Australian Government
Department of Health
Office of the Gene Technology Regulator

January 2018

Risk Assessment and Risk Management Plan
(consultation version)
for

DIR 156

Limited and controlled release of buffalo grass genetically modified for herbicide tolerance and dwarf phenotype

Applicant – Royal Melbourne Institute of Technology (RMIT) University

This RARMP is open for consultation until 1 March 2018.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

via mail to:	The Office of the Gene Technology Regulator MDP 54, GPO Box 9848, Canberra ACT 2601 or
via email to:	ogtr@health.gov.au

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications do **not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan (Consultation Version)

for

Licence Application No. DIR 156

Introduction

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. Licence conditions have been drafted for the proposed field trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application number	DIR 156
Applicant	The Royal Melbourne Institute of Technology (RMIT) University
Project title	Limited and controlled release of buffalo grass genetically modified for herbicide tolerance and dwarf phenotype
Parent organism	*Stenotaphrum secundatum* (buffalo grass)
Introduced genes and modified traits	• Gene encoding the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Arabidopsis thaliana* (thale cress) for tolerance to the herbicide glyphosate • Gene encoding the enzyme gibberellic acid 2-oxidase 3 from *Spinacia oleracea* (spinach) for shorter stature and slowed growth
Proposed location	One site in Victoria
Proposed release size	Up to 200 m^2
Proposed release dates	April 2018 – April 2019
Primary purpose	To assess agronomic characteristics of the GM buffalo grass plants

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release 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 which may result in harm that were considered included exposure of people or animals to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to other buffalo grass 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 buffalo grass is not a food crop which reduces human exposure, the GM plant material will not be used for animal feed and that the proposed limits and controls will effectively contain the GMOs and their genetic material and minimise exposure.

Risk management plan

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. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft 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 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	*Gene Technology Act 2000*
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FSANZ	Food Standards Australia New Zealand
GA	Gibberellin
GA 2-OX	Gibberellin 2-oxidase
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
m	metres
mm	millimetres
NLRD	Notifiable Low Risk Dealing
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PMV	Panicum mosaic virus
PC2	Physical Containment level 2
Qld	Queensland
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
USA	United States of America
USDA	United States Department of Agriculture

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 in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, 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. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

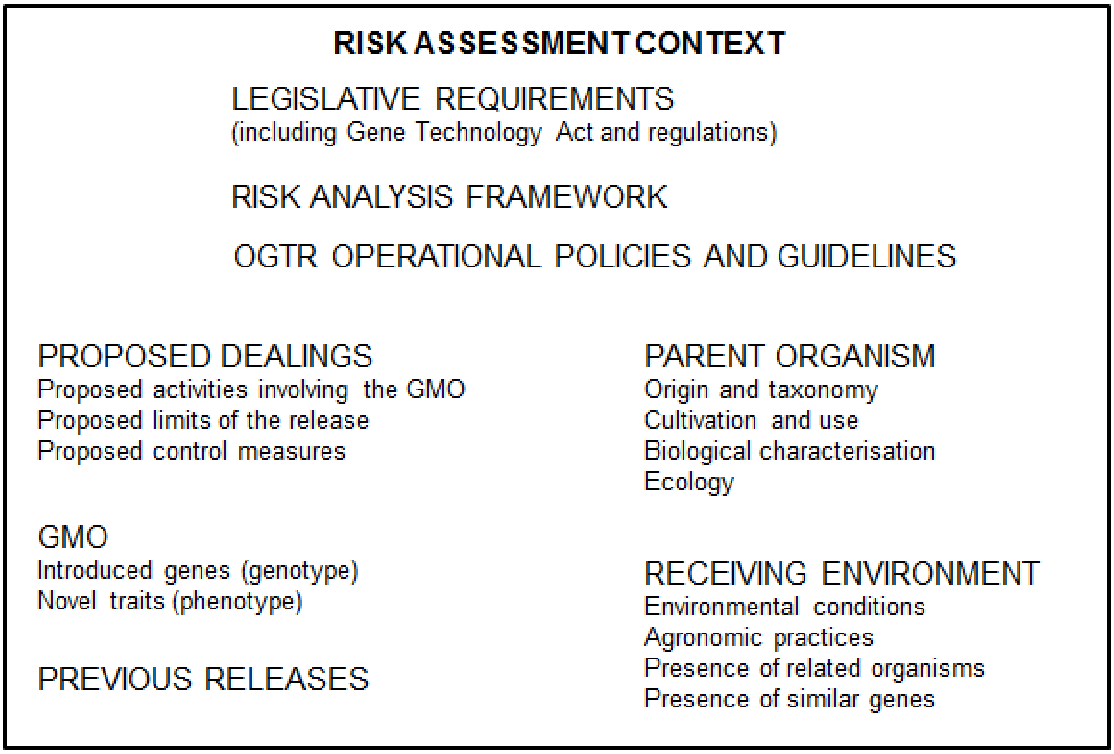

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the

environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public.

7. The *Risk Analysis Framework* (OGTR 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator 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, the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed dealings

9. The RMIT University proposes to release up to 20 lines of buffalo grass genetically modified for herbicide tolerance and dwarf phenotype into the environment under limited and controlled conditions. The purpose of the release is to assess agronomic characteristics of the GM buffalo grass plants under field conditions.

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

- conducting experiments with the GMOs,
- growing the GMOs,
- transporting the GMOs,
- disposing of the GMOs,

and possession, supply or use of the GMOs for the purposes of, or in the course of, any of the above.

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

11. The release is proposed to take place for one growing season, between April 2018 and April 2019. GM buffalo grass would be grown on one trial site with an area of up to 200 m^2. The trial site would be located in Bundoora, in the local government area of Whittlesea in Victoria.

12. Only trained and authorised staff would be permitted to deal with the GM buffalo grass.

3.2 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 buffalo grass and the introduced genetic material in the environment. These include:

- restricting the production of inflorescences on the GM buffalo grass by mowing,
- surrounding the planting area with a 2 m wide monitoring zone that will be kept bare of vegetation and be monitored weekly for the presence of volunteer plants or weeds,
- treating non-GM buffalo grass grown in the trial site the same as GM plants,
- cleaning equipment used with the GMOs before use for other purposes or removal from the trial site,

- enclosing the trial site within a fence with lockable gates to prevent animals from entering and restrict unauthorised access,
- destroying all GM plants after the completion of the trial,
- monitoring the trial site after completion of the trial every month for one year, destroying any buffalo grass volunteers,
- transporting and storing GM plant materials in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, and
- not allowing GM plant material to be used for human food or animal feed.

Section 4 The parent organism

14. The parent organism is buffalo grass (*Stenotaphrum secundatum* (Walt.) *Kuntze*) which is exotic to Australia. Buffalo grass is an ornamental turf grass grown in parks, gardens, residential and commercial properties, sporting venues and for land rehabilitation and landscape improvement purposes (HIA, 2016). NSW and Qld are the major producers but production occurs in all states and territories in Australia. It is a warm season grass so peak production is during spring and summer months. In the 2014-2015 season buffalo grass was the predominant species grown for turf with a production of 15 million m^2, 33% of the total turf grass production (HIA, 2016).

15. Detailed information about the parent organism is contained in the reference document *The Biology of* Stenotaphrum secundatum (*Walt.*) *Kuntze* (buffalo grass) (OGTR, 2018), which was produced to inform the risk analysis for licence applications involving GM buffalo grass. Baseline information from this document will be used and referred to throughout the RARMP.

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

5.1 Introduction to the GMOs

16. The applicant proposes to release up to 20 lines of GM buffalo grass. Each line contains two introduced genes, one gene to confer tolerance to the herbicide glyphosate and another gene to confer a dwarf phenotype. The introduced genes and regulatory sequences were sourced from plants (Table 1).

Table 1 Introduced genetic elements in the GM buffalo grass

Genetic element	Function in the GM plant	Source gene	Source organism
P-RUBI	Promoter	*UBIQUITIN*	Rice (*Oryza sativa*)
I-R-Act	Expression enhancer	*ACTIN 1*	Rice (*Oryza sativa*)
Resyn-A thal EPSPS	Confers tolerance to the herbicide glyphosate and also acts as selectable marker	*5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE* (*EPSPS*)	Thale cress (*Arabidopsis thaliana*)
ZmADH3'	Transcriptional terminator	*ALCOHOL DEHYDROGENASE 1*	Maize (*Zea mays*)
P-GOS2	Promoter	*GOS2*	Rice (*Oryza sativa*)
GA 2-Ox 3	Reduces plant stature and slows growth	*GIBBERELLIC ACID 2-OXIDASE 3* (*GA 2-OXIDASE 3*)	Spinach (*Spinacia oleracea*)

Genetic element	Function in the GM plant	Source gene	Source organism
SpH3’	Transcriptional terminator	*Histone H1*	Wild tomato species (*Solanum pennellii*)

17. The introduced genes in GM buffalo grass are under the control of constitutive promoters, which are promoters that drive expression of genes to high levels in most plant tissues throughout the life of the plant. Therefore, the proteins encoded by *EPSPS* and *GA 2-oxidase 3* are expected to be present in all parts of the GM buffalo grass. The introduced *EPSPS* gene is controlled by the *ubiquitin 1* (P-RUBI) constitutive promoter while *GA 2-oxidase 3* is under the control of the GOS2 promoter, both promoters are sourced from rice (*Oryza sativa*). Expression of the *EPSPS* gene in GM buffalo grass is also further increased by the enhancer I-R-Act, which is sourced from the rice *actin 1* gene.

18. The GM buffalo grass lines were produced using biolistic transformation (particle bombardment). Information about this transformation method can be found in the document *Methods of plant genetic modification* available from the OGTR Risk Assessment References page.

5.2 The introduced genes, encoded proteins and their associated effects

5.2.1 The EPSPS gene from Arabidopsis thaliana (thale cress)

19. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is the sixth enzyme in the shikimate pathway, which is essential for the synthesis of aromatic amino acids and other secondary metabolites in algae, higher plants, bacteria, and fungi (Padgette et al., 1995). Because the shikimate pathway is absent from mammals, which get the aromatic amino acids from their diet, EPSPS is an attractive target for the development of antimicrobial agents and herbicides (Schonbrunn et al., 2001). Glyphosate (N-phosphonomethyl glycine) is a potent and specific inhibitor of EPSPS and is successfully used as a herbicide (Amrhein et al., 1980). Glyphosate inhibits the activity of the EPSPS enzyme in plants, blocking the synthesis of aromatic amino acids and eventually leading to cell death.

20. EPSPS catalyses the transfer of the enolpyruvyl moiety from phosphoenol pyruvate to shikimate-3-phosphate, forming the products 5-enolpyruvyl-shikimate-3-phosphate and inorganic phosphate (Figure 2). EPSPS is not known to participate in other metabolic pathways in plants (Padgette et al., 1996). Glyphosate appears to compete with phosphoenol pyruvate for the same binding site in EPSPS, thus inhibiting the reaction (Schonbrunn et al., 2001).

