGT- UDP-glycosyl-transferase; DFR - dihydroflavonol reductase; ANS - anthocyanidin synthase; LAR - leucoanthocyanidin reductase; ANR - anthocyanidin reductase; IFS - isoflavone synthase; IFR - isoflavone reductase; DMID - 7,2'-dihydroxy, 4'-methoxyisoflavanol dehydratase; FS1 - flavone synthase 1; FS2 - flavone synthase 2.

41. The roles of plant CTs have been examined in great detail across a range of species and have been implicated in a range of functions including protection of plants from herbivores via antifeedant effects on both invertebrate and vertebrate herbivores, protection against pathogens, as well as potential protective effects against UV exposure (Winkel-Shirley, 2001; Barbehenn and Constabel, 2011). However, it should be noted that for significant protective effects against herbivores, CT levels of 5% of plant dry material are required (Barbehenn and Constabel, 2011).

42. Many plants, including species commonly used for food and animal feed, produce significantly higher levels of CTs than those likely to be produced in the GM white clover lines proposed for this release. Levels of 2% dry matter as CTs are biologically significant in forage species (Woodfield et al., 2019). Non-GM white clover plants produce tannins in a number of tissues, with highest levels generally produced in flowers (Burggraaf et al., 2006) and the phenolic subunits that are involved in the formation of CTs have been found in the flowers of white clover plants conventionally bred for high PAs in flowers (Foo et al., 2000). In forage, CTs can have anti-feedant effects when levels are above 5% of dry matter, whereby the CTs may bind proteins and reduce feed digestibility. However, animals which are routinely exposed to forage species will selectively graze the available food, consuming less of the forage species with high CT concentrations (van Cleef and Dubeux, 2019). Work examining the influence of tannins in *Eucalyptus spp.* on the fitness of marsupials such as possums and koalas feeding on them, showed indirect relationships through antifeedant effects or changes to digestibility of feed from high tannin levels in leaves consumed by the animals, rather than a direct effect of the tannins (Cork et al., 1983; DeGabriel et al., 2009). Where choices of feeds were available, possums susceptible to antifeedant effects chose to consume other foods with lower CT levels (Marsh et al., 2003).

#### 4.2.2 The introduced *TaMYB14-1* gene

43. The *TaMYB14-1* gene is an R2R3-MYB transcription factor (Roldan et al., 2020), an allelic variant of the *TaMYB14* gene isolated from *Trifolium arvense* L. (hare's-foot clover), which is involved in the regulation of PA biosynthesis pathways in legumes (Hancock et al., 2012).

44. Transcription factors are involved in the regulation of a wide range of cellular processes, by regulating functional genes involved in biosynthetic pathways and processes (Dubos et al., 2010; Ambawat et al., 2013). The MYB family of proteins is large and functionally diverse, represented across all eukaryotes, including a broad distribution in plants (Dubos et al., 2010; Ambawat et al., 2013; Liu et al., 2015) and most MYB proteins function as transcription factors (Ambawat et al., 2013). MYB transcription factors are involved in a range of functions including control of cell development, differentiation and function, regulation of plant development, responses to environmental – both abiotic and biotic – stress, and regulation of primary and secondary metabolism. Reviews, including detailed discussion of these functions are available (see for example Du et al., 2009; Dubos et al., 2010; Ambawat et al., 2013; Liu et al., 2015).

45. Of the four main groups of MYB proteins that have been recognised, plants mainly contain R2R3-MYB proteins that contain an N-terminal, conserved DNA binding domain and a diverse C-terminal modulator region, which is responsible for the regulatory activity of the MYB protein (Ambawat et al., 2013). These proteins contain two (R2 and R3) DNA binding domains in the conserved region, each of which form helices when bound to DNA (Du et al., 2009). Large numbers of R2R3-MYB proteins involved in the phenylpropanoid<sup>2</sup> pathway (of which the PA pathway is part) have been identified across a range of plant

<sup>2</sup> This pathway is involved in the biosynthesis of a wide range of plant secondary metabolites, including and the biosynthesis of lignin, as well as other important compounds, such as the flavonoids, coumarins, and lignans.

species including *Arabidopsis*, fruit and vegetable crops, grains, legumes, floral and tree species (Liu et al., 2015).

46. In glasshouse trials of GM white clover plants expressing the *TaMYB14* gene, PAs were present in foliar tissues, which in wild-type white clover do not contain PAs (Hancock et al., 2012). A number of enzymes involved in the PA biosynthesis pathway were upregulated in the GM white clover plants. The GM white clover line with highest *TaMYB14* expression had the highest concentration of PAs, as well as the highest expression of some, but not all, of the PA biosynthesis genes measured in this study (Hancock et al., 2012).

47. Transcriptome analysis of leaf tissue samples showed that a number of other MYB factors involved in secondary metabolism in white clover were equally expressed in GM white clover expressing *TaMYB14-1* compared with wild type (WT - non-GM) white clover leaf tissue. This indicates that expression of *TaMYB14-1* did not inhibit or silence biosynthesis of other secondary metabolites such as isoflavonoids or flavonols (Hancock et al., 2014). Secondary compounds produced by white clover are important in protection from disease, pathogens, and environmental stresses and as such it is important that expression of pathways involved in producing these compounds are not inhibited by the expression of *TaMYB14* (Hancock et al., 2014).

48. A number of enzymes involved in the parts of the pathway leading to production of flavan-3-ols, which are key to the formation condensed tannins (also known as proanthocyanidins), have been shown to be upregulated or expressed only in the GM white clover lines, as shown in Figure 2 (Hancock et al., 2012).

#### 4.2.3 Source organism for the *TaMYB14-1* gene

49. The donor organism for the *TaMYB14-1* gene is *Trifolium arvense* L., hare's-foot clover. This species is native to north Africa, Europe the Middle East and western Asia ([Weeds of Australia - Queensland Biosecurity Edition](#); [FloraBase the Western Australia Flora](#); [VICFLORA Flora of Victoria](#); all websites accessed 17 September 2020). It is broadly distributed across southeastern Australia, including Tasmania, and in southwestern Australia ([Atlas of Living Australia](#), accessed 6 July 2020;) and is widely naturalised in these areas and occasionally in south Australia and southeastern Queensland ([Weeds of Australia - Queensland Biosecurity Edition](#), accessed 17 September 2020). Randall (2017) rates *T. arvense* as a high weed risk, with most of the reports listed in the compilation referring to it as naturalised populations or an environmental weed (DSE Victoria, 2009; White et al., 2018). It is not listed as a Weed of National Significance ([Weeds of National Significance website](#); accessed 17 September 2020). In Victoria, it is described as highly invasive, but of low impact as a weed ([Biodiversity of the Western Volcanic Plains](#), accessed 17 September 2020) and is naturalised in most bioregions of Victoria ([VICFLORA Flora of Victoria](#) – accessed 17 September 2020). In WA, it is found in low rainfall areas and is adapted to low fertility soils. It has hard seed coat and shows low levels of germination under unfavourable conditions, but has no specialised structures for distribution of seed ([FloraBase the Western Australia Flora](#), accessed 17 September 2020).

#### 4.3 Toxicity/allergenicity of the protein associated with the introduced *TaMYB14-1* gene

50. As the GMOs are at an early stage of development, no toxicity or allergenicity studies have been conducted on the GM white clover plants or purified protein produced by the full-length *TaMYB14-1* gene. Bioinformatics searches for potential allergens can be conducted as a predictive tool for identifying biologically relevant sequences or structural similarities to known allergens, although the results are not definitive and in general serve to indicate proteins requiring further attention (Goodman, 2008). They provide a good tool at early stages to indicate whether further testing of particular proteins should be considered. The amino acid sequence of the protein expressed by the *TaMYB14-1* gene was compared to sequences of known allergens using the [AllergenOnline database](#), which contains data for over 2000 known allergens. No matches to protein allergens listed in that database, that met thresholds that would indicate protein identity or immunological similarity to known allergens, were found for the protein encoded by *TaMYB14-1* (information supplied by applicant). This suggests that expression of this gene is unlikely to produce any increase in allergenicity of the GM white clover.

#### 4.4 Characterisation of the GMOs

51. In preliminary glasshouse experiments on the white clover lines containing the *TaMYB14-1* gene, CT levels of 0.5% to 1.8% dry mass (DM) were reported, with a mean degree of polymerisation (mDP) of 6 in leaf extracts. These GMOs showed accumulation of PAs in epidermal cells of the leaves as well as in trichomes, whereas wild type plants only accumulated PAs in trichomes. Leaves of some GM plants appeared to have very high levels of PAs in epidermal tissues based on staining patterns, but these plants died before enough leaf material could be harvest for compositional analysis (Hancock et al., 2012).

52. Expression of selected genes in the PA pathway was examined in the GM white clover lines (see Figure 2). No consistent differences in expression were found between GM and WT white clover lines for *TrCHS*<sup>3</sup>, *TrF3H* and *TrFLS* genes, while *TrF3'5'H*, *TrDFR* and *TrANS* genes were upregulated, particularly the *TrF3'5'H* (more than 600-fold). *TrANR* and *TrLAR* were only expressed in GM lines. Additionally, this study examined genes coding for putative PA transporters (*TrMATE1* and *TrMATE2*), and showed that *TrMATE1* was not expressed in WT, but was highly expressed in GM lines, while *TrMATE2* was expressed at the same level in both WT and GM lines (Hancock et al., 2012).

53. Although these GM white clover lines are at an early stage of development, field trials have been conducted in the USA with the lines expressing the *TaMYB14-1* gene that showed high CT concentrations in glasshouse trials. The highest production of leaf CTs for these lines in field trials was just over 2% DM for a second generation (T<sub>2</sub>) transgenic homozygous plant (mean for homozygous T<sub>2</sub> plants was 1.87% DM), while backcross plants had concentrations below 1% (Woodfield et al., 2019). In the T<sub>2</sub> homozygous plants, there was a yield penalty, with statistically significant reductions in plant dry weight as compared to backcross plants and large, but not statistically significant, difference between T<sub>2</sub> homozygous and T<sub>2</sub> heterozygous or null segregant plants (Woodfield et al., 2019). The cause of reduced yields was not elucidated. No further characterisation of the GMOs has been provided.

54. The applicant has supplied information indicating that no vector backbone material was present in the two transformation events proposed for field trial and that there was a single copy of the gene, which is stably inherited in the GM white clover lines (information also supplied by applicant).

55. The applicant stated that no adverse responses have been reported for people working with the GM lines in glasshouse trials or in field trials in the USA.

## Section 5 The receiving environment

56. The receiving environment forms part of the context in which the risks associated with dealings with the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

57. White clover is cultivated in higher rainfall (at least 700 mm annual rainfall) areas of Australia, most commonly in Vic., Tas. and NSW, with a small amount grown in WA and south-eastern Queensland (Qld). It is generally grown as a short-term perennial pasture species in mixed pastures with perennial grasses and other legumes. Pasture mixes vary across different agricultural areas, as will the most suitable cultivars. Information relevant to the commercial cultivation and distribution of white clover in Australia, including key biotic and abiotic interactions in the white clover-growing environment, is presented in the white clover biology document (OGTR, 2020). Information relevant to the suitable conditions for white clover cultivation, variety selection and pasture mixtures can be found in relevant agricultural publications and websites as listed in sections below. Sections 5.1 to 5.3 summarise the key environmental and cultural factors for white clover cultivations, with information summarised from these publications except where otherwise attributed.

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<sup>3</sup> “Tr” in the designations of these genes indicates that they are the genes from *T. repens* (white clover).

## 5.1 Relevant biotic factors

58. Weeds impact white clover in a number of ways – reduced yield through competition for water, nutrient and space availability, contamination of seed resulting in rejection from certification or rejection from overseas markets, costs of weed control, herbicide usage-related issues including possible development of resistance and potential environmental and social problems (Riffkin et al., 2005). Main weeds of white clover in Australia are Annual ryegrass (*Lolium rigidum*), Sowthistle (*Sonchus oleraceus*), Maltese cockspur (*Centaurea melitensis*) and Jersey cudweed (*Pseudognaphalium luteoalbum*), with varying impacts at different stages of the white clover crop cycle (Riffkin et al., 2005). Control of weeds is generally through herbicide application.

59. Important invertebrate pests vary according to location and use of white clover for seed production or grazing. Pests affecting the establishment of white clover in Australia include red-legged mite (*Halotydeus destructor*), blue oat mite (*Penthaleus major*), lucerne flea (*Sminthurus viridis*), corbies (*Oncopera spp.*), pasture web worms (*Hednota spp.*) and related caterpillars, blackheaded pasture cockchafer (*Aphodius tasmaniae*), pink cutworm (*Agrotis munda*) and reticulated slug (*Deroceras reticulatum*). In seed crops, the main pests include native budworm (*Helicoverpa punctigera*), clover casebearer (*Coleophora frischella*) and bluegreen aphid (*Acyrtosiphon kondoi*) and in some cases the pea aphid (*Acyrtosiphon pisum*) (Berg, 1993; Seed Technology and Marketing Pty Ltd, 2007a, b, c).

60. Nematodes may significantly reduce white clover performance by reducing root growth and nitrogen fixation, and by stunting both leaf and stolon growth (Lane et al., 2000). In Australia, the root-knot nematode (*Meloidogyne spp.*) the clover cyst nematode (*Heterodera trifolii*) and a free-living nematode (*Helicotylenchus dihystra*) in sub-tropical areas (Zahid et al., 2001), as well as the stem nematode (*Ditylenchus dipsaci*), and root lesion nematodes. Breeding for resistance to invertebrate pests, including nematodes, has been an area of interest for white clover breeding in Australia and New Zealand for some time (Jahufer et al., 2002; Williams et al., 2007).

61. A range of fungal pathogens cause damage to taproots and stolons, which can lead to the subsequent death of these structures. Clover rot (*Sclerotinia trifoliorum*) is the most common fungal pathogen with frequent significant impacts on white clover productivity. Other minor fungal diseases include grey mould (*Botrytis cinerea*) and wart disease (*Physoderma trifolii*) (Clarke, 1999b). Fungal leaf spot diseases, including Pepper spot (*Leptosphaerulina trifolii*), Common leaf spot (*Pseudopeziza trifolii*), Black/Sooty spot (*Cymadothea trifolii*), Stemphylium leaf spot (*Stemphylium spp.*), Stagonospora leaf spot (*Stagonospora spp.*), Downy mildew (*Peronospora trifoliorum*) and Powdery mildew (*Erysiphe trifolii*), rarely cause significant losses (Clarke, 1999c).