21. One way to overcome glyphosate action is to increase the levels of EPSPS by introducing an extra copy of the *EPSPS* gene under the control of a constitutive promoter by means of gene technology. This overexpression of EPSPS increases the plant’s tolerance to glyphosate, which means that GM plants are able to withstand concentrations of glyphosate that normally kill non-GM plants. GM petunia and thale cress plants tolerant to glyphosate have been achieved by overexpressing their native EPSPS enzymes (Klee et al., 1987). The natural tolerance to glyphosate that many weed species display is in some instances the result of overproduction of EPSPS due to extra copies of the *EPSPS* gene (Gaines et al., 2010; Powles, 2010).

22. Natural variants of EPSPS enzymes also exist that have lower binding affinity for glyphosate and therefore are not inhibited or are less inhibited by this herbicide. One of these variants is the CP4 EPSPS enzyme from the soil bacterium *Agrobacterium* sp. strain CP4 (Padgette et al., 1995). This enzyme has been introduced in alfalfa, canola, cotton, maize, potato, soybean and sugar beet to generate commercial GM crops tolerant to glyphosate (International GM Approval Database).

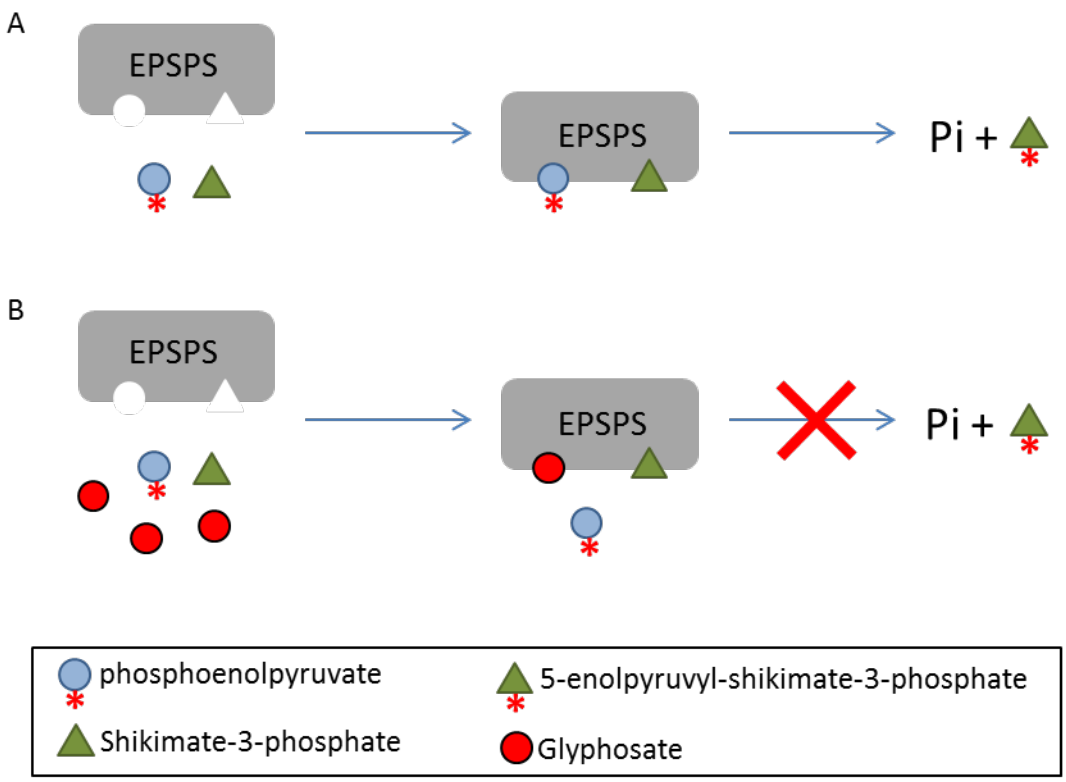

Figure 2. Glyphosate inhibition of EPSPS. (A) EPSPS catalyses the transfer of the enolpyruvyl moiety from phosphoenol pyruvate to shikimate-3-phosphate forming the products 5-enolpyruvyl-shikimate-3-phosphate and inorganic phosphate (Pi). (B) Glyphosate inhibits this reaction by binding EPSPS and precluding phosphoenol pyruvate binding.

5.2.2 The GA 2-oxidase 3 gene from spinach

23. GA 2-oxidase 3 is an enzyme that participates in the deactivation of the plant hormones gibberellins. Gibberellins (GAs) are plant hormones that are essential for many developmental processes in plants, including seed germination, stem elongation, leaf expansion, pollen maturation and the induction of flowering (reviewed in Daviere and Achard, 2013). Since its original discovery, many GAs have been identified in plants, although only a few GAs have biological activity. Non-bioactive GAs act as precursors for the bioactive forms or are de-activated metabolites (reviewed in Daviere and Achard, 2013). Biologically active gibberellins and their precursors are deactivated by the enzymes GA 2-oxidases. Three *GA 2-oxidase* genes are found in spinach. GA 2-oxidases 1 and 2 deactivate the biologically active gibberellin GA_1 and its immediate precursor GA_{20} (Lee and Zeevaart, 2002, 2005). GA 2-oxidase 3 deactivates early precursors in the gibberellin biosynthetic pathway, GA_{12} and GA_{53} (Lee and Zeevaart, 2005). Increased expression of *GA 2-oxidases* in GM thale cress or tobacco results in decreased levels of active GAs and a reduction of plant stature, darker green leaves, delayed germination and late flowering (Schomburg et al., 2003; Lee and Zeevaart, 2005). All these effects are reversed by exogenous application of gibberellin which suggests that these enzymes do not participate in other plant pathways. Overexpression of *GA 2-oxidase 3* from spinach did not affect flower and seed development in GM tobacco (Lee and Zeevaart, 2005).

5.3 Toxicity/allergenicity of the proteins associated with the introduced genes

24. The introduced genes are sourced from *Arabidopsis thaliana* and spinach (*Spinacia oleracea*). *Arabidopsis thaliana*, (thale cress or mouse-ear cress), is a small herbaceous annual flowering plant in the Brassicaceae (mustard family, which also includes cabbage and broccoli). It is the most widely used

model organism in plant biology and genetics (Koornneef and Meinke, 2010). Thale cress is edible by humans, and can be used similarly to other mustard greens, in salads or sautéed, but its use as an edible spring green is not widespread (Atlas of Living Australia). It is generally considered a weed, due to its widespread distribution in agricultural fields, roadsides, and disturbed lands. *Arabidopsis thaliana* is naturalised in Australia (Atlas of Living Australia). Spinach is a vegetable grown in Australia and regularly consumed by humans. On this basis the general public has been exposed to the introduced genes and their encoded proteins without any adverse effects.

25. The introduced genes are two enzymes with essential metabolic or developmental functions so homologues are widespread in the plant kingdom. Homologues of both enzymes are a common component of food and feed. A search of the AllergenOnline database did not list any proteins from thale cress or spinach as allergens and no reports were found of toxic proteins or toxic metabolites produced by enzymes from thale cress or spinach.

26. There have been numerous studies on the allergenicity and toxicity of the CP4 EPSPS introduced in many GM crops. No toxicity or allergenic effects associated with CP4 EPSPS have been found (Padgette et al., 1996; Kim et al., 2006; ILSI, 2016).

27. The GM buffalo grass lines have been grown by the applicant in glasshouse trials and workers handling the GM plants have not reported any adverse effects.

28. No studies on the toxicity or allergenicity of the GM buffalo grass lines have been undertaken to date as this is early-stage research.

5.4 Characterisation of the GMOs

29. The introduced genes specifically confer glyphosate tolerance and a dwarf phenotype. Semi-dwarf varieties of turf grasses are in demand mainly because they have low maintenance due to requiring less water and less frequent mowing (Chen et al., 2009). In some turf grass species, semi-dwarf varieties also show tolerance to stresses like shade, cold or drought or better turf qualities such as prostrate growth and thinner leaves (Chen et al., 2016; Li et al., 2016). Glyphosate tolerance facilitates weed management, as glyphosate can be applied to the GM buffalo grass at any time to kill weeds without causing any detrimental effect to the lawn.

30. All GM buffalo grass lines proposed for release have been grown in the glasshouse for 5 years. GM plants have shown a stable, dwarf phenotype that is shorter in height with less biomass than the parent plant. GM plants also grow more slowly than non-GM plants under the same environmental conditions.

Section 6 The receiving environment

31. The receiving environment forms part of the context in which the risks associated with dealings involving 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).

32. Information relevant to the growth and distribution of buffalo grass in Australia is discussed in *The Biology of* Stenotaphrum secundatum (*Walt.*) *Kuntze* (buffalo grass) (OGTR, 2018).

6.1 Relevant abiotic factors

33. The release is proposed to take place at the RMIT University Bundoora Campus, Victoria. The average temperatures in Bundoora range between 12.4-26.9°C in summer and 5.6-14.9°C in winter. The minimum temperature in Bundoora does not normally go below 0°C (Bureau of Meteorology website). Buffalo grass is a perennial, warm-season species that has optimum growth when temperatures are around 30°C and water is not limiting. Its minimum temperatures for growth are around 10-12 °C (Aldous et al., 2014). In the warm-temperate areas of Australia, including Bundoora,

buffalo grass growth occurs during spring and summer, slows down in autumn and ceases in the winter as the grass goes into dormancy (Aldous et al., 2014). Buffalo grass requires long days to flower (Genovesi et al., 2009) and flowering usually occurs during December to February in Victoria (HerbiGuide, 2014a).

34. Winter hardiness is a major limiting factor for buffalo grass. Laboratory-based freeze tests showed that the survival rates were 33.6% and 17.8% following treatment at -3 °C and -4°C, respectively. No regrowth was observed after treatment at -6°C (Kimball et al., 2017). In Bundoora, it is predicted that buffalo grass stops growth but endures winter conditions. Management practices suggest that so long as water supply is adequate, the plant can endure high temperatures (OGTR, 2018).

35. Buffalo grass is adaptable to varying levels of soil moisture, from moderate water deficit to temporary flooding and waterlogging. It commonly grows in areas of 1000-2000 mm annual rainfall. However, if adequate soil moisture can be maintained, it will colonise areas with an annual rainfall down to 750 mm (Cook et al., 2005). As the average annual rainfall in Bundoora is 672.5 mm (Bureau of Meteorology website), the applicant proposes to irrigate the GM buffalo grass using an automated sprinkler system.

6.2 Relevant biotic factors

36. Several pests and diseases can affect buffalo grass (The Buffalo Lawn Care). The caterpillars of various moth species will feed on leaves and could cause significant damage to the grass. The most common buffalo grass pests in Australia are the armyworms. Armyworms is a common name for several different caterpillar pests of the family Noctuidae, which include lawn grub (*Spodoptera* spp.), common armyworm (*Mythimna convecta*), southern armyworm (*Persectania ewingii*), and inland armyworm (*Persectania dyscrita*) (MacDonald, 1995; Ozbreed, 2011). The Sod webworm (*Herpetogramma licarsisalis*), which is the caterpillar of the Crambus moth, also feeds on buffalo grass (The Buffalo Lawn Care).

37. Pests that feed on buffalo grass roots are the larvae of the African black beetle (*Heteronychus arator*) and the nematode *Belonolaimus longicaudatus*. The African black beetle is a minor pest but can be problematic when present in high numbers (The Buffalo Lawn Care). The nematode *Belonolaimus longicaudatus* is the most destructive species of nematode and causes loss in root density in cultivars susceptible to infection (Aryal et al., 2015).

38. Ants will often damage turf by creating many nests across a lawn which brings soil to the surface. This often leads to poor lawn health and thinning out of the grass in the affected area (The Buffalo Lawn Care).

39. Fungal diseases and *Panicum mosaic virus* (PMV) have been responsible for the decline of buffalo grass in the Gulf Coast region of the USA (Cabrera and Scholthof, 1999). The PMV infection becomes visible when grass blades develop a mottled, chlorotic appearance and the disease can eventually spread across large sections of turf. Infected grass is weakened and becomes prone to weed invasions. The first report of PMV on buffalo grass in Australia dates back to 2008 and was found on one of the commercial turf farms in the Hawkesbury area, NSW (Thomas and Steele, 2011). The virus is spread through contaminated equipment, e.g. lawn mowers or stolons that are used for propagation. Biological vectors that could spread the virus are not known. There is no treatment for PMV but some varieties of buffalo grass are PMV-resistant (Duble, 2004).

40. Typical broadleaf weeds of buffalo grass in Australia include bindii (*Soliva pterosperma*), clover (*Trifolium* spp.), cats ear (*Hypochaeris radicata*) and dandelion (*Taraxacum officinale*). Grass weeds include paspalum (*Paspalum* spp.), winter grass (*Poa annua*), kikuyu (*Pennisetum clandestinum*), and couch grass (*Cynodon dactylon*) (OGTR, 2018). Buffalo grass lawns are highly resistant to weed infestation (Busey, 2003b) and a well-maintained sward of buffalo grass can suppress weed growth. Weed infestation is likely when the health of the sward is compromised, and ground cover becomes patchy (Busey, 2003a; Aldous et al., 2014).

6.3 Relevant agricultural practices

41. The GM buffalo plants to be used for the proposed trial are propagated vegetatively in PC2 glasshouses and will be planted by hand in the trial site, one plant per one square metre plot. Fertiliser will be applied in soluble form. Once established, the applicant proposes to mow the grass on a regular basis, when it grows to a height of 3-5 cm above the ground. The applicant will manage pests and disease by routine monitoring and chemical control if required

6.4 Presence of related plants in the receiving environment

42. Buffalo grass is a species grown in all states and territories in Australia and is used as a turf in gardens, parks, residential and commercial properties, sporting venues and for land rehabilitation and landscape improvement purposes (HIA, 2016). It is also a naturalised species in Australia, appearing in various bushland settings, particularly in coastal districts (Muyt, 2001). It is a very genetically diverse species. There are diploids, triploids, tetraploids and aneuploids (Busey, 2003b). In Australia diploids and triploids have been described. Diploids are fertile and produce viable seeds, while triploids are considered sterile (Busey, 2003b; Loch et al., 2009). Naturalised buffalo grass is mainly a triploid and is found throughout Australia (Sauer, 1972; Loch et al., 2009). There is also an area of NSW in the Hunter valley where diploid buffalo grass is naturalised. This diploid form is the origin of the Australian cultivars sold by turf companies in Australia (Loch et al., 2009). Fertile diploids are able to cross-pollinate. Crosses between diploids and triploids do not occur naturally but require *in vitro* techniques to succeed (Busey, 2003b).

43. There are seven species of *Stenotaphrum* (Sauer, 1972). Only *S. micrathum* has been described in Australia. This is found in islands at the Great Barrier Reef, but not in mainland Australia (Atlas of Living Australia; Sauer, 1972). No natural hybridization events have been reported between buffalo grass and *S. micrathum*, in Australia or elsewhere. It has been suggested that hybridisation between buffalo grass and *S. dimidiatum* might be possible as some buffalo grass varieties show phenotypic characteristics reminiscent of *S. dimidiatum* (Sauer, 1972; Busey, 2003b). However, *S. dimidiatum* is absent in Australia (Sauer, 1972). Thus, it is highly unlikely that buffalo grass could result in intrageneric crosses under natural conditions in Australia.

44. No published data was found to suggest that natural crossing occurs between buffalo grass and plants from other genera (OGTR, 2018).

6.5 Presence of similar genes and encoded proteins in the environment

45. The introduced genes are derived from thale cress and spinach, which are both plants present in the Australian environment. As discussed in Section 5, the introduced genes encode two enzymes with essential metabolic or developmental roles, so homologous genes are widespread in plants.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Approvals by the Regulator

46. None of the GM buffalo grass lines included in this application have previously been approved for release in Australia.

47. The Regulator has not received any previous applications for release of GM buffalo grass.

7.1.2 Approvals by other government agencies

48. There are no approvals of GM buffalo grass, or applications for GM buffalo grass under consideration, by other Australian authorities.

7.2 International approvals

49. GM buffalo grass with the same introduced genes was released for a field trial in the USA in July 2012. USDA determined that the GM buffalo grass plants were not considered regulated since the parent plant was not a pest, the introduced genetic material wasn't sourced from pest organisms and the method used to genetically engineer the plant did not involve plant pests (USDA letter re transgenic *Stenotaphrum*).

Chapter 2 Risk assessment

Section 1 Introduction

50. 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 2). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

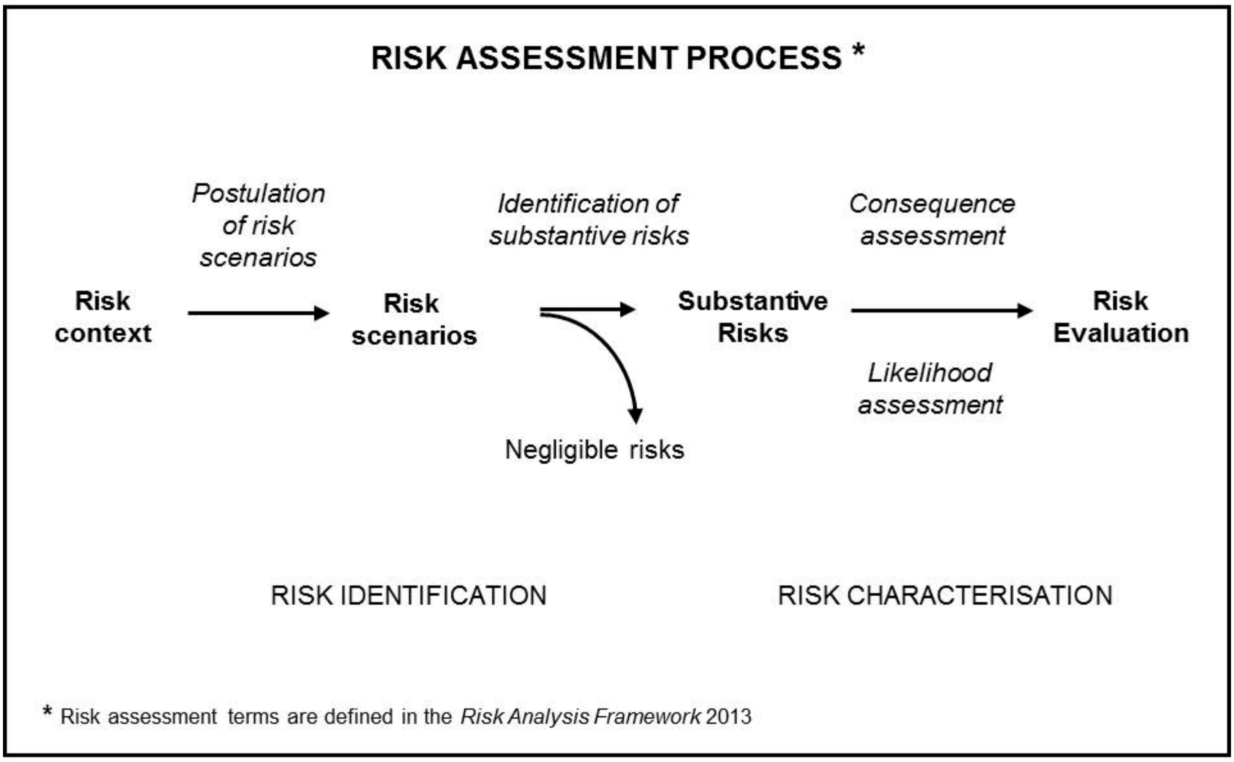

Figure 2. The risk assessment process

51. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO in the short or long term. These are called risk scenarios.

52. A number of risk identification techniques are used by the Regulator and staff of the OGTR, 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, GM traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al. 2014). Risk scenarios from previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

53. Risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

54. Substantive risks (i.e. those identified for further assessment) 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

55. Risk scenarios are comprised of three components:

i. the source of potential harm (risk source),

ii. a plausible causal linkage to potential harm (causal pathway), and

iii. a potential harm to people or the environment.

56. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these 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, and
- the characteristics of the parent organism(s).

2.1 Risk source

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

58. As discussed in Chapter 1, the GM buffalo grass lines have been modified by the introduction of two genes derived from the plants thale cress and spinach and designed to confer glyphosate tolerance and a dwarf phenotype. These introduced genes are considered further as potential sources of risk.

59. The introduced genes are controlled by introduced regulatory sequences. These were derived from rice, maize and the wild tomato plant *Solanum pennelli*. Regulatory sequences are naturally present in plants, and the introduced sequences are expected to operate in similar ways to endogenous sequences. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, the potential for harm from the regulatory sequences will not be further assessed for this application.

60. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, 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. 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). Plants generated by conventional breeding have a long history of safe use, and 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). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

61. 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 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 GMOs, (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 organisms,
- gene transfer by horizontal gene transfer (HGT), and
- unauthorised activities.

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

63. 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.

64. 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 for substantial penalties for unauthorised dealings with GMOs or non-compliance 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, unauthorised activities will not be considered further.

2.3 Potential harm

65. Potential harms from GM plants include:

- 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, and
- 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).

66. These harms are based on those used to assess risk from weeds (Keese et al. 2014; Standards Australia Ltd et al. 2006). 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 'versus' nature conservation.

2.4 Postulated risk scenarios

67. Five risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 2, and examined in detail in Sections 2.4.1 – 2.4.5. Postulation of risk scenarios considers impacts of the GM buffalo grass plants on people undertaking the dealings, as well as impacts on people and the environment if the GM plants or genetic material were to spread and/or persist.