62. A review of viral diseases of pasture in Australia lists 11 viruses found in white clover, of which Alfalfa mosaic virus (AMV) and White clover mosaic virus (WCMV) are regarded as most significant, with Clover yellow vein virus (CYVV) as potentially important along with Subterranean clover red leaf virus (SCRLV) (Jones, 2013). Of these, AMV, WCMV and CYVV are widespread in clover throughout Australia and have an impact on white clover productivity, with disease incidence and severity influenced by the viral species and the conditions under which studies were conducted (Garrett, 1991; McKirdy and Jones, 1995; Norton and Johnstone, 1998; Clarke, 1999; Jones, 2013). Reductions in white clover pasture production in response to viral infections result from reductions in foliage yield and quality, nitrogen fixing capacity and vegetative persistence (Kalla et al., 2001). AMV and CYVV are transmitted only by aphids, whereas WCMV is not, but is readily spread by machinery (Garrett, 1991). Most viruses that affect white clover are predominantly present in pastures, whereas CYVV is also present in natural environments (Godfree et al., 2004).

63. Additionally white clover can be affected by phyllody (unnatural development of floral tissues into leafy structures) caused by a mycoplasma (Reed, 2008).

64. White clover may affect the germination and survival of other plants due to allelopathic effects. A number of compounds, including plant flavonoid compounds, secreted by the roots or present in plant material can be leached into the surrounding soils (Macfarlane et al., 1982a, b; Carlsen and Fomsgaard, 2008; Carlsen et al., 2012; Weston and Mathesius, 2013). This may be regarded as a detrimental effect

when it limits the growth of other desired species, but may also be part of the effectiveness of white clover in suppressing weeds when used as a cover crop.

65. Short distance dispersal of white clover seeds may occur by dehiscence, stock trampling, worms, ants, and to a small extent by wind. Long distance dispersal of seeds occurs through human activities and by birds and grazing animals. Seeds can remain viable after passing through the digestive tracts of sheep, cattle and goats several days after consumption (Suckling, 1952; Yamada and Kawaguchi, 1971, 1972) and birds such as sparrows, pigeons, pheasants and rooks (Krach, 1959). Clover seed (*Trifolium* spp.) is eaten by species including crimson and Adelaide rosellas (*Platycercus elegans*) and galahs (*Elophus roseicapilla* syn. *Cacatua roseicapilla*) (Tracey et al., 2007). One study examined the potential for germination of viable seed after passage through the gut of birds and found that for bladder clover (*Trifolium spumosum*) seeds approximately 1% of fed seeds were able to germinate after digestion by birds (Twigg et al., 2009). However, this study examined seed feeding under caged conditions and germination of seeds was conducted under laboratory conditions. It is unknown whether this is representative of what would occur under field conditions. Additionally, no white clover seeds were included in this study, only other clover species that have considerably larger seeds than white clover, which could influence seed ingestion, digestion and germinability after digestion, making direct comparison difficult. Ingestion of white clover seeds by earthworms does occur and viable seed has been found in worm casts (McRill and Sagar, 1973). Ants have also been shown to carry white clover seeds in Australian pastures (Campbell, 1966). Kangaroos, rabbits and possums are pests known to decrease yield of improved pastures, so it is assumed that they feed on white clover, although this is not specifically documented and distribution by Australian animals and birds has not been studied.

66. Nitrogen fixation by pasture legumes has been estimated to provide a benefit of \$4 billion to Australia annually (Drew et al., 2014). The ability to fix atmospheric nitrogen is dependent on a symbiotic relationship of legumes with soil bacteria – *Rhizobium* spp. – and many legumes, including white clover, are inoculated with Rhizobia at planting to ensure adequate nodulation of roots for nitrogen fixation. White clover requires inoculation with ‘Group B’ inoculants (Rhizobial strain TA1, containing *Rhizobium leguminosarum* bv. *trifolii*) (Drew et al., 2014).

## 5.2 Relevant abiotic factors

67. The applicant has listed a range of areas from which the field trial sites may be selected, across NSW, Vic., Qld and WA. As such, these sites represent a broad range of climatic and agricultural areas.

68. White clover is tolerant of a wide range of soil types and is able to tolerate relatively acidic (NSW DPI, 2020) or alkaline (but not highly acidic or alkaline) soils, however is best suited to soils with neutral pH (5.5-6.5) (Jahufer et al., 2001). It can perform well on relatively infertile soils provided there is sufficient P and S, and is able to fix N, thus increasing N levels in soils (NSW DPI, 2020). It is tolerant of a wide range of soil types from clay to silty loam, provided moisture availability is sufficient (Jahufer et al., 2001). Main nutrient deficiencies affecting white clover are P, S, Mo and K (NSW DPI, 2020) and it may compete poorly with grasses for P, K and S (Harris, 1998).

69. White clover performs best in temperate climates, with a temperature ranges of 18-30°C (Reed, 2008) or 20-25 °C (Frame, 2003) described as optimal. High summer temperatures can limit production from white clover pastures, especially in conjunction with drought, which can occur at the same time (Smoliak et al., 2008; NSW DPI, 2020). Shallow root systems (most roots within 200 mm of the soil surface) may contribute to low tolerance to heat and cold (Jahufer et al., 2001). It is likely to survive heat better in mixed swards where grasses may provide protection from high solar radiation and temperature (Frame, 2003).

70. It grows best in higher rainfall areas – greater than 750 mm annual rainfall, but can be grown in areas with slightly lower rainfall if other conditions are suitable (Jahufer et al., 2001; NSW DPI, 2020).

71. Salinity and waterlogging will limit white clover production. It has been classified as salinity intolerant (Jahufer et al., 2001; Smoliak et al., 2008; Agriculture Victoria, 2020b), but with varying degrees of waterlogging tolerance depending on the cultivar. Waterlogging tolerance ranges from low tolerance,

requiring dry conditions but good access to water from moist or damp subsoil, to good tolerance where plants will cope with damp conditions growing in or near areas where the soil surface is saturated most of the time (Agriculture Victoria, 2020a, b).

72. Herbicides from a number of chemical groups are approved for use in controlling weeds in white clover – see the APVMA Public Chemical Registration Information System (PUBCRIS) for more information ([APVMA PUBCRIS search tool](#)) – and for control of white clover as weed in other crops or pastures. White clover does not compete well with grasses for nutrients such as P, K, and S (Harris, 1998), so in areas where grass growth is strong white clover may not compete well. However, white clover apparently copes better than grass under close grazing. Thus in areas where white clover is a weed, maintaining dense grass cover and limiting close cropping through grazing or mowing may help to control white clover. One of white clover's advantages is its ability to fix N through its relationship with Rhizobia, however, in areas with high N levels in the soil it may lose this competitive advantage, so maintaining good soil N may be helpful in controlling weedy white clover.

### 5.3 Relevant agricultural practices

73. White clover is commonly planted in a wide range of areas across south eastern mainland Australia, Tasmania, north eastern NSW, south eastern Qld and south western WA. This trial lists 117 possible LGAs in which the trial sites could be selected. These range throughout traditional growing areas with the exception of Tasmania.

74. The limits and controls of the proposed release are outlined in Section 2.1 and Section 2.2 of this Chapter. The applicant proposes to plant the GM clover lines using conventional planting methods to plant from seed, or using transplanted seedlings grown in propagating trays, while the non-GM clover and lucerne (*Medicago sativa*) used in the pollen traps and pollen buffer would be planted from seed. They have proposed that small areas would be planted by hand, while larger areas would be planted using commercial equipment. The applicant has also proposed that perennial ryegrass may be planted as part of mixed sward trials and both non-GM white clover and birdsfoot trefoil (*Lotus corniculatus*) may also be planted as a comparator species. Planting would mostly occur in autumn, but, in areas that are suitable, the applicant has indicated that they may plant in spring as well.

75. The proposed crop management practices would be similar to those used for commercial white clover production. Harvesting will occur either by hand or with commercial equipment. Irrigation may be used in planting areas if required to maintain crops when environmental conditions are not optimal. The applicant has indicated that herbicides and pesticides would be used to maintain crop health and that they would be applied using registered label rates and recommendations, by trained personnel wearing appropriate personal protective equipment (PPE).

76. Harvesting would be by hand or using commercial harvesting (mowing) equipment. The trial may include planting areas at the same site in consecutive years, however, the applicant proposes that fallow or rotation cropping is preferable, so in most situations a new planting area would be established, rather than planting over an existing planting area.

### 5.4 Presence of related plants in the receiving environment

77. As noted in 5.3, the trial sites will be selected from a range of sites across Vic., NSW, Qld and WA. These sites are within the commercial growing regions for white clover pastures in Australia. Although there are many species of clover present in Australia, white clover is only sexually compatible with other white clover plants, the only other related plants in the receiving area would be other populations of white clover.

### 5.5 Presence of similar genes and their products in the environment

78. The introduced genes and regulatory sequences were isolated from commonly occurring organisms that are already widespread in the environment (see Table 2, Section 4.1).

79. As discussed in Section 4, the gene inserted in the lines proposed for this trial is from the R2R3-MYB family of genes. MYB factors are widely conserved across eukaryotes and the R2R3-MYB genes are common across a number of plant species including common food and forage crops. Wild type (non-GM) white clover does contain a homologue of the *TaMYB14-1* gene (*TrMYB14-1*) and does produce low levels of condensed tannins, although generally not widespread in leaf tissues (Hancock et al., 2012). Other research has indicated that PAs are detectable at varying concentrations in white clover flowers during development, as are other compounds in the flavonoid pathway, and very low concentrations were detected in leaf tissues, localised in trichomes (Abeynayake et al., 2012).

80. The gene inserted in the GM white clover proposed for this trial is expected to result in the production of higher concentrations of CTs, which are present in a wide range of plants. Their roles in plant protection against herbivory and their effect on herbivorous invertebrates and mammals have been the subject of numerous studies (see, for example, Barbehenn and Constabel, 2011 and references therein). It is likely that humans and other organisms are exposed to a range of CTs in the environment.

81. The regulatory sequences that control expression of the genes inserted in the GM white clover are derived from microorganisms that are common in the environment (CaMV, and *A. tumefaciens*), as mentioned in Section 4.1.

82. The GM white clover plants also contain the *nptII* selectable marker gene derived from *E. coli*, a common bacterium that is widespread in human and animal digestive systems and/or in the environment. More information on marker genes is available in the document [Marker Genes in GM Plants](#).

## Section 6 Relevant Australian and international approvals

### 6.1 Australian approvals

83. There have been no approvals for commercial release of GM white clover in Australia. There have been two field trials of GM white clover in Australia, [DIR 047/2003](#) and [DIR 089](#). These licences authorised field trials of clover modified for resistance to infection by AMV.

### 6.2 International approvals

84. The applicant has indicated that GM white clover containing the *TaMYB14-1* gene has been the subject of field trials in the United States (United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) permit 17-111-102n – 2017). Results of a US field trial have been published (Woodfield et al., 2019).

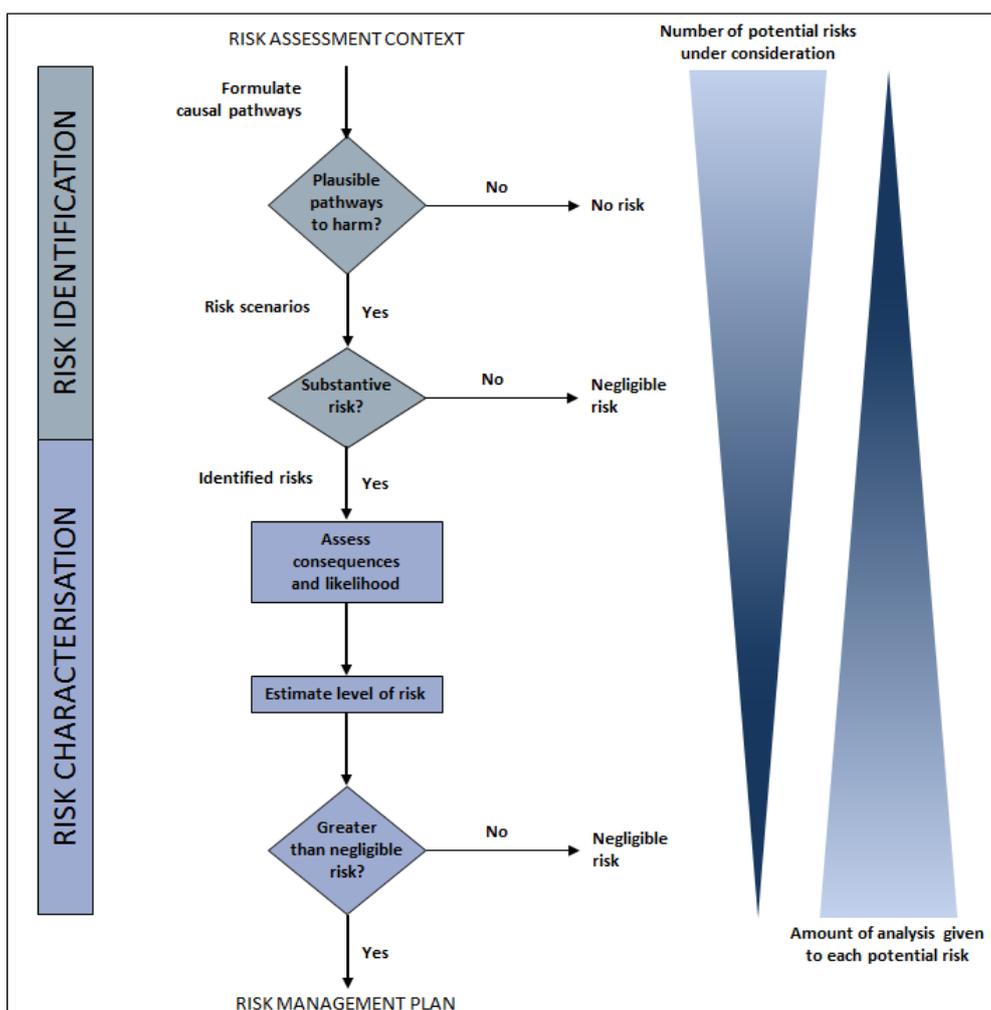
85. No general releases are recorded ([European Union GM Register](#); International Service for the Acquisition of Agri-Biotech Applications ([ISAAA](#)) [GM Approval database](#); [Biosafety Clearing House](#) (BCH) database; all accessed 21 August 2020).

86. None of the lines in the current application have been approved for release in any other country.

## Chapter 2 Risk assessment

### Section 1 Introduction

87. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 3). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



**Figure 3. The risk assessment process**

88. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

89. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or

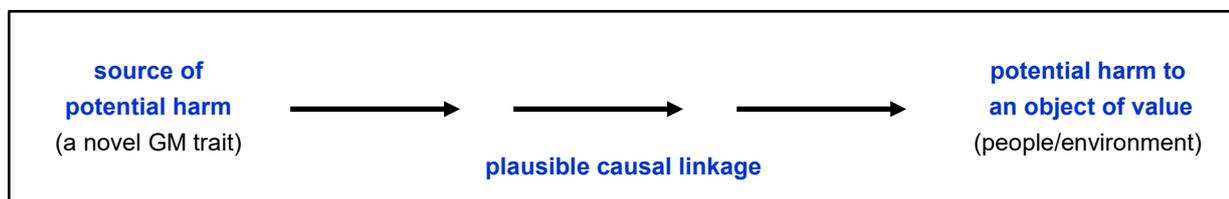
those that could not plausibly occur, do not advance in the risk assessment process (Figure 3) i.e. the risk is considered to be no greater than negligible.

90. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

## Section 2 Risk Identification

91. Postulated risk scenarios are comprised of three components (Figure 4):

- i. the source of potential harm (risk source)
- ii. a plausible causal linkage to potential harm (causal pathway)
- iii. potential harm to people or the environment.



**Figure 4: Risk scenario**

92. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO
- the characteristics of the parent organism(s).

### 2.1 Risk source

93. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

94. As discussed in Chapter 1, the GM white clover lines have been modified by the introduction of the *TaMyb14-1* gene derived from *T. arvense* L. The intended effect of insertion of this gene is to increase the condensed tannin content of white clover leaf tissues. This introduced gene is considered further as a potential source of risk.

95. The GM white clover also contains the marker gene *nptII* from *E. coli* that confers antibiotic resistance and was used as a selectable marker gene. This gene and its product have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document *Marker genes in GM plants* available from the [Risk Assessment References page](#) on the OGTR website. As the gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application.

96. The introduced genes are controlled by introduced regulatory sequences. These were derived from Cauliflower mosaic virus (CaMV) and *A. tumefaciens*. Regulatory sequences are naturally present in all plants and the introduced sequences are expected to operate in similar ways to endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary

DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

97. The genetic modifications have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding (Ladics et al., 2015; Schnell et al., 2015). Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015; Anderson et al., 2016). Plants generated by conventional breeding have a long history of safe use, with few documented cases where conventional breeding has resulted in an unacceptable level of a metabolite in a crop (Berkley et al., 1986; Seligman et al., 1987). There are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Current practices identify and remove harmful non-GM plants to protect domesticated animals and people (Steiner et al., 2013). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

## 2.2 Causal pathway

98. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- 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) on the properties of the organism
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the GM plants (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer
- unauthorised activities.

99. Although all of these factors are taken into account, some are not included in the risk scenarios below as they may have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

100. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for [DIR 108](#). Although the DIR 108 RARMP is for GM canola, the HGT considerations are the same for the current RARMP: plant HGT events rarely occur and the wild-type gene sequences or homologues are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

101. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for [DIR 117](#). In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides substantial penalties for unauthorised dealings with GMOs or noncompliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative

provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, risks from unauthorised activities will not be considered further.

### 2.3 Potential harm

102. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue, 2008; Keese et al., 2014) including:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

103. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

### 2.4 Postulated risk scenarios

104. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 3 and examined in detail in Sections 2.4.1 – 2.4.3.

105. In the context of the activities proposed by the applicant and considering both the short and long term, none of the three risk scenarios gave rise to any substantive risks.

**Table 3: Summary of risk scenarios from the proposed dealings with the GM white clover**

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene conferring increased condensed tannin concentration in leaves	Growing GM white clover at the field trial sites ↓ Expression of the introduced gene in GM plants ↓ Exposure of humans or other desirable organisms by ingestion of, or contact with, the plant material	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms	No	<ul style="list-style-type: none"> <li>• No matches were found for toxins or allergenic compounds in database searches for the expressed protein.</li> <li>• The pathway end products occur naturally in the environment and are not known to be toxic or allergenic to people or other desirable organisms at the levels potentially produced by these GMOs.</li> <li>• The small size of the trial and controls proposed for the trial would minimise exposure of people and other desirable organisms to the GM plant material.</li> <li>• No food or feed is to be produced from this trial.</li> </ul>

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced gene conferring increased condensed tannin concentration in leaves	Growing GM white clover at the field trial sites ↓ Dispersal of GM seed or vegetative material outside the trial limits ↓ GM seed germinates or vegetative material spreads ↓ Establishment of GM white clover plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants	No	<ul style="list-style-type: none"> <li>Proposed limits and controls minimise the likelihood of seed or viable plant material being dispersed outside the trial site.</li> <li>The introduced gene construct does not confer other characteristics that would enhance the spread and persistence of the GM white clover. As such, it is not expected to increase the weediness of the GM white clover lines.</li> <li>GM white clover could be controlled using conventional methods.</li> <li>Scenario 1 did not identify an increased risk of allergenicity or toxicity in the GM white clover.</li> </ul>
3	Introduced gene conferring increased condensed tannin concentration in leaves	Growing GM white clover at the field trial sites ↓ Fertilisation of sexually compatible plants outside the trial site by pollen from GM white clover plants ↓ Germination of GM hybrid seed ↓ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants	No	<ul style="list-style-type: none"> <li>Proposed limits and controls minimise the likelihood of pollen dispersal outside the trial site.</li> <li>There are no other sexually compatible species with which white clover can hybridise.</li> <li>Risk scenarios 1 and 2 did not identify toxicity, allergenicity or increased weediness of the GMOs as substantive risks.</li> </ul>

### 2.4.1 Risk scenario 1

<i>Risk Source</i>	Introduced gene conferring increased condensed tannin concentration in leaves
<i>Causal Pathway</i>	GM white clover planted at the field trial site ↓ Expression of the introduced gene in GM plants ↓ Exposure of humans or other desirable organisms by ingestion of, or contact with, the plant material ↓
<i>Potential Harm</i>	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms

#### **Risk source**

106. The source of potential harm for this postulated risk scenario is the introduced gene for increased condensed tannin concentration in the leaves of in GM white clover plants.

#### **Causal pathway**

107. The aim of the genetic modification in the GM white clover plants is to produce plants with increased condensed tannin (CT) concentration in leaf tissues. However, the inserted gene is under the control of a constitutive promoter, and so the encoded protein may potentially be expressed in all plant tissues. Whether increased expression occurs in other tissues, with the possibility of increased CT concentration in those tissues, has not yet been determined.

108. People may be exposed to GM plant material, the expressed protein and compounds of the PA pathway, either by direct contact with the plant material or through inhalation of pollen. This is most likely

at the trial site, but it could also occur during transport and handling of GM plant material. Other organisms such as livestock, rodents, marsupials, birds or invertebrates, including pollinators, may be exposed at the trial site through contact with, or ingestion of GM plant material.

109. The applicant has proposed a range of limits and controls that would reduce exposure of people and other animals to the GM white clover (detailed in Chapter 1, Section 2.2). The trial is limited in size and duration and the applicant has proposed measures to confine the GM clover to the trial sites. These include enclosing the planting area with pollinator-proof tents, or surrounding the planting area with an inner pollen trap (non-GM white clover), a pollen buffer crop (lucerne) and an outer pollen trap (non-GM white clover). They have also proposed surrounding parts of the trial with stock proof fences and lockable gates.

110. The trial is to be conducted at sites on private properties. The applicant has indicated that they will have access to and control of trial sites during the trial and only authorised people would be permitted to deal with the GM white clover.

111. Transport and storage of the GM plant material would be conducted according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs, thus limiting exposure of people during transport and storage of the GMOs. No material from this trial would be used for human food or animal feed. These proposed limits and controls would minimise the exposure of people or animals to the GM plants and their products.

### **Potential harm**

112. 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). Allergenicity is the potential of a substance 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).

113. Potentially, people exposed to the protein expressed by the introduced gene may show increased toxic reactions or increased allergenicity. Similarly, exposure to the protein expressed by the introduced gene, or the enzymes and products of the PA pathway that are upregulated as a result of gene expression, may lead to increased toxicity to other desirable organisms. From consideration of the causal pathway, including the proposed limits and controls, human exposure would be limited to staff involved in handling the GM white clover plants during the course of the field trial.

114. Although no toxicity or allergenicity studies have been performed on the GM plant material or the expressed protein, the applicant has stated that bioinformatic searches of the amino acid sequence for the expressed protein yielded no matches with known allergens.

115. As discussed in Chapter 1 (Section 3) and in the biology document (OGTR, 2020), white clover is primarily a pasture forage crop, grown as part of mixed-species pastures for animal production. Non-GM white clover produces some toxins and anti-nutritional factors, including cyanoglucosides. There is no reasonable expectation that the introduced gene expressed in the GM white clover or any upregulated enzymes in the PA pathway would affect the pathways producing known toxins or allergens or lead to the production of novel toxins or allergens. As outlined in Chapter 1, Section 3, there is only one reference reporting an allergy to white clover leaves in humans and one mention of an adverse food reaction in horses.

116. Additionally, as discussed in Chapter 1 (Section 3), a number of phytoestrogens, including isoflavones and coumesterol, which are produced as part of a different branch of the flavonoid biosynthesis pathway, but by a pathway that branches from the biosynthesis of CTs. Of the enzymes studied in relation to the GM white clover lines, the chalcone synthase (CHS) enzyme, involved in the shared part of these two pathways was upregulated in some GM lines expressing *TaMYB14-1* (Hancock et al., 2012). It is not clear whether this would result in higher levels of these compounds in GM white clover lines, but it should be noted that other non-GM legumes may contain higher levels of such compounds and these may still be consumed as stock feed and/or by humans.

117. The inserted gene is involved in regulating the PA pathway, resulting in higher concentrations of condensed tannins in the leaf tissue of GM white clover. This class of gene - transcription factors - is conserved across eukaryotes and the R2R3-MYB class, to which the inserted gene belongs, is common across a wide range of plants, with homologues of the inserted *TaMYB14-1* gene found in a number of species. Thus, such transcription factors are present in a range of organisms in the environment. Likewise, CTs are produced in a range of tissues across different plant species, often at levels much higher than those likely to be produced in the GM lines proposed for this trial. As such, humans and other beneficial organisms (including bees) routinely encounter these genes or homologues of these genes and their products through contact with plants or animals and food derived from them.

118. The applicant has also proposed that large animals would be excluded from the trial site whilst GM white clover is growing, by fencing parts of the trial site. Additionally, white clover is not commonly a source of human food and white clover from this trial will not be used for animal feed, thus further limiting the exposure of humans and other desirable organisms to the GM white clover.

**Conclusion**

119. Risk scenario 1 is not identified as a substantive risk due to limited exposure and the lack of toxicity or allergenicity of the introduced gene and its encoded protein to humans and lack of toxicity to other organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

**2.4.2 Risk scenario 2**

<i>Risk Source</i>	Introduced gene conferring increased condensed tannin concentration in leaves
<i>Causal Pathway</i>	↓ GM white clover planted at the field trial site ↓ Dispersal of GM seed or viable plant material outside the trial limits ↓ GM seed germinates or plant material survives and establishes ↓ Establishment of GM white clover plants in nature reserves, roadside areas or intensive use areas ↓
<i>Potential Harm</i>	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants

**Risk source**

120. The source of potential harm for this postulated risk scenario is the introduced gene for increased condensed tannin concentration in the leaves of in GM white clover plants.

**Causal pathway**

121. If GM white clover seed was dispersed outside the trial sites, or persisted at the trial sites after completion of the trial, this seed could germinate and give rise to plants expressing the introduced gene. These plants could spread and persist in the environment and establish populations of GM white clover, expressing genes for increased condensed tannin. This could increase the likelihood of exposure of people or other desirable organisms to the proteins expressed in the GM plants and the end products – condensed tannins.

122. Additionally, as white clover has the ability to reproduce vegetatively, movement of viable vegetative material may be a means of establishing white clover plants outside the trial area. Discussion of vegetative reproduction of white clover is limited to spread of stolons, which involves outward growth of vegetative material from the original plant and formation of roots where this material is in contact with the ground, rather than reproduction via movement of vegetative material from the original plant to a separate location. Although it is possible that vegetative material may provide a means of spread over longer

distances, there is little direct evidence of this (Herbiguide, 2014). It appears that seed is the most likely means of spread for white clover.

123. The seeds of white clover are numerous and very small - approximately 0.6-0.7 mg per seed (Jahufer et al., 2001; Frame, 2003). A percentage of seeds may be 'hard' seeds, which do not readily germinate and may survive in the soil for an extended period, however the percentage of hard seed varies widely based on environmental conditions and their influence on soil conditions, as well as other factors such as cultivar. In general, seeds ripening under dry conditions contain a higher percentage of hard seeds than those ripening under humid conditions. Seed survival in the soil varies depending on environmental conditions and cultural practices in the area of the seedbed. Reports of seed persistence vary greatly, however in many reports the viability of seed persisting in the seedbed is not assessed (see Chapter 1).

124. If any seeds survive and germinate, white clover will generally establish better in areas that have been disturbed, such as cropping areas, roadsides, and excavated areas (Godfree et al., 2004), and does not establish under closed shrub canopies (Garrett and Chu, 1997). White clover seed germinates better with an open canopy and it is regarded as being slow growing, especially during early stages of establishment, although its ability to compete with weeds increases with time (Frame, 2003). Limiting factors for white clover persistence and productivity in Australia are water stress in summer, viral infections, insect and nematode pests, poor grazing or fertiliser management and soil salinity (Jahufer et al., 2001; NSW DPI, 2020). It is regarded as a poor competitor with grass species for nutrients such as P, K and S (Harris, 1998).

125. Although white clover seeds may be spread long distances via human activities, the applicant has proposed limits and controls to prevent the spread of GM white clover seed from the trial site. Access to the site is restricted to authorised, trained staff. The applicant has proposed that white clover will be harvested using commercial equipment or by hand, depending on the size of the plot to be harvested, and has stated that all equipment used at the trial site would be cleaned before being used for any other purpose. All GM plant material would be transported in accordance with the Regulator's Transport, Storage and Disposal of GMOs guidelines, which would minimise the opportunity for dispersal of GM material and contact with any GM plant material during transport from the trial site to facilities for analysis.

126. White clover seed can also be spread via animals, including livestock and birds. They can survive passage through the ruminant digestive system and remain viable (Suckling, 1952; Yamada and Kawaguchi, 1971, 1972; Frame, 2003) and the same is true for seed consumed by some birds (Krach, 1959). Australian bird species do consume white clover seeds (Tracey et al., 2007) but it is not known whether they can spread viable seed. Although germination of bladder clover after digestion of birds is possible (Twigg et al., 2009), these studies were conducted with captive feeding and laboratory germination of digested seeds, using seeds that are considerably larger than white clover so direct comparison of these results to white clover field trials is tenuous. Likewise, although it is assumed that Australian mammals incidentally consume white clover seed with other pasture species, as they have been observed to damage sown pastures, their role in distribution of white clover seed has not been studied. It is also possible that rodents would also feed on white clover seeds and could transport them, although, again this is largely undocumented. Ants and earthworms have been reported to move white clover seeds over short distances. While other insects do feed on white clover and damage seeds, there are no reports of seed movement by these insects. As noted in Risk Scenario 1, the applicant has proposed excluding large animals from trial sites using fences.