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

Table 2 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
1	Introduced genes conferring herbicide tolerance and a dwarf phenotype	Growing GM buffalo grass plants at the trial site ⬇ Expression of introduced genes in GM plants ⬇ Exposure of people who deal with the GM plant material or of people in the vicinity of the trial site	Toxicity or allergenicity to people	No	• People do not eat buffalo grass. • The proposed limits and controls would restrict exposure of people to the GM plant material through skin contact or inhalation of pollen. • There were no adverse health effects on people handling the GM plants in glasshouse trials.
2	Introduced genes conferring herbicide tolerance and a dwarf phenotype	Growing GM buffalo grass plants at the trial site ⬇ Expression of introduced genes in GM plants ⬇ Exposure of animals eating GM plant material	Toxicity to desirable animals	No	• GM plant material would not be used as livestock feed. • The trial site is enclosed by a fence that would restrict access by grazing animals. • The small size and short duration of the proposed trial would minimise exposure of native animals to the GM plant material.
3	Introduced genes conferring herbicide tolerance and a dwarf phenotype	Growing GM buffalo grass plants at the trial site ⬇ Persistence of GM plants after completion of the trial ⬇ Establishment of volunteer GM plants in the environment ⬇ Expression of introduced genes in the volunteer plants	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants	No	• The proposed controls would minimise persistence of GMOs after completion of the trial. • Buffalo grass has limited ability to establish ongoing volunteer populations in the environment near the trial site.

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
4	Introduced genes conferring herbicide tolerance and a dwarf phenotype	Growing GM buffalo grass plants at the trial site ⬇ Dispersal of GM buffalo grass stolons and/or seeds outside the trial site ⬇ Establishment of volunteer GM plants in the environment ⬇ Expression of introduced genes in the volunteer plants	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants	No	• The proposed controls will reduce the number of GM seed produced by the plants in the trial. • The proposed controls would minimise dispersal of GM buffalo grass stolons and seeds. • Buffalo grass has limited ability to establish ongoing volunteer populations in the environment near the trial site.
5	Introduced genes conferring herbicide tolerance and a dwarf phenotype	Growing GM buffalo grass plants at the trial site ⬇ Pollen flow to non-GM buffalo grass crops/lawns or volunteers outside the trial site ⬇ Establishment of hybrid GM plants in the environment ⬇ Expression of introduced genes in the plants	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants	No	• The proposed controls would minimise pollen flow to non-GM buffalo grass outside the trial site. • Buffalo grass has limited ability to establish ongoing volunteer populations in the environment near the trial site.

2.4.1 Risk scenario 1

Risk source	Introduced genes conferring herbicide tolerance and a dwarf phenotype
Causal pathway	⬇ Growing GM buffalo grass plants at the trial site ⬇ Expression of introduced genes in GM plants ⬇ Exposure of people who deal with the GM plant material or of people in the vicinity of the trial site ⬇
Potential harm	Toxicity or allergenicity to people

Risk source

69. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and dwarf phenotype.

Causal pathway

70. GM buffalo grass expressing the introduced genes would be grown at the trial site. People could potentially be exposed to the GM plant material through skin contact or inhalation. People do not eat buffalo grass and, therefore, exposure through ingestion is not anticipated.

71. Only a few people would be expected to handle the GM buffalo grass due to the small scale of the proposed trial. The licence application proposes that only trained and authorised staff would be

permitted to deal with the GM buffalo grass. These people could be exposed to plant material through skin contact when handling the plants during cultivation, transportation or analysis. The applicant proposes a fence with lockable gates surrounding the trial site and monitoring zone, which would restrict unauthorised access to the trial. However, the field trial is situated within the RMIT University campus so people may pass the trial site at a distance of at least 2 m (i.e. the width of the proposed monitoring zone). Passers-by could be exposed to GM buffalo grass pollen.

72. Outcross pollination of buffalo grass is most probably mediated by wind (OGTR, 2018), as happens in most grasses (Whitehead, 1969). Buffalo grass flowers from November to February with the maximum number of inflorescences recorded in midsummer (Duff et al., 2009; HerbiGuide, 2014b). There is uncertainty about the amount of pollen produced by buffalo grass plants. Since buffalo grass plugs will be planted at a density of 1 plant per m^2, the plants will not completely cover the trial area until after at least three months (3 month-old non-GM buffalo grass plants planted in a similar way had a mean diameter of 106.1-167.4 cm (Duff et al., 2009)). It is important to consider that the GM buffalo grass has been modified for a dwarfing phenotype. This would mean that the time to reach complete coverage of the 200 m^2 would take longer than when planting non-GM buffalo grass. Therefore, the maximum number of flowers that might be produced in the trial area is not expected to be as high as in an established, confluent lawn.

73. The applicant has proposed measures to decrease the number of flowers that could be produced in the trial site. The plants would be mown frequently and this activity is expected to remove many developing inflorescences being produced by the buffalo grass. Frequent mowing also encourages vegetative growth over reproductive growth (Busey, 2003b). Stress due to low nutrition promotes flowering in buffalo grass (The Buffalo Lawn Care), as has been observed for other plant species (Takeno, 2016). The plants in the trial would be fertilised to avoid this stress response. These measures together with the small size of the trial and the low density of plants at planting are expected to result in low concentrations of GM buffalo grass pollen in the air.

74. The severity of allergic reactions to pollen is correlated with atmospheric pollen concentration. If pollen count is below a threshold level (typically around 30 grains/m^3 for grass pollen), this elicits no or minor symptoms even in people sensitive to the pollen allergens (Kiotseridis et al., 2013).

75. The proposed controls, including cultivation management, would make it unlikely for passers-by to be exposed to this threshold level. Exposure to pollen would only be possible during flowering, i.e. between November and February. These months would include the University summer holiday period during which lower pedestrian traffic is expected. This would further limit incidental exposure of passers-by.

Potential harm

76. 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).

77. The two introduced genes in the GM buffalo lines are derived from the plants thale cress and spinach both of which are safely consumed by humans without adverse effects. The introduced proteins are not expected to be toxic or allergenic (see chapter 1, section 5.3).

78. Pollen from non-GM buffalo grass has been reported to elicit allergic sensitivity in people in South Africa. However, Australian commercial suppliers claim that buffalo grass has low allergenicity due to the low pollen counts produced by the current cultivars (Lawn Solutions Australia; Ozbreed Environmental Turf and Plant Breeding). A study of the main tropical grasses inducing allergy did not identify buffalo grass as a clinically important source of pollen allergens (Davies, 2014).

79. The introduced traits may lead to an increase in pollen allergenicity in the GM buffalo grass. Pollen allergies are due to protein allergens present in pollen (Radauer and Breiteneder, 2006).

Overexpression of *EPSPS* is highly unlikely to affect allergens in pollen as EPSPS is a well-characterised enzyme involved in aromatic amino acid biosynthesis and is not known to be active in other metabolic pathways (Chapter 1, Section 5.2.1). However, overexpression of *GA 2-oxidase 3* leads to changes in the levels of gibberellins, which are hormones involved in many metabolic and developmental pathways (Chapter 1, Section 5.2.2). These changes could potentially lead to changes in the protein composition of pollen grains. If the quantity of a protein allergen was increased in the GM pollen grains, this would increase pollen allergenicity. This is an area of uncertainty for this risk assessment.

80. The licence applicant has grown the GM buffalo grass lines proposed for release in glasshouse trials (Chapter 1, Section 5.3). No adverse health effects were reported by people dealing with the GM plants in the glasshouse.

81. ***Conclusion***: Risk scenario 1 is not identified as a substantive risk because people do not eat buffalo grass, the proposed limits and controls would restrict exposure of people to the GM plant material through skin contact or inhalation of pollen, and there were no adverse health effects on people handling the GM plants in glasshouse trials. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Introduced genes conferring herbicide tolerance and a dwarf phenotype
Causal pathway	⬇ Growing GM buffalo grass plants at the trial site ⬇ Expression of introduced genes in GM plants ⬇ Exposure of animals eating GM plant material ⬇
Potential harm	Toxicity to desirable animals

Risk source

82. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and dwarf phenotype.

Causal pathway

83. GM buffalo grass expressing the introduced genes would be grown at the trial site. Animals entering the trial site could consume GM plant material.

84. Agricultural livestock are not expected to be exposed to the GM buffalo grass since the licence application proposes that the GM plant material will not be used for animal feed and there is a fence surrounding the trial site that would restrict access by grazing animals.

85. Native mammals could enter the trial site and feed on the GM buffalo grass plants. The fence surrounding the trial site would exclude some native mammals. Some bird species feed on turf grasses, e.g. ducks and geese (NSW Environment and Heritage). Birds and other animals are not expected to be attracted specifically to the trial site, since the trial is located in an urban area with high availability of other grasses in parklands and recreational lawns. The small size and short duration of the proposed trial, combined with the fence, would restrict the numbers of native animals, including birds that could be exposed to the GM plants.

86. Although non-native pest animals such as rabbits or rodents could be exposed to the GM buffalo grass through consumption, this will not be considered as a causal pathway leading to harm, as potential toxicity to these pests would not be an environmental harm.

87. Insects, including desirable species such as pollinators, could enter the trial site and feed on the GM buffalo grass. Organisms living in the soil such as earthworms would also contact root exudates of

the GM buffalo grass or decomposing plant material after harvest. The small size and short duration of the proposed trial would restrict the numbers of insects and soil organisms exposed to the GM plants.

Potential harm

88. As discussed in Risk Scenario 1, the introduced proteins conferring herbicide tolerance and a dwarf phenotype are sourced from plants that are regularly consumed by humans and animals without adverse effects, and are not known to be toxic. Desirable soil organisms are also regularly exposed to the introduced proteins and their degradation products.

89. Non-GM buffalo grass currently has a minor use as a forage grass (Busey, 1995; Mullen and Shelton, 1996). Non-GM buffalo grass plants naturally produce the toxin oxalic acid (Garcia-Rivera and Morris, 1955). Oxalic acid binds dietary calcium (Ca) or magnesium (Mg) to form insoluble Ca or Mg oxalate, which may lead to low serum Ca or Mg levels as well as to renal failure (Rahman et al., 2013). Concentrations of oxalic acid in non-GM buffalo grass are not detrimental to the health of cattle (OGTR, 2018) but may affect non-ruminant animals, which are more sensitive to oxalic acid (Rahman et al., 2013). There is some uncertainty whether the genetic modifications could alter the levels of natural toxins in the GM buffalo grass. Overexpression of *EPSPS* is unlikely to affect toxin production as EPSPS is a well-characterised enzyme involved in aromatic amino acid biosynthesis and is not known to be active in other metabolic pathways (Chapter 1, Section 5.2.1). However, overexpression of *GA 2-oxidase 3* changes the levels of gibberellins, which are hormones active in many developmental pathways (Chapter 1, Section 5.2.2). This may lead to changes in oxalic acid accumulation. This is an area uncertainty for the risk assessment.

90. ***Conclusion***: Risk scenario 2 is not identified as a substantive risk because the GM plant material would not be used as livestock feed, the proposed trial site is enclosed by a fence that would restrict access by livestock, and the small size and short duration of the proposed trial would minimise exposure of native mammals, birds, desirable insects or soil organisms to the GM plant material. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Introduced genes conferring herbicide tolerance and a dwarf phenotype
Causal pathway	⬇ Growing GM buffalo grass plants at the trial site ⬇ Persistence of GM plants after completion of the trial ⬇ Establishment of volunteer GM plants in the environment ⬇ Expression of introduced genes in the volunteer plants ⬇
Potential harm	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

91. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and dwarf phenotype.

Causal pathway

92. GM buffalo grass would be grown at the trial site. If the GMOs persisted at the trial site after completion of the trial either in the form of live GM plants, stolons or viable seed, this could lead to establishment of volunteer GM buffalo grass populations in the environment.

93. The applicant proposes to kill the GM buffalo grass at the end of the field trial by herbicide application, followed by manual removal of the plants and subsequent autoclaving of the plant material.

Considerations regarding the propagules of buffalo grass

94. Non-GM buffalo grass has been naturalised in Australia since the mid-1800s (Sauer, 1972). Buffalo grass has been identified as a weed of the natural environment, a weed escaped from cultivation and a weed of agriculture in Australia (Randall, 2017). In natural ecosystems, it is a weed known to be a minor problem warranting control at four or more locations within a State or Territory, while in agricultural ecosystems it is considered a minor problem, not important enough to warrant control (Groves et al., 2003). As a volunteer, buffalo grass can invade roadsides, gardens and lawns, grassland, river banks, swamps, coastal areas and disturbed sites (CABI, 2014). Weediness is linked with dispersal of stolons rather than seeds (Cook et al., 2005; CABI, 2014; HerbiGuide, 2014b).

95. Buffalo grass reproduces mainly asexually through the generation of stolons (Busey, 2003b). Stolons are branches that can develop roots when in contact with soil and hence either allow a plant to spread laterally or give rise to a new plant if the stolon detaches. It is possible that small fragments of stolons could survive the herbicide treatment at the end of the trial, remain in the soil and give rise to buffalo grass volunteers.

96. Some varieties of buffalo grass are also able to reproduce sexually (see Chapter 1, section 6.4). References describing seed production in buffalo grass are contradictory. Some report the number of seeds produced as low (Sauer, 1972; Busey, 2003b); however, others state that diploid varieties set a high percentage of viable seeds (Busey 2003b; Loch et al. 2009). Neither of these references quantifies the number of seeds. However, it is possible to estimate the number of seeds produced by fertile buffalo grass using information available in the literature. For example, the Australian variety 'Shademaster' was reported to have a high seed set and to produce 183.7 inflorescences per m^2 (Duff et al., 2009). Every inflorescence contains a minimum of 10-20 spikelets (Sauer, 1972), each containing one fertile floret (Cook et al., 2005; Clayton et al., 2006). If seed set is 60%, as reported for other fertile varieties in Busey (2003b), the high seed set variety 'Shademaster' is calculated to produce between 2,000 and 2,500 seeds per m^2.

97. The germination capability of buffalo grass seeds has been described to be poor or only reasonable in some references (The Buffalo Lawn Care; Beard, 2012) but also to be good in others (Busey, 2003b; Loch et al., 2009). There is uncertainty about the number of seeds, seed dormancy levels and the ability to germinate of the seed produced by the GM buffalo grass.

98. Buffalo grass volunteers could therefore also arise from seeds shed from the plants grown in the trial. However, the number of seeds remaining at the trial site would be expected to be low. As discussed in risk scenario 1, the trial would be small and only over one season, plants would be planted at a low density, maintained well and mowed frequently. The latter would remove most developing inflorescences and thereby reduce the number of seeds set during the trial.

Considerations regarding establishment of GM buffalo grass

99. The establishment rate of buffalo grass from stolons has been noted to be slow except in very warm climates (Aldous and Chivers, 2002), so in Victoria, buffalo grass may have a limited ability to establish.

100. Commercial propagation of buffalo grass is vegetative, therefore the available cultivars in the market are clones (Busey, 2003b). Self-pollination events or cross-pollination between plants belonging to the same cultivar produce inbred seed that give rise to seedlings with lower vigour

(Busey, 2003b; Kimball, 2015). Because all GM buffalo grass lines are the same cultivar, it is expected that most of the seed produced in the trial would be inbred and show inbreeding depression.

Persistence of GM buffalo grass

101. Even if volunteer plants survived initially, the GM buffalo grass may have limited ability to establish ongoing populations near the trial site. Buffalo grass is known to colonise areas of annual rainfall down to 750 mm (Cook et al., 2005). The average annual rainfall in Bundoora is 672.5 mm (Chapter 1, Section 6.1), and this may mean that GM buffalo grass volunteers in unirrigated areas would die during dry years.

102. However, the GM buffalo grass has a modified plant architecture due to the altered GA levels achieved by the overexpression of *GA 2-oxidase 3* (see Chapter 1, Section 5.2). Semi-dwarf varieties isolated from mutagenesis of other turf grass species are more tolerant to stresses such as shade and drought than the original varieties (Chen et al., 2016; Li et al., 2016). In these mutants, semi-dwarfism was linked to changes of GA endogenous levels or to the alteration of the GA perception pathway. It is therefore possible that the GM buffalo grass could be more tolerant to abiotic stresses. This is an area uncertainty for the risk assessment.

103. The applicant has proposed to destroy all plants after the trial, to monitor the trial site monthly for buffalo grass volunteers for 12 months after completion of the field trial, and to destroy any volunteers found. This is expected to minimise persistence of GM buffalo grass in the trial site.

Potential harm

104. A potential harm from volunteer GM buffalo grass populations would be toxicity or allergenicity to people. People do not consume buffalo grass plants, but they could be exposed to the GM buffalo grass through inhalation of pollen. As discussed in Risk Scenario 1, the risk to humans was assessed as negligible.

105. Another potential harm from volunteer GM buffalo grass would be toxicity to desirable animals. Volunteer GM buffalo grass plants could be eaten by livestock, native mammals, birds and insects. As discussed in Risk Scenario 2, the risk to desirable animals was assessed as negligible.

106. Volunteer GM buffalo grass plants are unlikely to reduce the establishment or yield of desirable plants to a greater extent than non-GM buffalo grass. In glasshouse experiments, the applicant has observed that the GM buffalo grass grows more slowly than non-GM plants under the same environmental conditions. Thus, the GM buffalo grass is expected to be less competitive than non-GM buffalo grass. The introduced glyphosate tolerance trait could allow GM buffalo grass volunteers to survive better than non-GM buffalo grass in areas where glyphosate application is used for weed management. However, GM buffalo grass volunteers can be controlled by a range of other herbicides as well as physical weed management techniques (Duff et al., 2009; HerbiGuide, 2014b).

Conclusion: Risk scenario 3 is not identified as a substantive risk because the proposed limits and controls would minimise the likelihood of persistence of the GMOs after completion of the trial, and buffalo grass has limited ability to establish ongoing volunteer populations in the geographic location of the trial. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	Introduced genes conferring herbicide tolerance and a dwarf phenotype
Causal pathway	⬇ Growing GM buffalo grass plants at the trial site ⬇ Dispersal of GM buffalo grass stolons and/or seeds outside the trial site ⬇ Establishment of volunteer GM plants in the environment ⬇

	Expression of introduced genes in the volunteer plants ⬇
Potential harm	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

107. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and dwarf phenotype.

Causal pathway

108. GM buffalo grass would be grown at the trial site and produce stolons and seeds. Stolons could spread outside the trial site by normal vegetative growth. Stolon fragments and seeds could be dispersed outside the trial site by wind or water, or by human or animal activity.

109. Buffalo grass spreads by branching stolons above the soil surface, with new stems and roots growing from nodes in the stolons (OGTR, 2018). The applicant has proposed that the trial site would be surrounded by a 2 m monitoring zone that is kept bare of vegetation and monitored weekly. Any buffalo grass stolons spreading into the monitoring zone would be destroyed. This measure would prevent spread of buffalo grass from the trial site by normal vegetative growth. If stolon fragments detached from the mother plant and were transported away, this could result in the dispersal of the GM buffalo grass outside the trial site.

110. The GM buffalo grass might be fertile to some extent. As discussed in risk scenarios 1 and 3, the proposed limits and controls would minimise the number of flowers on the GM buffalo grass plants, which would reduce the number of seeds produced in the trial site.

111. Upon maturation, buffalo grass seeds remain attached to the inflorescence called a rachis. The rachis breaks at branching nodes into squarish segments each one containing seeds (Busey, 2003b). These rachis segments could be spread by flooding. Although there is no information regarding seed dispersal by fresh water, the rachis of buffalo grass has been shown to float in salt water for up to 10 days and flotation in ocean currents has been proposed as the way of long-distance dispersal of the species (Sauer, 1972).

112. Stolon fragments and seeds could potentially be dispersed outside the trial site by human activity, by animal activity or by wind or water.

Potential for spread by human activity

113. GM buffalo grass plant material would be taken from the trial site for testing, and volunteer GM buffalo grass found in the monitoring zone or after completion of the trial would be removed for disposal. The trial plants would be regularly mown to simulate end user practices and remove inflorescences. The mowing debris, i.e. leaf clippings, may contain small stolon fragments or seeds (GMOs) that would be able to regenerate a plant if permitted to establish elsewhere. The applicant proposed that all GM plant material not required for testing would be destroyed. The equipment used in contact with the GMOs would be also cleaned before removal from the trial site or use for other purposes. These measures would minimise the likelihood of dispersal of GM buffalo grass by stolons and seeds by human activity.

Potential for spread by animal activity

114. Birds will rip buffalo grass to look for either grubs or the bulbs of some weed types (The Lawn Care Advice Site; Buffalo Lawn Care). This occurs if the grass is infested with pests or weeds. By foraging the lawn, birds could generate stolon fragments that could disperse by other means. The

applicant proposed to monitor the trial for pests and weeds and to carry out management when needed. The possibility of animals feeding on buffalo grass and dispersing stolons is unlikely as the proposed fence would restrict animal access, grass eaten would be digested by the animals and stolons do not possess structures facilitating animal dispersion. Also, since the trial site would be used to establish buffalo grass, there would be no complete buffalo grass coverage of the trial site for some time, particularly as the proposed planting density is lower than that typically used for lawn establishment and the GM buffalo grass has been modified for a dwarfing phenotype.

115. Although the fence surrounding the trial will restrict access by big grazing animals, birds and burrowing animals such as rabbits, rodents and seed-eating ants could enter the trial site to feed on buffalo grass and may ingest inflorescences containing seeds.

116. No scientific literature was found about the dispersal of buffalo grass seed by animals. Ants may transport seeds to nest sites over distances that are typically between tens of centimetres and a few metres (Gómez & Espadaler 1998). The seeds of buffalo grass do not have awns or hooks (OGTR, 2018), so they are not expected to adhere to animal fur or bird plumage. If buffalo grass seed is on the soil surface and conditions are wet, mud containing seeds could possibly stick to animal or bird feet and be transported outside the trial site. There is uncertainty about the ability of the seed to withstand digestion and be dispersed by endozoochory. However, the small size and duration of the trial will limit the number of animals in contact with buffalo grass seed and the proposed controls would reduce the number of seeds available for dispersal.

Potential for spread by wind or water

117. Stolon fragments from human or animal activity would be too heavy to be transported over long distances by wind but it is possible that GM buffalo grass rachis segments could be dispersed by high winds if a severe storm occurred while mature seed was present on plants or the soil surface. Stolon fragments and seeds could be transported by water during heavy runoff or flooding after a storm. However, the proposed trial site would be located away from any natural waterway, which would minimise the potential for stolon and seed dispersal through flooding. A standard licence condition would require the applicant to report any severe weather events.