127. The proposed trial sites are small and the period during sowing via seed (rather than the alternative option of transplanting seedlings) and when ripe seeds are present at the trial site, is short. The period immediately after harvest when animals could consume or spread viable seeds is similarly limited. The applicant has stated that any white clover seed production during trial would be undertaken in insect-proof tents, and in all other planting areas no seed would be allowed to set and be deposited on the ground. The applicant has proposed that planting areas using tents would be surrounded by a monitoring zone, which would be inspected for white clover volunteers during flowering of the GMOs in the planting areas, continuing until the planting area and any other areas requiring cleaning, are cleaned. This would reduce the amount of seed from the crop being available for spread by animals.

128. Dispersal of seed by wind is possible, but limited, and dispersal of seed by water, while likely (as inferred by the presence of white clover along watercourses and in riparian zones) is largely undocumented. The only report providing information about white clover seed survival in water indicated that seed could germinate after ten days in water (Morinaga, 1926). The applicant has proposed locating the outer edge of trial sites – i.e. the outer edge of the outer pollen trap or the outer edge of the monitoring zone, as applicable to the individual trial setup – at least 50 m from natural waterways.

129. In addition to the measures to control seed spread during the trial, the applicant has proposed measures to inspect the trial sites after cleaning to ensure that any volunteers germinating from seed remaining at the site are detected and destroyed before flowering. These measures further reduce any remaining opportunities for seed spread during this period.

130. White clover can also spread vegetatively via stolons. Any tented planting areas are proposed to be surrounded by a monitoring zone, which would be inspected regularly as detailed in paragraph 126. Thus, while GM white clover is growing in the planting area, any plants spreading via stolons into this area would be removed or prevented from flowering. The monitoring zone proposed extends 10 m from the outer edge of the planting area.

### **Potential Harm**

131. If GM plants were able to establish outside the trial site they could potentially cause increased toxicity or allergenicity to humans or increased toxicity to other desirable organisms through increased exposure. However, as discussed in Chapter 1 (section 4.3) and in Risk Scenario 1, there is no reasonable expectation that the GM white clover and the products would be any more toxic or allergenic than non-GM white clover.

132. Establishment of GM white clover outside the trial site could potentially reduce the establishment or yield of desirable agricultural crops; reduce establishment of desirable native vegetation; reduce utility of roadsides, drains, channels and other intensive use areas; or provide a reservoir for pathogens or pests.

133. In order to increase weediness by comparison with the non-GM parent, any characteristics which provided a selective advantage would need to be coupled with other mechanisms that increase spread and persistence in the environment, through changes in dispersal, establishment and survival. These characteristics would not reasonably be expected to change as a result of the introduced genes, either in individual lines or in a hybrid background.

134. As discussed in Chapter 1 (Section 3) and in *The Biology of Trifolium repens L. (white clover)*, non-GM white clover is regarded as a weed in Australia (Groves et al., 2003; Randall, 2017) and weedy populations are commonly found outside cultivated areas. As noted in Chapter 1, the WRA for non-GM white clover concludes that white clover has some weedy characteristics, and medium ability to establish in existing plant communities, but in general it has limited ability to reduce establishment of desired plant species. It is most often a weed of disturbed areas, but it is also found in natural ecosystems and in some areas is an environmental weed (DSE Victoria, 2009; White et al., 2018). In subalpine areas of south eastern Australia it has been noted as threatening some endangered species and native plant communities ([Weeds of Australia - Queensland Biosecurity Edition](#); accessed September 2020).

135. The GM white clover proposed for this trial expresses a gene that is expected to increase the concentration of CTs in leaves. When CT concentrations are higher than 5% DM they may have a number of effects such as antifeedant effects on invertebrate and vertebrate herbivores, thereby protecting plant tissue from consumption and potentially improving the plant's survival and productivity. As discussed in Chapter 1 (Section 4), the highest production of leaf CTs for the lines in field trials so far is just over 2% DM and in plants with the highest CT concentrations there was a yield penalty (Woodfield et al., 2019). It is also unlikely that the GM lines proposed for release in this trial will produce concentrations of CTs in leaf tissues high enough to provide protective effects. Thus, it is unlikely that the GM white clover plants would have any increased fitness compared to non-GM white clover and in fact may have reduced fitness due to reduced dry matter production.

136. While white clover naturally produces compounds that have allelopathic effects on other plants, pathways for these compounds diverge from the CT pathway. Although there is no data available, it is unlikely that concentrations of these compounds would be increased in the GMOs.

137. The introduced trait is not likely to change the susceptibility of the GM white clover lines to conventional controls. Thus, if required, the GM white clover plants proposed in this trial could be controlled by standard weed control measures, such as cultivation or the use of herbicides.

138. White clover establishment and survival is limited by a number of other factors, such as disease, poor ability to compete with grasses for nutrients, sensitivity to highly acidic or alkaline soils, mineral toxicities and sensitivity to certain classes of herbicides. Optimal white clover production is generally achieved only with human intervention such as weed control and inoculation with rhizobia, so growth and yields of plants growing outside cultivation are likely to be reduced. Despite these limitations, the presence of weedy populations established in a number of states indicates that white clover can survive outside cultivation and in some circumstances may pose a risk to other plant species, as noted above. However, as noted in paragraph 130, the genetic modification in this application is unlikely to increase the weediness of the GM white clover compared to non-GM white clover.

139. The limits and controls outlined in Risk Scenario 1 reduce the potential amount of seed available for dispersal outside the trial site and the opportunities for spreading seeds, as well as the opportunities for spread of viable vegetative material.

### Conclusion

140. Risk scenario 2 is not identified as a substantive risk due to the lack of toxicity or allergenicity of the introduced gene and its encoded protein; the fact that the GMO is not likely to be more weedy than non-GM white clover; the proposed limits and controls designed to restrict dispersal of seed or vegetative material; and the susceptibility to standard weed control measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

### 2.4.3 Risk scenario 3

<i>Risk Source</i>	Introduced gene conferring increased condensed tannin concentration in leaves
<i>Causal Pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">GM white clover planted at the field trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Fertilisation of non-GM white clover plants inside or outside the trial site by pollen from GM white clover plants</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Germination of GM hybrid seed</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas</p> <p style="text-align: center;">↓</p>
<i>Potential Harm</i>	<p style="text-align: center;">Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced establishment and yield of desirable plants</p>

### Risk source

141. The source of potential harm for this postulated risk scenario is the introduced gene for increased condensed tannin concentration in the leaves of in GM white clover plants.

### Causal pathway

142. Pollen from GM white clover lines could fertilise sexually compatible plants either inside or outside the trial sites. Hybrid plants carrying the inserted gene could form the basis for spread and dispersal of the gene in other varieties of white clover. People and other desirable organisms could then be exposed to the proteins expressed by the introduced genes through ingestion, contact with plant material or inhalation of pollen from hybrid plants.

143. It should be noted that vertical gene flow per se is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. Baseline information on vertical gene transfer associated with non-GM white clover plants can be found in the white clover biology document (OGTR, 2020) and a summary is provided in Chapter 1, Section 3 of this RARMP. As outlined, there are no other sexually compatible species for white clover, so outcrossing occurs between different individuals of the same population or between individuals of different white clover populations and/or cultivars. Thus, no gene flow could occur with the other species, including those grown as part of this trial.

144. White clover is generally regarded as self-incompatible and is therefore essentially an obligate outcrossing plant. The proposed trial consists of up to six lines of GM white clover, each containing the same gene, introduced through one of two transformation events, using the same construct, as well as non-GM white clover or birdsfoot trefoil grown within the trial as comparators. In addition to this, the GM white clover may be grown as part of a mixed sward with perennial ryegrass. The applicant is proposing to perform crosses between GM white clover lines and elite non-GM clover lines. It is possible that there could be pollen flow between GM white clover lines containing the different events, or that they could pollinate the non-GM white clover grown as part of the trial, including white clover plants grown as comparator lines, or plants in the inner or outer pollen trap.

145. The interaction of bees with GM white clover was discussed in detail in the RARMP for [DIR 089](#). In this trial, the applicant has proposed that beehives would be brought in for pollination of white clover and that all bees, honey and pollen from the hives would be destroyed after pollination. In tented sites, the presence of the insect-proof tents would prevent movement of bees from the site during pollination. In sites where planting areas are not tented, the proposed pollen trap and pollen buffer crops are expected to flower at the same time as GM white clover in the planting area and to attract bees that may have collected pollen from GM white clover in the planting areas, such that pollen movement outside the trial site is minimised.

146. The suitability of the pollen trap and pollen buffer crops with the same makeup as those proposed for this trial, has also been discussed in detail in the RARMP for [DIR 089](#). The use of the pollen traps and pollen buffer to contain pollen from the GMOs is consistent with the literature discussed in '*The Biology of Trifolium repens L. (white clover)*', which suggests that although bees may travel large distances, they will forage in a much smaller area where abundant food sources are available. In general, pollen will be deposited most often on the plants visited soon after pollen is gathered, and fertilisation is most effective from pollen collected on recently-visited plants (OGTR, 2020). Additionally, although bees from outside the trial site could access the GMOs at sites where planting areas are not tented, the presence of the pollen traps and pollen buffer to provide an abundant source of attractive food, would limit the likelihood of bees visiting the planting area and carrying pollen from the GMOs back to external hives. This would minimise the potential for gene flow and limit the likelihood of GM pollen being present in honey from external hives.

147. If pollen flow between white clover lines containing different events occurred, it could result in lines containing each of the events and therefore two copies of the *TaMYB14-1* gene. The applicant has stated that they will treat any non-GM white clover plants grown at the site as though they were GMOs, thus all white clover at the site will need to be destroyed at the end of the trial and the areas on which they were grown will have to be cleaned. In addition, there are requirements for any volunteers at the trial site to be destroyed before flowering, so in the very rare case that a hybrid plant occurred, it would not be allowed to remain and set seed.

148. The proposed limits and controls for this trial would reduce the likelihood of pollen flow from the trial to non-GM white clover outside the trial site. As mentioned in Risk Scenario 1, the applicant has proposed two options for trial sites in this trial. In Option 1, the GMOs would be grown in planting areas enclosed in an insect-proof tent, which would be surrounded by a monitoring zone and an isolation zone. In Option 2, the planting area would not be tented, and would be surrounded by an inner pollen trap crop, a pollen buffer crop and an outer pollen trap crop, which would be surrounded by an isolation zone. While the GMOs are being grown, the applicant proposes inspection requirements for volunteer white clover in the monitoring zone or the pollen trap and pollen buffer crops, and requirements to ensure that any

volunteers are destroyed or prevented from flowering. In the isolation zone, the applicant has proposed that no white clover may be intentionally planted while the GMOs are growing in the planting area. These measures would greatly reduce the potential for pollen flow from the trial to white clover planted outside the trial sites.

149. The applicant proposes postharvest monitoring of the sites for any volunteer GM white clover and destroying any volunteers, to prevent production of plants that could hybridise with other white clover through pollen flow.

### **Potential Harm**

150. If pollen from GM white clover lines was dispersed, resulting hybrids could spread and persist in the environment, leading to increased exposure and potentially increased toxicity or allergenicity to humans or increased toxicity to other beneficial organisms. Hybrids expressing the introduced gene could also reduce the establishment and yield of desired plants and subsequently reduce biodiversity.

151. If hybrids between two GM white clover lines were to occur, they could contain two copies of the inserted gene for increased CT concentration in leaves. The possible outcome from such a cross could be higher concentrations of CTs in the leaves of hybrids, although this has not been confirmed. It is also quite likely that any plants producing further increased concentrations of CTs would carry a significant yield penalty, as was observed for homozygous T<sub>2</sub> plants in field trials in the USA (Woodfield et al., 2019). They would not be expected to produce any novel products or show any difference in toxicity or allergenicity from either GM parent. It is also unlikely that they would produce levels of CTs higher than those found in other common plants. Hybrids between GM white clover and non-GM white clover would result in progeny with the same gene for increased CTs in leaves as the GM parent. However, there is no reason to believe that hybrid plants would possess a level of toxicity or allergenicity greater than that of either parent. Nor is it likely that such hybrids would possess a level of weediness greater than that of either parent.

152. In the event of vertical transfer from the GM white clover lines to non-GM white clover lines, it is expected that the introduced genes would confer the same properties in the hybrid as the GM parent. Thus, as discussed in Risk scenarios 1 and 2, the introduced gene products, are not expected to be toxic to humans or other organisms, nor are they likely to make the white clover lines more weedy. These characteristics are not expected to differ in a hybrid background.

153. The proposed isolation distances, together with the proposed inspection requirements, greatly restrict the possibility of pollen flow and subsequent vertical gene transfer of the genes from the GM lines to any plants outside the trial planting area. If any gene transfer occurred between the GMOs and pollen trap crops, the proposed treatment of any non-GM white clover planted within the trial site as if they were GMOs ensures that any plants in these areas would be destroyed at the end of the trial and the areas would be cleaned following this. Thus, any hybrids which may have been produced would be destroyed.

### **Conclusion**

154. Risk scenario 3 is not identified as a substantive risk due to the limited possibility of pollen flow for white clover. In addition, Risk scenarios 1 and 2 did not identify toxicity, allergenicity or increased weediness of the GMOs as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

## **Section 3      Uncertainty**

155. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

156. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

157. As field trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a field trial application. However, field trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.

158. For DIR 176, uncertainty is noted particularly in relation to:

- potential increased toxicity of GM white clover to people or animals or increased allergenicity to people
- whether there are increases in CT levels in tissues other than leaves
- potential for increased concentrations of other flavonoid compounds in GMOs, such as those with oestrogenic or allelopathic activity
- potential for the genetic modification to provide improved resistance to pests, pathogens or abiotic stresses, or that could lead to increased spread and persistence of the GMOs, such as altered flowering or seed production
- potential yield penalty incurred as a result of the introduced gene or any other phenotypic effects when higher tannin concentrations are achieved
- potential gene flow from GM white clover via pollen transfer to non-GM white clover.

159. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.

160. Chapter 3, Section 4, discusses information that may be required for future release.

## Section 4 Risk evaluation

161. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

162. Factors used to determine which risks need treatment may include:

- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

163. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- the introduced gene and its expressed proteins and products are unlikely to be toxic or allergenic
- no GM plant material would enter human food or animal feed
- limits on the size and duration of the proposed release
- suitability of proposed controls to restrict the spread and persistence of the GM white clover and its genetic material
- the introduced gene and its expressed proteins and products are unlikely to increase weediness of the GM white clover
- GM white clover volunteers could be controlled by conventional weed control measures.

164. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM white clover plants into the environment are considered negligible. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

## Chapter 3 Risk management plan

### Section 1 Background

165. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator’s decision-making process and is given effect through licence conditions.

166. 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.

167. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors 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 must also be reported to the Regulator.

168. The licence is also 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 to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

### Section 2 Risk treatment measures for substantive risks

169. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM white clover. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed containment measures (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

### Section 3 General risk management

170. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions are imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in full in the licence.

#### 3.1 Licence conditions to limit and control the release

##### 3.1.1 *Consideration of limits and controls proposed by PTM*

171. Sections 2.1 and 2.2 of Chapter 1 provide details of the limits and controls proposed by PTM in their application. Many of these are discussed in the three risk scenarios considered for the proposed release in Chapter 2. The appropriateness of these limits and controls is considered further in the following sections.

172. The applicant proposed that the release would take place at up to four sites per year. Sites would be chosen from the LGAs listed in Chapter 1 (Section 2.1), across NSW, Vic., Qld and WA. The trial would run for five and a half years (from April 2021 until December 2026). At most sites, a single planting would be made in each year, and the white clover would be harvested as an annual crop. However, the applicant has also indicated that at some sites where conditions are suitable, two plantings – autumn and spring – may be made at the same site. Additionally, at some sites the white clover may be managed as a perennial crop and remain at the planting site for more than one year. The applicant has also indicated that more than one planting area may be established at a site. The maximum area planted across all sites would be one ha per year, with a maximum of 0.3 ha at any single site. The small size and short duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk Scenario 1).

173. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions included in the licence state that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions. These measures would limit the exposure of people to the GM white clover (Risk Scenario 1).

### **3.1.2 Consideration of proposed controls to manage exposure to the GMOs**

174. The applicant proposed not allowing the GMOs or GM products to be used for human food or animal feed. A licence condition states that GM plant material must not be used as food for humans or feed for animals. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenario 1).

175. The applicant has proposed that sites would be located on private property with controlled access, for example within a fenced paddock, near internal farm fence lines, but away from gates. Sites would not be near external boundaries with neighbours or crown land. The applicant has proposed two site setup options (see below for details). Where pollen traps and pollen buffer crops are proposed, they have indicated that a fence with lockable gates would be located around the outer pollen trap. A condition has been included in the licence requiring the trial sites to be fenced (more information is included in paragraphs 200 and 201). Standard conditions have been included in the licence that require that only authorised people are permitted to undertake any activity authorised by the licence and that all people dealing with the GMOs must be trained and informed of the relevant licence conditions. These measures are considered appropriate to limit the potential exposure of people to the GMOs (Risk Scenario 1) and would limit the opportunity for seed spread outside the trial area (Risk Scenario 2).

### **3.1.3 Consideration of proposed controls to manage pollen flow from the GMOs**

176. The potential for outcrossing of white clover has been discussed in Chapter 1 and in Risk Scenario 3. As noted there, outcrossing for this release of GM white clover is limited to other white clover only as there are no other sexually compatible species.

177. The applicant has proposed a number of containment measures for the GM white clover with two possible site setup options. For Option 1, they propose that the planting area would be enclosed in an insect-proof tent while the GMOs are flowering. This would be surrounded by a 10 m monitoring zone. They have indicated that the monitoring zone may be planted to pasture grasses. This would be surrounded by an isolation zone, extending to a distance of 100 m from the outer edge of the planting area, in which no other white clover could be intentionally planted. The licence has included this site set up (a tented planting area, monitoring zone and isolation zone) as one of the planting options.

178. Under Option 1, the applicant has proposed that any GM white clover found outside the insect-proof tent will be destroyed before flowering, thus limiting the availability of GMO pollen to be spread by pollinators outside the tent. The applicant has proposed that the monitoring zone would be inspected every 35 days (commencing 14 days before expected flowering of the GMOs and continuing until the site is cleaned) for volunteers or related species. If detected, these would be destroyed or prevented from flowering. The isolation zone is proposed to be inspected at the same frequency, commencing at the same time as for the monitoring zone and continuing until the GMOs finish flowering, to ensure no white clover

has been intentionally planted. If found, these would be destroyed before flowering or prevented from flowering, or the GMOs would be destroyed. Inspection of the monitoring and isolation zones is considered important for identifying plants with which the GM white clover could outcross. However, GM white clover could outcross with any white clover plants, whether these plants were intentionally planted or not. Therefore, to limit pollen flow, conditions requiring inspection for any white clover plants in both the monitoring zone and isolation zone, and the removal/destruction of these plants are included in the licence (Risk Scenarios 2 and 3). A number of climatic factors can influence the time from emergence to flowering (Chapter 1, paragraph 28). Although flowering times may be as long as nine weeks (FAR, 2005), and the applicant has indicated that five weeks is common, it may be as short as four weeks from emergence (FAR, 2009). Given the potentially broad range of locations proposed for the trial and as a consequence, the varying climatic conditions under which the trials may be planted, time from plant emergence to flowering may also vary markedly across trial sites and may be shorter than the proposed inspection frequency of 35 days. Therefore, an inspection frequency of at least once every 28 days is considered appropriate, as this would ensure that volunteers would not progress to flowering - and seed set - without being detected.

179. Under Option 1, the applicant proposed that the monitoring zone would be planted to grasses, however they have not indicated how these would be managed. In order to ensure detection of volunteers, a condition is included in the licence requiring that the monitoring zone be maintained in a manner to allow detection of volunteer white clover plants. This may be achieved by keeping the area free of vegetation or maintaining vegetation in a manner that allow detection of any white clover plants (Risk Scenarios 2 and 3).

180. For Option 2, the applicant proposed that the planting area would not be enclosed by a tent, but instead would be surrounded by a 1 m inner pollen trap planted to non-GM white clover. The inner pollen trap would be surrounded by a 35 m pollen buffer crop of lucerne, and then by a 1 m outer pollen trap crop of non-GM white clover. This would be surrounded by an isolation zone extending to a distance of 200 m from the outer edge of the inner pollen trap. The applicant proposed that no white clover or related species could be intentionally planted in the isolation zone while the GMOs are growing. A diagonal access track 2 m wide would be maintained through the pollen traps and the pollen buffer to allow vehicle access to the planting area. The applicant indicated that 1 m gap of cleared soil would be maintained between the planting area and the inner pollen trap and between the pollen traps and the pollen buffer to delineate these areas.

181. Under Option 2, the purpose of the pollen trap crops is to ensure that any viable pollen from the GMOs is deposited within these crops, not spread outside the site. The suitability of the pollen trap and pollen buffer crops for managing pollen flow from GM white clover has been discussed in detail in Risk Scenario 3 as well as in the RARMP for [DIR 089](#). The applicant has stated that the pollen trap crops are to be planted to non-GM white clover of Mainstay and Legacy cultivars, which flower between November and February, thus these would flower at the same time as the GM white clover. The applicant has indicated that they will plant a range of lucerne varieties in the pollen buffer crop in order to produce a spread of flowering times and to ensure that flowering occurs in the crop across the whole period of GM white clover flowering in the planting areas. This would provide a food source attractive to bees, thus providing an area in which any pollen gathered from GM white clover lines could be deposited.

182. The applicant has also proposed that the pollen traps and pollen buffer crop would be inspected every 35 days, starting 14 days prior to the expected commencement of flowering, to determine the proportion of flowering present in these areas. If less than 25% of these areas were flowering while the GMOs are flowering, two options are proposed. First, flowers would be removed from the GMOs prior to pollen formation if this is possible. If flowering of the GMOs is at a rate that means removal of flowers is not feasible, the applicant proposes to destroy the GM white clover in the planting area.

183. Pollen traps and pollen buffer crops are considered a suitable way to manage pollen flow from the GMOs in the planting area. However, assessment of the percentage of flowering may not, on its own, be the most appropriate means of ensuring the pollen traps and pollen buffers provide an appropriate buffer to pollen movement. Therefore, conditions are imposed requiring the use of pollen traps and a pollen buffer that are dense and vigorous, that are flowering at the same time as the GMOs and that form a continuous barrier of at least 37 m in all directions from the planting area to significantly reduce pollen

flow. Managing the pollen traps and pollen buffers in this manner is considered an effective means to reduce potential pollen flow. Conditions included in the licence also require inspection of the pollen traps and pollen buffer every 28 days and remedial actions if these requirements are not met. If the remedial action chosen is to prevent the GMOs from flowering, inspections of the planting area must be conducted every 14 days (Risk Scenario 1 and Risk Scenario 3). A 2 m access track through the pollen traps and pollen buffer is permitted under the conditions in order to provide vehicle access to the planting area(s), as this is considered a measure that would not impact the performance of the pollen traps and pollen buffer.

184. The available literature indicates that white clover is almost entirely outcrossing, due to self-incompatibility mechanisms in this species. Production of certified<sup>4</sup> seed for white clover are produced under various seed production schemes, which specify, among other conditions, isolation requirements to ensure seed purity. For crops of under 2 ha, the Seed Certification Scheme (Australia) (Seed Services Australia, 2013) requires a minimum of 200 m (Basic) or 100 m (Certified) from other white clover crops, as does the NZ Ministry for Primary Industries Seed Field Production Standards (Ministry for Primary Industries New Zealand, 2014). The Organisation for Economic Co-operation and Development (OECD) Seed Scheme requires the same distance for non-hybrid legumes, but requires 400 m for hybrid seed production (OECD, 2018). In Canada, isolation distances of 300 m, 150 m or 50 m are required for Foundation, Registered or Certified seed, respectively (CSGA, 2018), and in the USA a distance of 900 ft (274 m), 450 ft (137 m) or 165 ft (50 m) is required from any flowering white clover for Foundation, Registered or Certified seed, respectively (CCIA, 2019).

185. Although the spread of pollen from the site is limited by the use of either insect-proof tents or pollen traps and pollen buffer, isolation from other white clover plants will also manage pollen flow. Information regarding spread of pollen is, as mentioned, quite variable and dependent on a number of factors. The applicant has suggest isolation distances of 100 m for tented planting areas and 200 m for planting areas with pollen traps and pollen buffers. A previous releases ([DIR 089](#)) has required a 500 m isolation zone (in which no white clover plants may be present), and seed schemes, as detailed above, require distances of 50 – 400 m depending on the scheme and the grade of seed. Taking into account this information, the experience of the OGTR and the level of uncertainty about pollen (and seed) spread, a conservative approach is imposed for this release, with isolation distances of 100 m for tented planting areas and 500 m for non-tented planting areas. In addition, as white clover may persist over many seasons once established, the condition in the licence requires that the isolation zone must be inspected for any white clover, not only intentionally planted white clover as proposed by the applicant, while the GMOs are flowering.

186. The applicant has proposed inspection of the planting area and the inner and outer pollen traps for related species, and inspection of the pollen buffer for white clover. These inspections are proposed to be conducted every 35 days, commencing 14 days prior to expected flowering and continuing until the planting area and the pollen traps and pollen buffer are cleaned. As discussed above, they propose that the isolation zone would be inspected for any intentionally planted white clover or related species at the same frequency, commencing at the same time and until the GMOs finish flowering. However, as there are no other sexually compatible species for white clover, the licence only requires inspection for white clover in the lucerne pollen buffer. However, it should also be noted that licence conditions require that plants in the pollen trap crops must be treated as though they are GMOs, so if any GM white clover were present in the pollen trap crops, they would be destroyed with the pollen trap crop. Additionally, the licence conditions require that pollen trap and pollen buffer plants must be destroyed at the end of the flowering season for GM white clover, and replanted the following season. Consistent with the condition imposed for Option 1, a condition requiring inspection of the isolation zone for the presence of any white clover plants is imposed for Option 2 in the licence, rather than intentionally planted white clover as proposed by the applicant. Appropriate remedial actions for any white clover detected during these inspections are also included in the licence (Risk Scenarios 2 and 3).

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<sup>4</sup> Different jurisdictions use different names for seed classes, - for simplicity, the term 'certified' is used here to signify any class of seed which must be produced under a certification scheme.

187. If multiple planting areas were established at a site, the applicant has indicated that all planting areas would be contained within the same surrounding zones (pollen traps, pollen buffer and isolation zone). The licence contains diagrams of possible site setup options including multiple planting areas (see figure 1C in the licence). For sites with multiple planting including non-tented planting areas, any land within the inner edge of the inner pollen trap that is not a planting area, is defined as a monitoring zone. Conditions for management of monitoring zones to minimise opportunities for pollen spread (or seed spread) from any volunteers are discussed in paragraph 203. Additional conditions related to sites with multiple planting areas are therefore included in the licence (Risk Scenarios 2 and 3).

188. The applicant has also proposed that any non-GM white clover, as well as any non-GM lucerne, perennial ryegrass and birdsfoot trefoil grown as part of the trial would be treated as they were GMOs. In the case of non-GM white clover, this will prevent spread of hybrid seed (see following discussion in paragraph 191). In the case of lucerne, ryegrass and birdsfoot trefoil, although these species are not sexually compatible with white clover, pollinators visiting the GM white clover may subsequently visit these plants and deposit pollen. However, it is unlikely that any such pollen deposited in the pollen buffer lucerne crop would survive for an extended period. However, because the perennial ryegrass and birdsfoot trefoil are grown in the planting area, they could contain GM white clover material, whereas the lucerne is unlikely to. Thus, the licence contains a condition requiring that all non-GM plants grown in the planting area or in a pollen trap (but not the pollen buffer), must be treated as though they were GMOs and must be destroyed at the end of the trial by approved methods.

189. The applicant has proposed that beehives would be placed in the planting areas while plants in the planting area, pollen trap crops and pollen buffer are flowering and that once pollination is complete, the bees, honey and pollen in the beehive would be destroyed. These measures are considered sufficient to ensure that any pollen on bees from these hives that had visited the GM white clover plants and any honey potentially containing pollen from the GMOs would not be spread outside the trial site, thus limiting both pollen dissemination and exposure of beneficial organisms to pollen from the GMOs (Risk Scenarios 1 and 2). This would also limit the exposure of people to the GMO by ensuring that any honey is not available for human consumption (Risk Scenario 1). As such, a condition requiring destruction of bees, pollen and any honey in the beehive is included in the licence.

### **3.1.4 Consideration of proposed controls to manage persistence of the GMOs**

190. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or future plantings. In order to manage persistence of GMOs, it is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed. Licence conditions require that the planting area must be cleaned (which would destroy any surviving GM plants) within 14 days after harvest, and that harvested GM seed or plant material not required to conduct experiments or for future planting, must be destroyed as soon as practicable. This condition also requires cleaning of the monitoring zone, pollen traps and pollen buffer (where used) within 14 days of harvest of the planting area.

191. As noted in paragraph 188, the applicant proposes that any non-GM white clover, as well as non-GM lucerne, perennial ryegrass and birdsfoot trefoil as part of the trial would be treated as though they were GMOs. While the other species are not sexually compatible with white clover, non-GM white clover grown at the trial site may be cross-pollinated by GM white clover and bear hybrid seeds. It is therefore appropriate to require non-GM white clover to be treated in the same manner as GM white clover, to manage persistence of the GMOs, and this measure is included in the licence. There is also a condition in the licence that requires harvest of the GM white clover to be separate from any other crops.