118. As discussed in risk scenario 3, the GM buffalo grass is expected to have limited ability to establish ongoing volunteer populations in the environment near the trial site.

Potential harm

119. The potential harms from Risk Scenario 4 are the same as for Risk Scenario 3, which considered harms that may be caused by volunteer GM buffalo grass populations in the environment.

Conclusion: Risk scenario 4 is not identified as a substantive risk because the proposed controls would minimise the potential for dispersal of GM stolons and seeds, and buffalo grass has limited ability to establish ongoing volunteer populations in the geographic location of the trial site. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.5 Risk scenario 5

Risk source	Introduced genes conferring herbicide tolerance and a dwarf phenotype
Causal pathway	Growing GM buffalo grass plants at the trial site ⬇ Pollen flow to non-GM buffalo grass crops/lawns or volunteers outside the trial site ⬇ Establishment of hybrid GM plants in the environment ⬇ Expression of introduced genes in the plants
Potential harms	Toxicity or allergenicity to people OR Toxicity to desirable animals

	OR Reduced establishment or yield of desirable plants

Risk source

120. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and dwarf phenotype.

Causal Pathway

121. GM buffalo grass would be grown at the trial site and may produce pollen. If the GM pollen fertilised non-GM buffalo grass plants that flowered simultaneously, which could be turf crops, lawns or volunteers, the non-GM plants could produce hybrid GM seed. The seed could grow into volunteer GM buffalo grass plants in the environment (outside the trial site).

122. There is very little information available regarding pollination of buffalo grass. It can be inferred from published literature that buffalo grass can both self-pollinate (Genovesi et al., 2009) and outcross (Busey, 2003b). Outcrossing is most probably mediated by wind (OGTR, 2018), as happens in most grasses (Whitehead, 1969). There is no information available about the amount of pollen produced by this species or about how outcrossing rates decrease with distance.

123. Outcrossing of GM buffalo grass with most naturalised buffalo grass in Australia is not possible, since most naturalised buffalo grass belongs to the Cape deme race that is triploid and mostly sterile (Sauer, 1972; Loch et al., 2009). GM buffalo grass could outcross with fertile Australian and American cultivars of buffalo grass grown in Australia as turf crops and residential/urban lawns (Loch et al., 2009). There are no other species within or outside the *Stenotaphrum* genus that are sexually compatible with buffalo grass in Victoria (see Chapter 1, Section 6.4).

124. The overexpression of *GA 2-oxidase 3* in GM buffalo grass may delay flowering time as has been observed in GM-*Arabidopsis* and *Nicotiana* overexpressing *GA 2-oxidases* (see Chapter 1, Section 5.2.2,). This could mean that the flowering periods of GM buffalo grass and non-GM buffalo grass would not be synchronised. However, as non-GM buffalo grass flowers between December to February in Victoria (HerbiGuide, 2014a), there is likely to still be some overlap in flowering periods.

125. As discussed in risk scenario 1, the applicant proposes to fertilise and frequently mow the GM buffalo grass plants to reduce the number of flowers present in the trial. These measures together with the small size and limited duration of the trial will ensure that only small quantities of GM pollen are produced. Due to pollen competition, any non-GM buffalo grass growing in a nearby lawn would be far more likely to be self-pollinated or pollinated by neighbouring non-GM lawn plants than by a small amount of GM pollen that might be available to the plants in the lawn.

126. Any flower remaining in the GM buffalo grass after mowing would be close to the ground which would restrict the wind dispersal of pollen over long distances. The distance achieved by wind dispersal depends on the height of the pollen source, the terminal velocity of pollen, mean wind horizontal speed and turbulence (Burd and Allen, 1988; Kuparinen, 2006). Higher release points increase pollen-flight distances because they allow more horizontal movement relative to settling, wind speeds and turbulence are higher and there is less interference by vegetative structures that may intercept and stop pollen (Burd and Allen, 1988; Friedman and Barrett, 2009). In contrast, wind speed decreases closer to the ground due to the friction exerted on the air flow by the underlying surface (Burd and Allen, 1988; Okubo and Levin, 1989). In the case of the frequently mown GM buffalo grass, pollen producing flowers would be at the same height as the vegetative structures of the plant which would be highly likely to intercept the shed pollen. Therefore, it is expected that the pollen shed by the GM buffalo grass will not travel a long distance. The applicant has stated that the closest grasses to the field trial are located 30 m away.

127. Buffalo grass crops and lawns that may be recipients of the GM buffalo grass pollen are expected also to be mown regularly which will remove many of the receptive flowers from the plant.

In commercial turf production buffalo grass is mown to encourage vegetative growth (OGTR, 2018) while in lawns plants are mown for aesthetics.

128. Volunteer buffalo grass in urban areas may not be managed and may flower. If these volunteer plants are derived from Australian cultivars, they may be somewhat fertile.

129. Even if GM pollen reached other buffalo grass plants and fertilised some flowers, buffalo grass has a limited ability to establish from seed, as discussed in risk scenario 4.

130. Given that buffalo grass reproduces mainly asexually, even if a few GM volunteers were present in the environment, it is highly unlikely that the GM traits would introgress into larger buffalo grass populations.

131. As discussed in risk scenario 3, GM buffalo grass would have limited ability to establish ongoing volunteer populations in the environment near the trial site.

Potential harm

132. If hybrid GM buffalo grass seeds grew into volunteer plants in the environment, the potential harms would be the same as discussed in Risk Scenario 3.

Conclusion: Risk scenario 5 is not identified as a substantive risk because the proposed controls would minimise pollen flow to non-GM buffalo grass outside the trial site and buffalo grass has limited ability to establish ongoing volunteer populations in the environment near the trial site. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

133. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis[1].

134. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data,
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity, and
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity,
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

135. 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.

136. 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.

137. For DIR 156, uncertainty is noted particularly in relation to:

[1] A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

- potential for increased toxicity and allergenicity of the GM buffalo grass,
- potential for increased tolerance to abiotic stresses of the GM buffalo grass, and
- sexual reproduction of the GM buffalo grass, including:
  - (a) pollen number and outcrossing rates,
  - (b) number of viable seeds produced per plant, and
  - (c) seed dormancy and longevity.

138. 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.

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

Section 4 Risk evaluation

140. 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.

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

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

142. Five risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls 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:

- none of the GM plant material would enter human food or animal feed,
- no adverse health effects were observed in people handling the GM plants in glasshouse trials,
- buffalo grass has limited ability to establish ongoing volunteer populations in the environment near the trial site,
- limits on the size and duration are proposed for the release, and
- the controls proposed by the applicant to restrict the spread and persistence of the GM buffalo grass plants and their genetic material are suitable.

143. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM buffalo grass plants into the environment are considered to be 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 additional 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

144. 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.

145. 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.

146. 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 are also required to be reported to the Regulator.

147. 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

148. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM buffalo grass. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 3.1), the proposed containment measures (Chapter 1, Section 3.2), and the receiving environment (Chapter 1, Section 6), 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

149. 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 have been drafted 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 detail in Chapter 4 (the draft licence).

3.1 Draft licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by the Royal Melbourne Institute of Technology (RMIT) University

150. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by RMIT University in the application. These are taken into account in the five risk scenarios postulated for the proposed release in Chapter 2. Many of the proposed control measures are considered standard for

GM crop trials and have been imposed by the Regulator in previous DIR licences. The appropriateness of these controls is considered further below.

Limits

151. The applicant proposes that the duration of the field trial would be limited to one year and the size of the proposed planting area is 200 m^2. The small size and short duration of the trial would limit the potential exposure of people and desirable animals to the GMOs (risk scenarios 1 and 2).

152. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions require all people dealing with the GMOs to be informed of relevant licence conditions. These measures would limit the potential exposure of people to the GMOs (risk scenario 1).

153. The applicant proposes that no GM plant material would be used for human food or animal feed. This would minimise exposure of desirable animals to the GM buffalo grass by consumption (risk scenario 2). It should be noted that buffalo grass is not consumed by humans.

Controls for persistence or dispersal

154. The applicant proposes that any non-GM buffalo grass plants grown in the planting area would be treated as if they were GMOs. This is necessary as the non-GM buffalo grass plants could be fertilised by GM buffalo grass pollen and bear GM seed. This standard licence condition helps to minimise persistence or dispersal of GM buffalo grass seed (risk scenarios 3 and 4).

155. The applicant proposed to have a 2 m monitoring zone surrounding the planting area that will be kept bare of vegetation. This monitoring zone will be inspected every week during the trial to remove any stolons spreading across this area. Since the growth rate of non-GM buffalo grass is 35.4-55.8 cm/month in the peak growing season (Duff et al., 2009), a 2 m monitoring zone will be enough to ensure the GM buffalo grass plants do not spread beyond the proposed trial area.

156. The applicant proposed to frequently mow the buffalo grass plants to reduce the number of flowers present in the trial. The mowing residues or leaf clippings may contain GM buffalo grass stolons and seeds that could disperse in the environment (risk scenario 4). Buffalo grass can spread when garden waste containing seeds and stolons is dumped in areas where these propagules may establish, i.e. in bushland (HerbiGuide, 2014b; Brisbane City Council, 2016). A draft licence condition imposes that leaf clippings must be collected and disposed of in a way that ensures the destruction of stolons and seeds.

157. The applicant proposed that all equipment used with the GMOs would be cleaned before use for other purposes or removal from the trial site. The applicant also proposed to transport, store and destroy the GMOs in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. This would include any leaf clippings which may contain stolons and/or seeds. These controls would restrict the potential for dispersal of GMOs by people (risk scenario 4).

158. The applicant proposed that the field trial site would be located away from natural or artificial waterways. This is expected to manage the possibility of dispersal of GM buffalo grass stolons and seeds by flooding (risk scenario 4). Although the applicant did not mention a distance between the trial site and waterways, a standard licence condition requires that the outer edge of a planting area be at least 100 m away from waterways. Another consideration is that buffalo grass seed or stolons could be locally dispersed by high winds or heavy runoff in the event of a severe storm (risk scenario 4). A standard licence condition requires notification of any extreme weather condition affecting the trial site while GMOs are growing and until the site is signed off to allow assessment and management of any risks.

159. The applicant proposes that the planting area would be enclosed in a fence with lockable gates. This would minimise the potential for dispersal of GM seeds or vegetative parts from the planting areas by livestock and humans (risk scenario 4) as well as the exposure of livestock to the GMOs by consumption (risk scenario 2).

160. Buffalo grass is a perennial plant that can reproduce vegetatively. The applicant proposes to destroy all GMOs at the trial site at the end of the trial by herbicide application, removal from soil and autoclaving before disposal. A draft licence condition requires all GM plants in the field to be destroyed within 14 days after completion of the trial. This will help to restrict persistence of GM buffalo grass on the trial site (risk scenario 3).