192. Although there is strong observational information about white clover persistence, as discussed in Chapter 1 and in *'The Biology of Trifolium repens L. (white clover)'* (OGTR, 2020), there is little documented evidence about how it may persist in different Australian environments and how long seeds remain viable. This trial may be conducted at sites with a wide range of soil and climatic conditions, both of which may influence the persistence of any seed. The focus of this trial is to examine the field performance of white clover with increased condensed tannins in leaf tissue. The applicant has confirmed that seed set would only be permitted in GM white clover in areas where seed production from promising lines is undertaken in

insect-proof tents. In planting areas where seed production from promising lines is not being undertaken, they intend to prevent seed set and dispersal of seed into the soil. However, white clover seeds are very small so if any flowering material is present it is possible that viable seed is present and, as such, could be deposited in the soil at trial sites. Therefore, it is likely that some seed would still be produced at these sites and so the applicant has proposed a number of measures to manage any seed remaining at all trial sites.

193. Following harvest, the applicant has proposed that the site would be inspected for volunteers at least every 35 days for at least 24 months for tented sites (Option 1) and for at least 36 months for non-tented (Option 2) sites. Any volunteers found would be destroyed before flowering. They also propose that in the 12 months prior to request for site sign-off, the inspections would show that there were no white clover volunteers. If any volunteers were detected during that 12 month period, they would be removed and undergo molecular analysis, to determine whether they were GM white clover volunteers or non-GM white clover.

194. The time from planting to flowering may vary across locations and different varieties; under Australian conditions, white clover usually flowers within 9 weeks of the appearance of the first leaves, however as noted in paragraph 178, flower emergence may be as early as four weeks. The applicants have indicated that they expect viable seed approximately five weeks after first flowering. A postharvest inspection frequency of at least once every 28 days, together with a requirement that any volunteers must be destroyed prior to flowering is regarded as sufficient to prevent seed set on volunteers and this is included as a condition in the licence. To ensure that any seedbank is depleted before a site can be signed off, the licence imposes a 36 month (three year) postharvest inspection period for this trial, with a 12 month period immediately prior to the sign off request when no GM white clover volunteers are detected. The latter requirement would be satisfied if there are no volunteers at all present during this period, or if any volunteers detected were analysed and shown to be non-GM white clover plants.

195. The applicant has also proposed that, during the postharvest period, the planting area would receive shallow tillage in autumn and spring each year when conditions are conducive to germination of volunteers, as well as irrigation to encourage germination if soil moisture conditions were not sufficient for germination. As discussed in Chapter 1 and in Risk Scenario 2, white clover seeds may be designated as hard seed, which will persist in the soil for extended periods and will only germinate when conditions are suitable, or soft seed, which germinates readily. The proportion of hard and soft seeds is variable and largely dependent on the environmental conditions under which the seed ripens. Shallow tillage of the trial site during the postharvest period would promote suitable conditions for seed germination, provided there is adequate soil moisture. Promotion of germination of seeds in the seedbed then facilitates detection and destruction of volunteers, thus removing seed remaining at the site. Therefore, licence conditions are included requiring shallow tillage in autumn and spring during the postharvest period in all areas that have been cleaned following harvest, together with watering if soil moisture is not sufficient to provide conditions conducive to germination.

196. The applicant has proposed that postharvest monitoring would include the planting area and any areas that have been cleaned. Conditions in the licence require that for Option 1, with tented planting areas, the planting area and the monitoring zone must be cleaned within 14 days of harvest. For Option 2, the planting area, pollen traps and pollen buffer must be cleaned with 14 days of harvest. Any areas outside the planting area where the GMOs may have been dispersed in the course of dealings under this licence, or any equipment used in connection with GMOs, must be cleaned as soon as practicable and before use for any other purpose. These conditions are considered suitable to manage risks associated with persistence of seeds at the trial site and have been included in the licence.

197. The applicant has proposed that GM white clover would be destroyed using one or more of the following methods: destructive analysis, uprooting, root cutting and shredding/mulching, tillage, herbicide application, burning/incineration, autoclaving, or burial to a depth of at least 1 m. All of these methods are considered effective in destroying one or more life stages of the GM white clover, so are included in the licence. The applicant also proposed that the burial site would be in a pit located in the pollen buffer area and that only vegetative material would be destroyed by burial. However, as white clover material may be disposed of at different times through the growing season the applicant does not propose to cover the

plant material to a depth of 1 m of soil until the pit has reached its capacity or until the site is cleaned. They have proposed this material would be compacted and covered with soil immediately, and the burial pit would be covered with a solid cover, able to carry the weight of a person to ensure it would not be damaged if accidentally stepped on. They have proposed monitoring of the burial site during postharvest monitoring periods and until the site is signed off, for the presence of volunteers or for any disturbance. They have not proposed inspections of the site while it is in use.

198. This method of destruction has been used in previous releases (DIR 089). The use of a solid cover while the burial pit remains in use would prevent access to the pit other than for intentional addition of plant material to the pit. Additionally, in order to promote decomposition of plant material in the burial pit, any plant material must be thoroughly wet at the time of addition to the burial pit in addition to compaction and covering with soil. Conditions have been included in the licence requiring that all plant material destroyed by burial is treated in this manner and that the pit is covered to a depth of 1 m with soil once the final addition of plant material has been made to the pit. The applicant has stated that if plants have reached flowering and seed set, other methods of destruction such as herbicide treatment would be used prior to burial of plant material. A condition in the licence therefore states that only vegetative material may be destroyed by burial and that any plant material which has reached flowering must only be buried after destruction by a method which is suitable for destruction of seeds. In addition, as white clover seeds are very small, it is possible that some seed may inadvertently be included in material buried in the pit, and so a condition is included requiring that the burial pit should be inspected at least once every 28 days, beginning from when the first material is added to the pit. This inspection for any volunteer white clover or any disturbance, is in addition to the post-cleaning inspections imposed for the burial pit.

### **3.1.5 Consideration of proposed controls to manage dispersal of the GMOs**

199. The applicant has proposed that all equipment, tools, shoes and other clothing would be inspected for GM seeds or stoloniferous material and cleaned before using it for any other purpose. Such measures are considered appropriate to ensure seed or viable vegetative material is not unintentionally dispersed by equipment. The licence contains a condition that requires any equipment used in connection with the GMOs must be cleaned as soon as practicable after use and before use for any other purpose. Requirements for cleaning of equipment associated with transport and storage of the GMOs would need to be conducted according to the requirements set out in the Regulators [Guidelines for the Transport, Storage and Disposal of GMOs](#).

200. The applicant has stated that the trial sites, located on private land, would be within fenced paddocks and away from boundary fences. The applicant has also proposed that additional fencing would be erected to exclude stock at the trial sites planted using Option 2. They propose that the fence would be positioned around the edge of the outer pollen trap and would be inspected for damage while stock are present on the farm during the trial or during postharvest monitoring.

201. Spread of viable seed by livestock has been documented (see Chapter 1). Although it is possible that other land-based animals could consume and spread white clover seed, this has not been documented. It is unlikely that the trial planting areas would be large enough to provide an attractive food source to larger wild mammals. The proposal to locate trial sites away from external fences and crown land would also reduce the likelihood of large populations of some wild mammals close to the sites, thus minimising the likelihood of opportunistic grazing and spread of either seed or vegetative propagules (Risk Scenario 2). There is also no indication that the GM white clover would be more toxic to mammals than non-GM white clover if it were consumed (Risk Scenario 1). Additionally, populations of rabbits are likely to be present in farmland, so measures to prevent access by small mammals such as rabbits are warranted. For Option 1, the insect-proof tent would restrict access of smaller land-based animals from the planting area. If livestock were present on the properties, they could damage the tents and access the planting areas. Additionally, tents are only required during flowering, so at other times land-based animals could access the planting area. Therefore, the presence of fences is an effective means of excluding most large animals, including livestock, for both Option 1 and Option 2 planting layouts. Likewise, a fence around the planting area is considered suitable to prevent access by small mammals. Therefore, conditions have been included in the

licence requiring the use of fences around sites, whether tented or not, to prevent access by livestock and around planting areas to prevent access by small mammals.

202. The potential for seed spread by birds has been considered in Chapter 1, Section 3. The applicant has proposed that where seed production is required in this trial, seed production would be conducted in insect-proof tents (which would prevent access by birds) and that in other areas no viable seed would be allowed to set. Even if not all viable seed was able to be removed, it is likely that only very small amounts of seed would be available at the trial site for spread by birds or animals. Considering the small seed size and limited availability of seed in the planting area, as well as the relative abundance of non-GM white clover and lucerne in the surrounding pollen trap crops and pollen buffer, any GM white clover seed is unlikely to be an attractive source of food for birds. Thus, it is considered unnecessary to impose additional measures to control access of birds to the planting area (Risk Scenario 2). Additionally, there is no indication that the GM white clover would be more toxic to birds than non-GM white clover, thus no specific restriction of access by birds is considered necessary in light of discussion in Risk Scenario 1.

203. Recent licences for grain crops include conditions requiring the use of measures to control rodents in the planting area while GMOs are being grown and until the planting area has been cleaned. These measures include, but are not limited to, the use of rodent baiting or trapping. This condition is included in the licence to minimise the risks associated with rodent activity. In addition, these licences included a condition which requires that the monitoring zone must be maintained in a manner that allows detection of white clover volunteers and related species while the GMOs are being grown and until the area has been cleaned. Such measures not only provide conditions suitable for detection of volunteers, but also provide conditions that do not attract or harbour rodents (Risk Scenario 2). A condition requiring management of the monitoring zone in this manner has been included in this licence.

204. The applicant has proposed a distance of 50 m from the trial site (from the outer edge of the outer pollen trap in Option 2) to any natural waterway and obtaining confirmation from landholders that any possible sites are not prone to flooding. These conditions would reduce the likelihood of any plant material, including seeds or viable vegetative material, being removed from the planting area by water (Risk Scenario 2) and have been included in the licence conditions. A condition has also been imposed requiring immediate notification of any extreme weather event affecting the trial site during the release to allow assessment and management of any risks.

205. The applicant has indicated that seed and stolon material from this trial may be used for planting in later seasons of the trial. No information has been provided regarding the handling of seed immediately following harvest, although the applicant proposes that seed or stolon material may be transported and used for experimental analysis in PC2 laboratories under appropriate Notifiable Low Risk Dealings (NLRDs) authorisation. Any seed or vegetative material that would not be required for analysis or further planting would be destroyed by the methods listed in the licence. Licence conditions require that if seed harvested from the GMOs is threshed other than in accordance with NLRD requirements, it must be threshed separately from any other crop, and threshing must take place on a planting area or in a facility approved in writing by the Regulator.

206. The applicant has proposed that any GM plant material would be transported to approved facilities for analysis or destruction according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. If seed required storage onsite before transport, it must be stored according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. Any material remaining after analysis must be stored in an approved facility for subsequent use, or destroyed by autoclaving or another method approved by the Regulator. These are standard conditions in the licence relating to the handling of GMOs, to minimise exposure of people and other desirable organisms to the GMOs (Risk Scenario 1), dispersal into the environment and gene flow (Risk Scenario 2 and 3).

### **3.1.6 Summary of licence conditions to be implemented to limit and control the release**

207. A number of licence conditions have been included to limit and control the release, based on the above considerations. These include requirements to:

- limit the duration of the release to a maximum of five and a half years, until December 2026
- limit the release to four locations per year, with a maximum of 0.3 ha per location
- limit the release to a maximum total area of 1 ha per year
- locate trial sites at least 50 m from any natural waterways
- where planting areas are within an insect-proof tent:
  - surround the planting area with a monitoring zone of at least 10 m, maintained in a manner that allows detection of volunteers and prevention of volunteers flowering; and
  - surround the monitoring zone with a 90 m isolation zone in which no white clover may be grown
- where planting areas are not within an insect-proof tent:
  - surround the planting area with an inner pollen trap of at least 1 m, planted to non-GM white clover; and
  - surround the inner pollen trap with a pollen buffer of at least 35 m, planted to lucerne; and
  - surround the pollen buffer with an outer pollen trap of at least 1 m, planted to non-GM white clover; and
  - surround the outer pollen trap with an insolation zone of 464 m, in which no white clover may be grown
- surround sites with a fence suitable to exclude livestock
- implement measures including rodent baits and/or traps to control rodents within the planting area
- harvest the GM white clover separately from other crops
- clean the areas after use including the planting area, pollen traps and pollen buffer (where used) and any area in which seed has been dispersed
- clean any equipment used before use for any other purpose
- apply measures to promote the germination of any white clover seeds that may be present in the soil after harvest, including irrigation and shallow tillage
- monitor for at least 36 months after harvest and destroy any white clover plants that may grow and until no GM volunteers have been detected for a continuous 12 month period prior to the end of monitoring
- monitor any site used to bury seed for at least 24 months to detect any disturbance or volunteers
- destroy all GMOs not required for further analysis or future trials
- transport and store the GMOs in accordance with the Regulator's guidelines
- not allow the GM plant material to be used for human food or animal feed.

### 3.2 Other risk management considerations

208. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

#### 3.2.1 Applicant suitability

209. 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, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- 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 capacity of the applicant to meet the conditions of the licence.

210. The licence conditions include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

211. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

### **3.2.2 Contingency plan**

212. The licence requires that PTM submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM white clover outside permitted areas.

213. Before planting the GMOs, PTM is required to provide the Regulator with a method to reliably and uniquely detect the GMOs or the presence of the genetic modifications in a recipient organism.

### **3.2.3 Identification of the persons or classes of persons covered by the licence**

214. 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. Prior to growing the GMOs, PTM is required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

### **3.2.4 Reporting requirements**

215. The licence requires 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.

216. A number of written notices are also required under the licence regarding dealings with the GMOs, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- details of site management choice – tented or not tented
- expected and actual dates of planting
- details of areas planted to the GMOs
- expected dates of flowering
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

### **3.2.5 Monitoring for compliance**

217. 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.

218. 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.

219. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides 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 4 **Issues to be addressed for future releases**

220. Additional information has been identified that may be required to assess an application for a commercial release of these GM white clover lines, or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM white clover lines, particularly with respect to potential for increased toxicity and allergenicity, allelopathy or oestrogenic effects
- additional phenotypic characterisation of the GM white clover lines, particularly with respect to increased insect or disease tolerance, abiotic stress tolerance, changes in flowering and seed production or other characteristics that may contribute to weediness
- additional phenotypic characterisation of the GM white clover lines, particularly with respect to concentrations of condensed tannins in leaves and other tissues and any yield penalty incurred as a result of expression of the inserted genes
- additional data on pollen flow and resulting gene transfer to non-GM white clover.