161. Buffalo grass reproduces asexually through the generation of stolons and also sexually through the production of seeds. The applicant proposes to monitor the planting area for buffalo grass volunteers for 12 months after the end of the trial and to remove any volunteers. Removed volunteers would be autoclaved before disposal. A monitoring period of 12 months would be enough to detect and destroy any volunteers arising from stolons (risk scenario 3). Spring time will commence five months after the end of the trial, when buffalo grass volunteers will be easily detected due to their fast growth rate. Australian buffalo grass plants planted in September in Queensland had a mean plant diameter ranging from 106.1-167.4 cm three months after planting, which translates into a growth rate of 35.4 - 55.8 cm/month (Duff et al., 2009). Even if GM buffalo grass volunteers grow at half this rate in the next spring and summer after the trial they will still be easily detected and removed by people monitoring the area.

162. However, buffalo grass also produces seed and there is uncertainty about the dormancy and longevity of the seed in soil. Therefore, a draft licence condition will require monitoring the trial for at least 12 months and until the site is free of volunteers for at least nine consecutive months at the end of the monitoring period. In addition, another draft licence condition will require the applicant to irrigate the planting area every 35 days in Spring and Summer to promote germination and reduce persistence of GM seed. The proposed period of post-harvest monitoring, combined with the irrigation requirements, is considered appropriate to minimise persistence of GM buffalo grass (risk scenario 3).

163. The applicant proposed to perform post-harvest inspections monthly. This frequency is considered adequate to detect volunteers before flowering. Volunteers arising from seeds or small stolon fragments are unlikely to flower within one month and even if volunteers developed inflorescences as discussed in risk scenario 5, gene flow is highly unlikely. A draft licence condition requires post-harvest inspections to occur at least every 35 days to ensure that volunteers are found and destroyed.

Controls for pollen dispersal

164. The applicant proposes to manage pollen flow from the GM buffalo grass by mowing the buffalo grass frequently. The inflorescence of buffalo grass is a modified panicle with the branches partially embedded in hollows on one face or the sides of a corky rachis (Busey 2003b). As a result, the inflorescences look like thick spikes that stick out above the vegetative parts of the buffalo grass plants (The Buffalo Lawn Care). These inflorescences are 50-100 mm long and 5-10 mm wide (Sauer, 1972). Commercial suppliers recommend mowing buffalo grass to a height of 25-70 mm depending on the conditions where the grass is growing (The Buffalo Lawn Care; Lawn Solutions Australia; The Lawn Guide). In order to remove the most number of flowers, buffalo grass plants should not be permitted to grow taller than 50 mm. This is critical when the grass is likely to flower, i.e. from November to February (HerbiGuide, 2014b). This coincides with the time when buffalo grass grows most quickly. In summer, buffalo grass lawn can grow in height at a rate of 75 mm/week and it is recommended to mow the grass every 5-7 days (The Buffalo Lawn Care). A draft licence condition proposes that once the buffalo grass plants are established they have to be monitored weekly and mown to keep the plant height below 50 mm. This would reduce the number of flowers present at the trial and keep pollen levels low, preventing allergic reactions to pollen (risk scenario 1), outcrossing (risk scenario 5) and seed set (risk scenarios 3 and 4).

165. Buffalo grass can respond to stress by increasing its production of inflorescences and lengthening the period of flowering (The Buffalo Lawn Care). Stress levels can increase in buffalo grass lawns if the plants are suffering from lack of adequate water or nutrition or from other severe

conditions such as excessive cold, winds etc. The applicant proposed to frequently irrigate and fertilise the buffalo grass plants to avoid stress due to low water and nutrient levels. This will keep the number of flowers produced by the plants to normal physiological levels and in combination with mowing, decrease the likelihood of outcrossing (risk scenario 5) and reduce the number of seeds produced in the trial site (risk scenarios 3 and 4).

166. As discussed in risk scenario 5, no literature was found about the outcrossing rates of buffalo grass and how these decrease with distance. Both theoretical and experimental data for wind pollinated plants demonstrate that for an elevated pollen source, the maximum rate of deposition occurs some distance downwind that is roughly proportional to the square of the height of the source and that concentration then drops rapidly almost inversely proportional to the square of the distance (Whitehead, 1969). In a highly simplified model, the ballistic equation can be used to estimate the average dispersal distances of air-borne particles (Kuparinen, 2006). The distance a particle disperses from the release point to the deposition place x_d equals the height of the particle multiplied by the horizontal wind velocity under which the particle is released divided by the terminal velocity of the particle. Assuming a release height of 5 cm, a wind speed of 3 m/s (10.8 km/h, the annual average wind speed for Bundoora – Australian Bureau of Meteorology) and a pollen terminal velocity of 4.2 cm/s (average settling velocity of grass pollen particles in Borrell (2012)), the average dispersal distance of the GM buffalo grass pollen that is not intercepted by vegetative structures is estimated as 3.6 m. If the wind speed is 19.5 m/s (70 km/h, the strongest wind recorded in Bundoora in 2017 – WillyWeather), the estimated average dispersal distance is 23 m. This equation does not take into account vertical turbulence that is responsible for long distance travel of pollen particles; however long distance travel events are rare (Kuparinen, 2006). The applicant has stated that the closest grasses to the field trial are located 30 m away. An isolation distance of 30 m from other buffalo grass plants is expected to greatly reduce the potential for pollen flow. A draft licence condition proposes an isolation zone of 28 m surrounding the 2 m wide monitoring zone in which no non-GM buffalo grass must be grown. Combined with the measures aimed at reducing the potential for pollen production, the distance is considered adequate to reduce pollen flow to buffalo grass plants outside the trial.

3.1.2 Summary of draft licence conditions to be implemented to limit and control the release

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

- limit the duration of the release to between April 2018 and April 2019,
- limit the size of the release to 200 m^2 at one site in the local government area of Whittlesea, Victoria,
- locate the proposed field trial site at least 100 m away from the nearest natural or artificial waterway,
- surround the planting area with a monitoring zone of 2 m that is kept bare of vegetation. The monitoring zone is to be inspected weekly for the presence of any plants which must be destroyed,
- surround the monitoring zone with an isolation zone of at least 28 m free from other buffalo grass,
- monitor the buffalo grass weekly once established and mow before the grass grows taller than 50 mm,
- surround the trial site with a fence capable of excluding livestock and limiting unauthorised human access,
- clean the equipment prior to use for other purposes or removal from the trial site,
- collect all leaf clippings and dispose them by means preventing stolon and seed dispersal,

- transport, store and destroy GMOs including leaf clippings, in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs,*
- destroy all GMOs at the trial site at the end of the trial,
- irrigate the planting area after the removal of the GMOs to promote germination of volunteers,
- monitor the planting area and monitoring zone for at least 12 months and until the site is free of volunteers for at least 9 months, and
- not allow the GM plant material to be used as human food or animal feed.

3.2 Other risk management considerations

168. 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, and
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

169. 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, and
- the capacity of the applicant to meet the conditions of the licence.

170. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

171. 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

172. If a licence were issued, RMIT University would be required to 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 buffalo grass outside permitted areas.

173. RMIT University would also be required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. This methodology would be required before planting the GMOs.

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

174. If a licence were issued, the persons covered by the licence would be 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, RMIT University would be 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

175. If issued, the licence would require 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, and
- any unintended effects of the trial.

176. A number of written notices would also be required under the licence to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting,
- details of areas planted to the GMOs,
- expected dates of flowering,
- expected and actual dates of the end of the trial,
- dates of cleaning the planting areas, and
- details of inspection activities.

3.2.5 Monitoring for compliance

177. 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 site.

178. 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.

179. 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

180. Additional information has been identified that may be required to assess an application for a commercial release of this GM buffalo grass line or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM buffalo grass, particularly with respect to potential for increased toxicity or allergenicity,
- additional phenotypic characterisation of the GM buffalo grass plants, particularly with respect to potential for increased competitiveness and survival under abiotic stress,
- information regarding potential for seed production and seed dormancy of the GM plants, and
- information regarding potential for long distance pollen flow from buffalo grass.

Section 5 Conclusions of the consultation RARMP

181. The RARMP concludes that the proposed limited and controlled release of GM buffalo grass 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.

182. If a licence were issued, conditions would be 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.

Chapter 4 Draft licence conditions

Section 1 Interpretations and definitions

1. In this licence:

(a) unless defined otherwise, words and phrases used have the same meaning as they do in the Act and the Regulations;

(b) words importing a gender include any other gender;

(c) words in the singular include the plural and words in the plural include the singular;

(d) words importing persons include a partnership and a body whether corporate or otherwise;

(e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

(f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

(g) specific conditions prevail over standard conditions to the extent of any inconsistency.

2. In this licence:

'Act' means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State law under which this licence is issued.

'**Bare Fallow**' means land where no plants are grown intentionally, and the few plants that may grow unintentionally do not impede identification of Buffalo grass.

'Buffalo grass' means plants of the species *Stenotaphrum secundatum.*

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

(a) in relation to an area specified in this licence as requiring Cleaning, the Destruction of the GMOs in that area, to the reasonable satisfaction of the Regulator; or

(b) in relation to Equipment, the removal and/or Destruction of the GMOs, to the reasonable satisfaction of the Regulator.

'Contingency Plan' means a written plan detailing measures to be taken in the event of the unintended presence of the GMOs outside an area that must be inspected. A Contingency Plan must include procedures to:

(a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event; and

(b) recover and/or Destroy the GMOs; and

(c) inspect for and Destroy any Volunteers that may exist as a result of the event.

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

(a) manual uprooting;

(b) treatment with herbicide;

(c) burning/incineration;

(d) autoclaving;

(e) a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of plants with mature seed heads still attached, treatment with herbicide would not be appropriate as it would not destroy viable seeds.

'Equipment' includes, but is not limited to, lawn mowers, spray irrigation equipment, transport equipment (e.g. bags, containers), clothing and tools.

'Flowering' is taken to begin when any plant of the class of plants referred to in a particular condition first flowers, and is taken to end when all plants in the class of plants no longer have flowers.

'GM' means genetically modified.

'GMOs' means the genetically modified organisms that are the subject of the dealings authorised by this licence. GMOs include live plants, stolons that are able to grow into live plants, and viable seed.

'Harvest' means removal of the GMOs from the Planting Area.

'Isolation Zone' means an area of land extending outwards at least 28 m in all directions from the outer edge of the Monitoring Zone, as indicated in Figure 1.

'Logbook' means a written or electronic record containing information required to be collected and maintained by this licence and which is able to be presented to the Regulator on request.

'Monitoring Zone' means an area of land extending outwards 2 m from the edge of the Planting Area, as indicated in Figure 1. Land defined as a Monitoring Zone while the field trial is in place continues to be defined as a Monitoring Zone until Sign-off.

'OGTR' means the Office of the Gene Technology Regulator.

'Personal Information' means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

(a) whether the information or opinion is true or not; and

(b) whether the information or opinion is recorded in a material form or not.

'Planting Area' means an area of land where the GMOs and non-GM Buffalo grass are planted and grown pursuant to this licence.

'Plant Material' means any part of the GM or non-GM Buffalo grass plants grown at a Planting Area, whether viable or not, or any product of these plants.

'Regulations' means the Gene Technology Regulations 2001 (Commonwealth) or the corresponding State law under which this licence is issued.

'Regulator' means the Gene Technology Regulator.

'Sign-off' means a notice in writing from the Regulator, in respect of an area, that post-harvest obligations no longer apply in respect of that area.