## Section 5 **Conclusions of the RARMP**

221. The RARMP concludes that the proposed limited and controlled release of GM white clover poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

222. Conditions are imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

## References

- Abeynayake, S.W., Panter, S., Chapman, R., Webster, T., Rochfort, S., Mouradov, A., and Spangenberg, G. (2012). Biosynthesis of proanthocyanidins in white clover flowers: Cross talk within the flavonoid pathway. *Plant Physiology* *158*, 666-678.
- Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., and Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. *Journal of Plant Physiology* *168*, 204-212.
- Agati, G., Stefano, G., Biricolti, S., and Tattini, M. (2009). Mesophyll distribution of 'antioxidant' flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance. *Annals of Botany* *104*, 853-861.
- Agriculture Victoria (2020a). Salinity indicator plants - A guide to spotting soil salting. (State of Victoria (Agriculture Victoria)) Accessed: 26 June 2020.
- Agriculture Victoria (2020b). White Clover. (State of Victoria (Agriculture Victoria)) Accessed: 26 June 2020.
- Ambawat, S., Sharma, P., Yadav, N.R., and Yadav, R.C. (2013). MYB transcription factor genes as regulators for plant responses: an overview. *Physiology and Molecular Biology of Plants* *19*, 307-321.
- Anderson, J.E., Michno, J.M., Kono, T.J., Stec, A.O., Campbell, B.W., Curtin, S.J., and Stupar, R.M. (2016). Genomic variation and DNA repair associated with soybean transgenesis: a comparison to cultivars and mutagenized plants. *BMC Biotechnology* *16*, 41.
- Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical Reviews in Toxicology* *36*, 219-251.
- Ayres, J., and Lane, L. (2008). Trophy white clover – a new cultivar for dryland pastures. Report No. Primefact 821. (NSW Department of Primary Industries).
- Ayres, J.F., Davies, H.L., Farquharson, R.J., and Murison, R.D. (2000). The contribution of pasture research for animal production from legume-based pastures in temperate Australia. *Asian Australasian Journal of Animal Sciences* *13 (suppl. 2000B)*, 1-4.
- Ballhorn, D.J., and Elias, J.D. (2014). Salinity-mediated cyanogenesis in white clover (*Trifolium repens*) affects trophic interactions. *Annals of Botany* *114*, 357-366.
- Barbehenn, R.V., and Constabel, P.C. (2011). Tannins in plant–herbivore interactions. *Phytochemistry* *72*, 1551-1565.
- Berg, B.P., Majak, W., McAllister, T.A., Hall, J.W., McCartney, D., Coulman, B.E., Goplen, B.P., *et al.* (2000). Bloat in cattle grazing alfalfa cultivars selected for a low initial rate of digestion: A review. *Canadian Journal of Plant Science* *80*, 493-502.
- Berg, G. (1993). Invertebrate pests of white clover. In *White Clover* (Melbourne: Dairy Research and Development Corporation), pp. 91-94.
- Berkley, S.F., Hightower, A.W., Beier, R.C., Fleming, D.W., Brokopp, C.D., Ivie, G.W., and Broome, C.V. (1986). Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery. *Annals of Internal Medicine* *105*, 351-355.
- Burggraaf, V.T., Woodward, S.L., Woodfield, Thom, E.R., Waghorn, G.C., and Kemp, P.D. (2006). Morphology and agronomic performance of white clover with increased flowering and condensed tannin concentration. *New Zealand Journal of Agricultural Research* *49* 147-155.
- Campbell, M.H. (1966). Theft by harvesting ants of pasture seed broadcast on unploughed land. *Australian Journal of Experimental Agricultural and Animal Husbandry* *6*, 334-338.

- Carlsen, S.C.K., and Fomsgaard, I.S. (2008). Biologically active secondary metabolites in white clover (*Trifolium repens* L.) – a review focusing on contents in the plant, plant-pest interactions and transformation. *Chemoecology* 18, 129-170.
- Carlsen, S.C.K., Pedersen, H.A., Spliid, N.H., and Fomsgaard, I.S. (2012). Fate in soil of flavonoids released from white clover (*Trifolium repens* L.). *Applied and Environmental Soil Science* 2012, 1-10.
- CCIA (2019). White Clover Crop Standards. (Davis, California: California Crop Improvement Association).
- Clarke, R. (1999). Diseases of white clover - 1: virus diseases. Report No. Agriculture Notes AG0731. (Agriculture Victoria).
- Cork, S.J., Hume, I.D., and Dawson, T.J. (1983). Digestion and metabolism of a natural foliar diet (*Eucalyptus punctata*) by an arboreal marsupial, the koala (*Phascolarctos cinereus*). *Journal of Comparative Physiology* 153, 181-190.
- CSGA (2018). Canadian Regulations and Procedures for Pedigreed Seed Crop Production. (Canadian Seed Growers' Association).
- D'Hondt, B., Brys, R., and Hoffmann, M. (2010). The incidence, field performance and heritability of non-dormant seeds in white clover (*Trifolium repens* L.). *Seed Science Research* 20, 169-177.
- De Lucas, J.A., Forster, J.W., Smith, K.F., and Spangenberg, G.C. (2012). Assessment of gene flow in white clover (*Trifolium repens* L.) under field conditions in Australia using phenotypic and genetic markers. *Crop and Pasture Science* 63, 155-163.
- DeGabriel, J.L., Moore, B.D., Foley, W.J., and Johnson, C.N. (2009). The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology* 90, 711-719.
- Donald, G.E. (2012). Analysis of Feed-base Audit. (Meat & Livestock Australia Limited).
- Drew, E., Herridge, D., Ballard, R., O'Hara, G., Dealer, R., Denton, M., Yates, R., *et al.* (2014). Inoculating legumes: A practical guide. (Canberra: Grains Research & Development Corporation).
- DSE Victoria (2009). Advisory list of environmental weeds of the Ranges bioregions of Victoria. (Melbourne: Victorian Government Department of Sustainability and Environment).
- Du, H., Zhang, L., Liu, L., X.F., T., Yang, W.J., Wu, Y.M., Huang, Y.B., *et al.* (2009). Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry (Moscow)* 74, 1-11.
- Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., and Lepiniec, L. (2010). MYB transcription factors in *Arabidopsis*. *Trends in Plant Science* 15, 573-581.
- Ellison, N.W., Liston, A., Steiner, J.J., Williams, W.M., and Taylor, N.L. (2006). Molecular phylogenetics of the clover genus (*Trifolium*-Leguminosae). *Molecular Phylogenetics and Evolution* 39, 688-705.
- FAR (2005). White Clover - Understanding growth & development. Report No. 44. (Lincoln, New Zealand: Foundation for Arable Research).
- FAR (2009). White clover. A growers guide. Report No. Issue 3. (Lincoln, New Zealand: Foundation for Arable Farming).
- Felsot, A.S. (2000). Insecticidal genes part 2: Human health hoopla. *Agrichemical & Environmental News* 168, 1-7.
- Foo, L.Y., Lu, Y., Molan, A.L., Woodfield, D.R., and McNabb, W.C. (2000). The phenols and prodelphinidins of white clover flowers. *Phytochemistry* 54, 539-548.
- Frame, J. (2003). *Trifolium repens* L. (Food and Agriculture Organization of the United Nations (FAO)).
- Garrett, R.G. (1991). Impact of viruses on pasture legume productivity. Paper presented at: Department of Agriculture Victoria White Clover Conference.

- Garrett, R.G., and Chu, P.W.G. (1997). White clover expressing the coat protein of alfalfa mosaic virus: field trial issues. Paper presented at: Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations (Canberra: Bureau of Resource Sciences).
- Godfree, R.C., Chu, P.W.G., and Woods, M.J. (2004). White clover (*Trifolium repens*) and associated viruses in the subalpine region of south-eastern Australia: implications for GMO risk assessment. *Australian Journal of Botany* 52, 321-331.
- Goodman, R.E. (2008). Performing IgE serum testing due to bioinformatics matches in the allergenicity assessment of GM crops. *Food and Chemical Toxicology* 46 Suppl 10, S24-S34.
- Groves, R.H., Hosking, J.R., Batianoff, G.N., Cooke, D.A., Cowie, I.D., Johnson, R.W., Keighery, G.J., *et al.* (2003). Weed categories for natural and agricultural ecosystem management (Bureau of Rural Sciences, Canberra).
- Hancock, K., Collette, V., Chapman, E., Hanson, K., Temple, S., Moraga, R., and Caradus, J. (2014). Progress towards developing bloat-safe legumes for the farming industry. *Journal of Crop and Pasture Science* 65, 1107-1113.
- Hancock, K.R., Collette, V., Fraser, K., Greig, M., Xue, H., Richardson, K., Jones, C., *et al.* (2012). Expression of the R2R3-MYB transcription factor *TaMYB14* from *Trifolium arvense* activates proanthocyanidin biosynthesis in the legumes *Trifolium repens* and *Medicago sativa*. *Plant Physiology* 159, 1204-1220.
- Harris, S.L. (1998). White Clover - How Much and How to Get it. Paper presented at: 50th Ruakura Farmers' Conference (Dairying Research Corporation).
- Harris, W. (1987). Population dynamics and competition. In *White Clover*, M.J. Baker, and W.M. Williams, eds. (Wallingford: CAB International), pp. 203-278.
- Hart, A.L. (1987). Physiology. In *White Clover*, M.J. Baker, and W.M. Williams, eds. (Wallingford: CAB International), pp. 153-183.
- Herbiguide (2014). *White clover Trifolium repens L.*
- Hughes, M.A. (1991). The cyanogenic polymorphism in *Trifolium repens L.* (white clover). *Heredity* 66, 105-115.
- Hyde, E.O.C. (1954). The function of the hilum in some *Papilionaceae* in relation to the ripening of the seed and the permeability of the testa. *Annals of Botany* 18, 241-250.
- Jahufer, M.Z.Z., Cooper, M., Ayres, J.F., and Bray, R.A. (2002). Identification of research to improve the efficiency of breeding strategies for white clover in Australia - a review. *Australian Journal of Agricultural Research* 53, 239-257.
- Jahufer, Z., Rogers, H., and Rogers, M. (2001). *White clover*. (Department of Natural Resources and Environment, Victoria).
- Jones, R.A.C. (2013). Virus diseases of perennial pasture legumes in Australia: incidences, losses, epidemiology, and management *Crop and Pasture Science* 64, 199-215.
- Jones, R.M. (1982). White clover (*Trifolium repens*) in subtropical south-east Queensland. I. Some effects of site, season and management practices on the population dynamics of white clover. *Tropical Grasslands* 16, 118-127.
- Jovanovic, M., Mimica-Dukic, N., Poljacki, M., and Boza, P. (2003). Erythema multiforme due to contact with weeds: a recurrence after patch testing. *Contact Dermatitis* 48, 17-25.
- Kalla, R., Chu, P., and Spangenberg, G. (2001). Molecular breeding of forage legumes for virus resistance. Paper presented at: 2nd International Symposium, Molecular Breeding of Forage Crops (Lorne and Hamilton, Victoria, Australia: Kluwer Academic Publishers).
- Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* 7, 123-149.

- Keese, P.K., Robold, A.V., Myers, R.C., Weisman, S., and Smith, J. (2014). Applying a weed risk assessment approach to GM crops. *Transgenic Research* 23, 957-969.
- Kooyers, N.J., Hartman Bakken, B., Ungerer, M.C., and Olsen, K.M. (2018). Freeze-induced cyanide toxicity does not maintain the cyanogenesis polymorphism in white clover (*Trifolium repens*). *American Journal of Botany* 105, 1224-1231.
- Krach, K.E. (1959). Excretion of undigested seeds of clover, grasses and weeds of birds and effect of passage through stomach and intestine on their viability. *Zeitschrift für Acker- und Pflanzenbau* 107, 405-434.
- Ladics, G.S., Bartholomaeus, A., Bregitzer, P., Doerr, N.G., Gray, A., Holzhauser, T., Jordan, M., *et al.* (2015). Genetic basis and detection of unintended effects in genetically modified crop plants. *Transgenic Research* 24, 587-603.
- Lane, L.A., Ayres, J.F., and Lovett, J.V. (2000). The pastoral significance, adaptive characteristics, and grazing value of white clover (*Trifolium repens* L.) in dryland environments in Australia: A review. *Australian Journal of Experimental Agriculture* 40, 1033-1046.
- Liu, J., Osbourn, A., and Ma, P. (2015). MYB transcription factors as regulators of phenylpropanoid metabolism in plants. *Molecular Plant* 8, 689-708.
- Macfarlane, M.J., Scott, D., and Jarvis, P. (1982a). Allelopathic effects of white clover 1. Germination and chemical bioassay. *New Zealand Journal of Agricultural Research* 25, 503-510.
- Macfarlane, M.J., Scott, D., and Jarvis, P. (1982b). Allelopathic effects of white clover 2. Field investigations in tussock grasslands. *New Zealand Journal of Agricultural Research* 25, 511-518.
- Marsh, K.J., Foley, W.J., Cowling, A., and Wallis, I.R. (2003). Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *Journal of Comparative Physiology B* 173, 69-78.
- McKirby, S.J., and Jones, R.A.C. (1995). Occurrence of alfalfa mosaic and subterranean clover red leaf viruses in legume pastures in Western Australia. *Australian Journal of Agricultural Research* 46, 763-774.
- McRill, M., and Sagar, G.R. (1973). Earthworms and seeds. *Nature* 244, 482.
- Ministry for Primary Industries New Zealand (2014). MPI Seed Varietal Certification Programme/ Appendix 1: Seed field production standards. (Ministry for Primary Industries New Zealand).
- Morinaga, T. (1926). Germination of seeds under water. *American Journal of Botany* 13, 126-140.
- Norton, M.R., and Johnstone, G.R. (1998). Occurrence of alfalfa mosaic, clover yellow vein, subterranean clover red leaf, and white clover mosaic viruses in white clover throughout Australia. *Australian Journal of Agricultural Research* 49, 723-728.
- NSW DPI (2012). Pasture varieties used in NSW 2012 - 2013. (New South Wales Department of Primary Industries).
- NSW DPI (2020). White clover. Accessed: 18 June 2020.
- OECD (2018). OECD seed schemes 2018: OECD schemes for the varietal certification or the control of seed moving in international trade. (Organisation for Economic Co-operation and Development).
- OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra, Australia: Office of the Gene Technology Regulator).
- OGTR (2020). The biology of white clover (*Trifolium repens* L.). (Canberra, Australia: Office of the Gene Technology regulator.).
- Olsen, K.M., Hsu, S.-C., and Small, L.L. (2008). Evidence on the molecular basis of the Ac/ac adaptive cyanogenesis polymorphism in white clover (*Trifolium repens* L.). *Genetics* 179, 517-526.
- Olsen, K.M., Kooyers, N.J., and Small, L.L. (2013). Recurrent gene deletions and the evolution of adaptive cyanogenesis polymorphisms in white clover (*Trifolium repens* L.). *Molecular Ecology* 22, 724-738.

- Olsen, K.M., Sutherland, B.L., and Small, L.L. (2007). Molecular evolution of the Li/li chemical defence polymorphism in white clover (*Trifolium repens* L.). *Molecular Ecology* 16, 4180-4193.
- Pali-Schöll, I., De Lucia, M., Jackson, H., Janda, J., Mueller, R.S., and Jensen-Jarolim, E. (2017). Comparing immediate-type food allergy in humans and companion animals—revealing unmet needs. *Allergy* 72, 1643-1656.
- Randall, R.P. (2017). *A Global Compendium of Weeds*, 3rd edn (Perth, Western Australia).
- Reed, K.F.M. (2008). *White Clover*. (AWI, GRDC, MLA, RIRDC, Dairy Australia.).
- Reed, S.M., Bayly, W.M., and Sellon, D.C. (2010). *Equine Internal Medicine*, 3rd edn (St. Louis: Saunders, An Imprint of Elsevier).
- Richards, A.J., and Fletcher, A. (2002). The effects of altitude, aspect, grazing and time on the proportion of cyanogenics in neighbouring populations of *Trifolium repens* L. (white clover). *Heredity* 88, 432-436.
- Riffkin, P., Moerkerk, M., Kearney, G., Jahufer, Z., and Argall, R. (2005). *Effective weed control for the Australian White Clover Seed Industry*. Report No. 05/089. (Canberra: Rural Industries Research and Development Corporation).
- Roldan, M.B., Cousins, G., Fraser, K., Hancock, K.R., Collette, V., Demmer, J., Woodfield, D.R., *et al.* (2020). Elevation of condensed tannins in the leaves of *Ta-MYB14-1* white clover (*Trifolium repens* L.) outcrossed with high anthocyanin lines. *Journal of Agricultural and Food Chemistry* 68, 2927-2939.
- Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C., *et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research* 24, 1-17.
- Seed Services Australia (2013). *Seed certification manual*. (Urrbrae, Australia: Division of Primary Industries & Resources South Australia (PIRSA)).
- Seed Technology and Marketing Pty Ltd (2007a). *White clover - haifa*. (Seedmark).
- Seed Technology and Marketing Pty Ltd (2007b). *White clover - quest*. (Seedmark).
- Seed Technology and Marketing Pty Ltd (2007c). *White clover - waverley*. (Seedmark).
- Seligman, P.J., Mathias, C.G.T., O'Malley, M.A., Beier, R.C., Fehrs, L.J., Serrill, W.S., and Halperin, W.E. (1987). Phytophotodermatitis from celery among grocery store workers. *Archives of Dermatology* 123, 1478-1482.
- Smoliak, S., Ditterline, R.L., Scheetz, J.D., Holzworth, L.K., Sims, J.R., Wiesner, L.E., Baldridge, D.E., *et al.* (2008). *White Clover (Trifolium repens)*. (Montana State University).
- Society of Toxicology (2003). *Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology*. *Toxicological Sciences* 71, 2-8.
- Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., *et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. *Plant Physiology* 161, 1587-1594.
- Suckling, F.E.T. (1952). Dissemination of white clover (*Trifolium repens*) by sheep. *New Zealand Journal of Science and Technology* A33, 64-77.
- Thomas, R.G. (1987). Reproductive development. In *White Clover*, M.J. Baker, and W.M. Williams, eds. (Wallingford: CAB International), pp. 63-123.
- Toole, E.H., and Brown, E. (1946). Final results of the Duvel buried seed experiment. *Journal of Agricultural Research* 72, 201-210.
- Tracey, J., Bomford, M., Hart, Q., Saunders, G., and Sinclair, R. (2007). *Managing bird damage to fruit and other horticultural crops*. (Canberra: Bureau of Rural Sciences, Australian Government).

- Twigg, L.E., Lowe, T.J., Taylor, C.M., Calver, M.C., Martin, G.R., Stevenson, C., and How, R. (2009). The potential of seed-eating birds to spread viable seeds of weeds and other undesirable plants. *Austral Ecology* *34*, 805-820.
- van Cleef, F., and Dubeux, J. (2019). Condensed tannins in forage legumes. Report No. SS-AGR-440. (Gainesville, Florida: University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS)).
- Vickery, P.J., Wheeler, J.L., and Mulcahy, C. (1987). Factors affecting the hydrogen cyanide potential of white clover (*Trifolium repens* L.). *Australian Journal of Agricultural Research* *38*, 1053-1059.
- Virtue, J.G. (2008). SA weed risk management guide. (Adelaide: Government of South Australia: Department of Water, Land and Biodiversity Conservation).
- Voisey, C.R., White, D.W.R., Dudas, B., Appleby, R.D., Ealing, P.M., and Scott, A.G. (1994). *Agrobacterium*-mediated transformation of white clover using direct shoot organogenesis. *Plant Cell Reports* *13* 309-314.
- Weston, L.A., and Mathesius, U. (2013). Flavonoids: Their structure, biosynthesis and role in the rhizosphere, including allelopathy. *Journal of Chemical Ecology* *39*, 283-297.
- White, M., Cheal, D., Carr, G.W., Adair, R., Blood, K., and Meagher, D. (2018). Advisory list of environmental weeds in Victoria. Report No. 287. (Heidelberg, Victoria: Department of Environment, Land, Water and Planning (Victoria)).
- Williams, W.M. (2014). *Trifolium* interspecific hybridisation: widening the white clover gene pool. *Crop & Pasture Science* *65*, 1091-1106.
- Williams, W.M., Eastom, H.S., and Jones, C.S. (2007). Future option and targets for pasture plant breeding in New Zealand. *New Zealand Journal of Agricultural Research* *50*, 223-248.
- Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology* *126*, 485-493.
- Woodfield, D.R., Clifford, P.T.P., Baird, I.J., and Cousins, G.R. (1995). Gene flow and estimated isolation requirements for transgenic white clover. Paper presented at: 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms (The University of California, Division of Agriculture and Natural Resources).
- Woodfield, D.R., Roldan, M.B., Voisey, C.R., Cousins, G.R., and Caradus, J.R. (2019). Improving environmental benefits of white clover through condensed tannin expression. *Journal of New Zealand Grasslands* *81*, 195-202.
- Yamada, T., and Kawaguchi, T. (1971). Dissemination of pasture plants by livestock 1. Recovery and viability of some pasture plant seeds passed through digestive tract of goats. *Journal of Japanese Society of Grassland Science* *17*, 36-47.
- Yamada, T., and Kawaguchi, T. (1972). Dissemination of pasture plants by livestock 2. Recovery, viability and emergence of some pasture plant seeds passed through the digestive tract of the dairy cow. *Journal of Japanese Society of Grassland Science* *18*, 8-15.
- Zahid, M.I., Gurr, G.M., Nikandrow, A., Hodda, M., Fulkerson, W.J., and Nicol, H.I. (2001). Survey of fungi and nematodes associated with root and stolon diseases of white clover in the subtropical dairy region of Australia. *Australian Journal of Experimental Agriculture* *41*, 1133-1142.

## Appendix A Summary of submissions from prescribed experts, agencies and authorities

Advice received by the Regulator from prescribed experts, agencies and authorities<sup>5</sup> on the consultation RARMP is summarised below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator’s decision to issue the licence.

Submission	Issues raised	Comment
1	<p>Does not support the proposed trial of GM white clover.</p> <p>Notes community concern for negative environmental impacts including risk of spread resulting in modification of indigenous flora.</p> <p>In response to petitions, Council has passed a motion the Shire does not support the use of GM crops which:</p> <ul style="list-style-type: none"> <li>- acknowledges that the Shire has an interest in genetic modification, but that has no jurisdiction over GM crop regulation, lacks scientific knowledge available to decision makers to reach conclusions on harm to human health or the environment;</li> <li>- acknowledges significant opposition to and fear of GM crops and foods derived from them, within the Shire;</li> <li>- notes the belief that negative perceptions concerning GMOs have potential to harm marketing of organic and other local produce.</li> </ul>	<p>Noted.</p> <p>The Gene Technology Regulator (the Regulator) is required to assess GMO applications in accordance with the Gene Technology Act 2000, the object of which is to protect the health and safety of people and the environment. This is a limited and controlled release with limited duration and size. The release has licence conditions to manage risks of spread of the GM white clover and to ensure that it does not persist at trial sites following completion of the trial. The GM white clover is unlikely to be more competitive than non-GM white clover, therefore is not expected to have any greater impact on indigenous plant communities than non-GM white clover (Chapter 2, Risk scenario 2; Chapter 3). Additionally, white clover is not sexually compatible with any other plant species and therefore the modification will not transfer to indigenous flora.</p> <p>Noted.</p> <p>No food or feed use is permitted for this trial. The Regulator’s considerations are limited to risks to the health and safety of people and the environment. Social issues, such as fear of GM crops and derived food, cannot be considered by the Regulator when making a decision.</p> <p>Marketing and trade issues, including segregation and coexistence regimes, are outside the scope of assessments conducted by the Regulator. These issues are the responsibility of the State and Territory governments and industry.</p>

<sup>5</sup> Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Submission	Issues raised	Comment
2	<p>Reiterated opposition to GMOs as detailed in the earlier response.</p> <p>States that consultation via electronic or print media was not adequate to consult with the local community.</p> <p>Requested copies of the call for submissions in order to distribute this to the local community.</p>	<p>Noted.</p> <p>Please see the response to the previous submission.</p> <p>A response was sent to this submitter during the consultation period, providing information about the availability of documents related to this application on the OGTR website, together with a link to the OGTR website. The response included an invitation to share this link with any interested persons, enabling wider circulation in the local community.</p>
3	<p>All plausible risk scenarios have been identified.</p> <p>No additional information that should be considered was identified.</p> <p>Agree with the overall conclusions of the RARMP.</p>	Noted.
4	<p>Overall, the application has negligible risks to the health and safety of people and the environment.</p> <p>Satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.</p>	Noted.
5	<p>Agrees with the overall conclusions of the Risk Assessment and Risk Management Plan.</p> <p>Believes that the proposed release of GM white clover in a controlled setting is unlikely to pose a risk to the environment due to the controls that are expected to be in place during the trial that limit dispersal and spread of GM clover.</p> <p>The RARMP should discuss:</p> <ul style="list-style-type: none"> <li>- the potential for the GM clover to have increased weediness, including data comparing GM and non-GM parent plants to support conclusions on weediness. If data are not available, then the uncertainty regarding this risk should be made clear</li> <li>- further potential pathways to harm such as seed-mediated dispersal by birds.</li> <li>- further elaborate factors that may limit seed dispersal and spread of GM clover plants, in particular dispersal of seed by birds or wind.</li> </ul>	<p>Noted.</p> <p>This field trial is investigating the agronomic performance and the production of condensed tannins in the GM white clover lines under field conditions. As such, data comparing non-GM parental lines with GM white clover lines under Australian field conditions is not yet available.</p> <p>Uncertainty around a number of areas has been identified in the RARMP (Chapter 2, Section 3). Additionally, Chapter 3 (Section 4) of the RARMP lists a number of issues to be addressed for future applications including phenotypic characterisation of the GM white clover plants. The RARMP has been modified to add specific detail to each of these lists to ensure that other contributors to increased weediness such as abiotic stress and increased flowering are mentioned.</p> <p>Whilst there is some information available about consumption of seeds by birds and potential for survival of viable seed following digestion, there is limited data available under field conditions. Studies which have been conducted under laboratory conditions (for both feeding and seed germination) do provide some evidence of seed survival following digestion but may not represent the potential for seed survival and germination in the field. Additionally, although one study</p>

Submission	Issues raised	Comment
6	Broadly supportive of DIR 176. No specific comments regarding this application.	<p>mentioned in this submission does examine the effects of bird digestion on seed germination for a number of plant species, white clover was not included, and the seeds examined were all substantially larger than white clover seeds. Hence, conclusions from this study are not directly relevant. However, this has now been clarified in the RARMP (Chapter 1, Section 5.1 and in Chapter 2, Risk Scenario 2).</p> <p>Where insect-proof tents are used, it is expected that they would provide some protection against bird feeding on GM white clover and this has been noted in the RARMP. Not all field experiments will be conducted inside insect proof tents, however the applicant has indicated that no seed would be allowed to set in planting areas that do not have insect-proof tents (Chapter 2, Risk Scenario 3), although the risk assessment has assumed that some seed set will still occur.</p> <p>Seed dispersal by wind has been noted for short distance movement, but there is little or no documented evidence that this would occur (RARMP Chapter 1, Sections 3 and 4). In the current trial, the presence of either an insect proof tent or pollen trap and pollen buffer crops surrounding the planting area would significantly limit the likelihood of seed movement by wind (Chapter 2, Risk Scenario 2).</p>

## Appendix B Summary of submissions from the public on the consultation RARMP

The Regulator received four submissions from the public on the consultation RARMP. The issues raised in the submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator’s decision to issue the licence.

Submission	Issues raised	Comment
1	White clover is attractive to honey bees. Will the trial affect bees & consumers of honey? Will apiarists in the area of the trials be informed?	<p>The GM white clover produces increased levels of condensed tannins in leaves. These compounds are already produced in non-GM white clover flowers and seeds, and many other plants produce higher levels than those expected in the trial. Therefore, it is highly likely that bees are already exposed to these compounds. The condensed tannins are unlikely to be produced at a level which would have any effect on honey bees and this is addressed in Risk Scenario 1 of the Risk Assessment and Risk Management Plan (RARMP).</p> <p>Controls for this trial include the use of pollen traps and a pollen buffer crop. These provide abundant alternative sources of attractive food, so bees from outside the trial are likely to access these sources in preference to the GM plantings. If beehives are used specifically for the trial, bees from these hives would be destroyed at the end of the trial, as would any honey produced from the hives.</p> <p>The location of all field trial sites are notified on the OGTR website.</p>
2	I think we should stop messing with nature	The functions of the Gene Technology Regulator (the Regulator) are defined by the <i>Gene Technology Act 2000</i> (the Act) which is legislation passed by the Parliament of Australia. The Regulator must consider each application for a licence for dealings with GMOs based on criteria listed in the Act.
3	If this GM white clover is not be used for human food or animal feed, what's the point of it?	<p>The aim of this current trial is to study the agronomic performance, nutritional analysis, compositional analysis, molecular analysis and genetic stability of the GM white clover under field conditions. The applicant has not sought use of the GMOs for food or feed for this particular trial. The GM white clover contains an introduced gene that is expected to increase the concentration of condensed tannins in leaves.</p> <p>Condensed tannins are associated with improved animal production in pastoral agricultural systems and potential reduce the occurrence or bloat. Therefore, although the ultimate aim may be to use the GM white clover as animal feed, a new application and assessment would be required to determine any potential risks before being allowed as animal feed.</p>
4	It is a waste of breath as genetic modification is being allowed daily. It is affecting us and the environment.	The object of the Act is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

Submission	Issues raised	Comment
		<p>The RARMP prepared by the Regulator assessed risks to people and the Australian environment from the proposed field trial of GM white clover. The RARMP concluded that the proposed release poses negligible risks to people and the environment. Consultation on the RARMP includes experts and other agencies, including the Department of Agriculture, Water and the Environment. Their advice was considered in the preparation of the final RARMP, which informs the Regulator’s decision on whether to approve the licence for the application.</p>