'Volunteers' means GM or non-GM Buffalo grass plants that have not been intentionally grown.

'Waterways' means all permanent natural waterways and man-made waterways that flow into natural waterways.

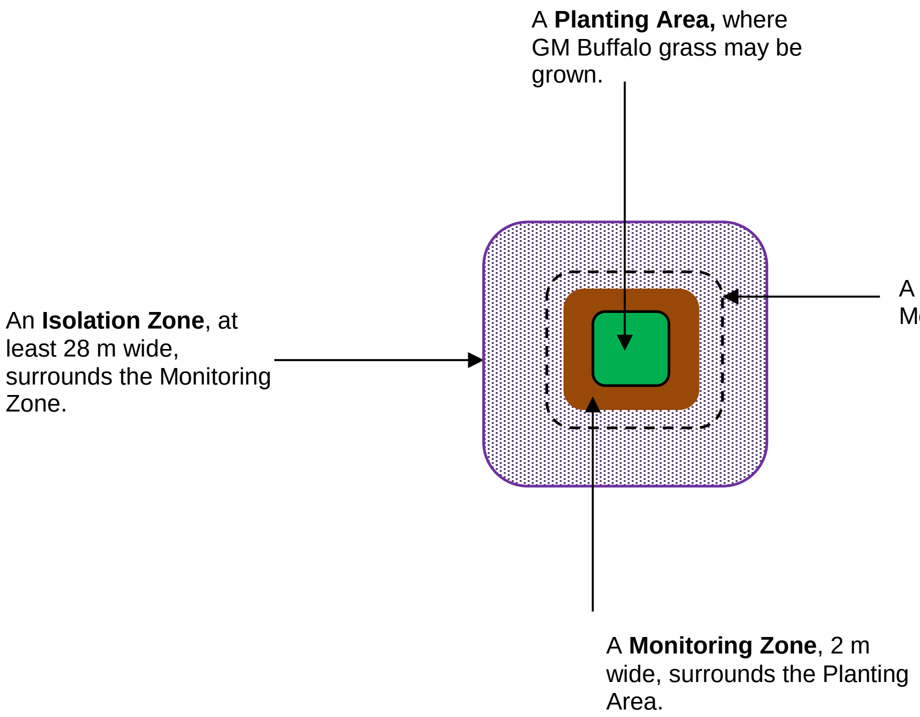

Figure 1. Diagram showing the relationships between the Planting Area, Monitoring Zone and Isolation Zone.

Section 2 General conditions and obligations

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

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

5. The holder of this licence ('the licence holder') is the Royal Melbourne Institute of Technology (RMIT) University.

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

7. The dealings authorised by this licence are to conduct experiments with the GMOs, grow the GMOs, transport and dispose of the GMOs, and possession, supply or use of the GMOs in the course of any of these dealings.

Obligations of the Licence Holder

8. The licence holder must notify the Regulator in writing as soon as practically possible if any of the contact details of the project supervisor change from those notified in the licence application or subsequently.

Note: address correspondence to ogtr.applications@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following conditions address ongoing suitability of the licence holder.

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

10. The licence holder must:

(a) inform the Regulator immediately in writing, of:

i. any relevant conviction of the licence holder occurring after the commencement of this licence; and

ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; and

iii. any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it; and

(b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested, within the stipulated timeframe.

11. The licence holder must be able to access and control the Planting Area, Monitoring Zone, Isolation Zone and approved facilities (if any) to the extent necessary to comply with this licence, for the duration of the licence.

The following conditions seek to ensure that persons conducting the dealings are aware of the licence conditions and appropriate processes are in place to inform people of their obligations.

12. Prior to conducting any dealings with the GMOs, the licence holder must provide to the Regulator:

(a) names of all organisations and persons or functions or positions of the persons who will be covered by the licence, with a description of their responsibilities; and

Note: Examples of functions or positions are 'project supervisor', 'site manager', 'farm labourer' etc.

(b) detail of how the persons covered by the licence will be informed of licence conditions; and

(c) detail of how the licence holder will access and control the Planting Area, Monitoring Zone, Isolation Zone and approved facilities (if any) for the duration of the licence; and

Note: this may include a description of any contracts, agreements, or other enforceable arrangements.

(d) written methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism; and

(e) a Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected.

13. Any changes to the information provided under the immediately preceding condition must be communicated in writing to the Regulator within 14 days of the changes occurring.

14. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

(a) the particular condition (including any variations of it); and

(b) the cancellation or suspension of the licence; and

(c) the surrender of the licence.

15. The licence holder must not permit a person covered by this licence to conduct any dealing with the GMOs unless:

(a) the person has been informed of any applicable licence conditions, including any variation of them; and

(b) the licence holder has obtained from the person a signed and dated statement that the person:

i. has been informed by the licence holder of the licence conditions including any variation of them; and

ii. has understood and agreed to be bound by the licence conditions, or variation.

16. The licence holder must:

(a) inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator; and

(b) provide the Regulator, if requested, with copies of the signed and dated statements referred to in the immediately preceding condition.

Provision of new information to the Regulator

Licence conditions are based on the Risk Assessment and Risk Management Plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment or risk management is communicated to the Regulator.

17. The licence holder must inform the Regulator if the licence holder becomes aware of:

(a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

(b) any contraventions of the licence by a person covered by the licence; or

(c) any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

(a) the licence holder will be taken to have become aware of additional information of a kind mentioned in paragraph 17(a) if he or she was reckless as to whether such information existed; and

(b) the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in paragraph 17 (b) or 17(c) if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note: Contraventions of the licence may occur through the action or inaction of a person. For example if it is a condition of the licence that Volunteers are Destroyed prior to Flowering and a Volunteer flowers, then the person responsible for controlling Volunteers will have contravened that licence condition.

18. If the licence holder is required to inform the Regulator under the immediately preceding condition, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of the incident via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours. Notification without delay will allow the Regulator to conduct a risk assessment on the incident, determine appropriate risk management measures and attend the location, if required.

19. If the licence holder informs the Regulator under the immediately preceding condition and the Regulator requests further information, such information must be provided in a manner, and within the time period, stipulated by the Regulator.

Obligations of persons covered by the licence

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

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

Section 3 Limits and control measures

Limits on the release

The following licence conditions maintain the risk assessment context within which the application was assessed, by imposing limits on where and when the GMOs may be grown, and on other activities that can be undertaken.

22. The only plants that may be intentionally grown at a Planting Area are:

(a) the GMOs covered by this licence as described in Attachment A of the licence; and

(b) non-GM Buffalo grass plants.

Note: Attachment A is not included in the draft licence as the plants are described in the Risk Assessment and Risk Management Plan.

23. Planting and growing of the GMOs may only occur within the following limits:

Period	Maximum number of Planting Areas per year	Maximum size of Planting Area	Local Government Area in which Planting Area may be located
April 2018 – April 2019	1	200 m^2	Whittlesea

24. Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in its use as food for humans or feed for animals.

Control measures

The following licence conditions maintain the risk assessment context within which the application was assessed by restricting spread and persistence of the GMOs.

Controls to minimise pollen, stolon and seed dispersal during cultivation

25. The outer edge of any Planting Area must be at least 100 m away from Waterways.

26. Any extreme weather event that is expected to affect or has already affected a Planting Area or associated areas, while the GMOs are growing or while the Planting Area is subject to inspection requirements, must be notified in writing to the Regulator as soon as practically and reasonably possible.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 46).

27. Non-GM Buffalo grass plants grown in a Planting Area must be handled as if they were the GMOs.

28. The Planting Area must be surrounded by a 2 m wide Monitoring Zone that is kept Bare Fallow. Any Buffalo grass in the Monitoring Zone must be Destroyed as soon as possible.

29. The Monitoring Zone must be surrounded by an Isolation Zone in which no Buffalo grass must be present.

30. The Monitoring Zone must be surrounded by a fence with lockable gates capable of restricting unauthorised access and excluding livestock.

31. The Buffalo grass plants in the Planting Area must not be permitted to grow taller than 50 mm.

32. All mowing clippings must be collected and Destroyed as soon as possible to prevent dispersal of the GMOs.

Note: If the mowing clippings are transported off the Planting Area for Destruction then transport must comply with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

33. The Buffalo grass plants in the Planting Area must be irrigated and fertilised as required to minimise the induction of a stress response in the plants.

34. Any Equipment used in the Planting Area must be Cleaned before removal from the Planting Area.

35. Inspections must be conducted by people trained to recognise Buffalo grass, and actions taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
(a) Planting Area	First inspection must occur at least 14 days prior to the expected commencement of Flowering of any GMOs*, and inspections must continue until all GMOs in the Planting Area have finished Flowering	At least once every 7 days	The height of the Buffalo grass	Mow Buffalo grass to keep its height below 50 mm
(b) Monitoring Zone	First inspection must occur 7 days after planting and until the GMOs have been Destroyed.	At least once every 7 days	Plants	Destroy
(c) Isolation Zone	From 14 days prior to the expected commencement of Flowering of any GMOs*, and until all GMOs in the Planting Area have finished Flowering	At least once every 7 days	Buffalo grass	Destroy
(d) Fence	First inspection must occur 7 days prior planting and until the GMOs have been Destroyed.	At least once every 14 days	Damage that may permit livestock or unauthorised persons to enter	Repair as soon as practicable

**Condition 48 (b) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.*

Note: Details of any inspection activity must be recorded in a Logbook as detailed in Condition 48(f).

36. All Buffalo grass in the Planting Area must be Destroyed by the end of April 2019.

Controls during processing or experimentation with GMOs

37. If experimentation or analysis with the GMOs is not conducted under a NLRD authorisation, such activities may only be undertaken within:

(a) the Planting Area before Cleaning; or

(b) a facility approved in writing by the Regulator.

Note: Dealings conducted under a NLRD authorisation must be assessed by an Institutional Biosafety Committee before commencement, must comply with the requirements of the Regulations, and are not subject to the conditions of this licence.

38. Within a facility approved under the immediately preceding condition, any area that is used for processing, experimentation or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

Controls during transport or storage of the GMOs

39. If transport or storage of the GMOs is not conducted under a NLRD authorisation, such activities must:

(a) only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation; and

(b) be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* for PC2 GM plants as current at the time of transportation or storage; and

(c) comply with all other conditions of this licence.

Note: Condition 15 requires signed statements for persons transporting or disposing of the GMOs.

40. Methods and procedures used to transport GMOs must be recorded, and must be provided to the Regulator, if requested.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 46).

Controls regarding the persistence of the GMOs

41. The Planting Area and Monitoring Zone must be Cleaned within 14 days after completion of Harvest.

42. After Cleaning, the Planting Area and associated areas of land must be inspected by people trained to recognise Buffalo grass. Inspections must cover the entirety of areas to be inspected. Actions must be taken as follows:

Area of land	Period of inspection	Inspection frequency	Inspect for	Action
(a) Planting Area (b) Monitoring Zone (c) Any other area where GMOs have dispersed during planting, growing or Destruction	From the day of completion of Cleaning of the Planting Area and Monitoring Zone, until the Regulator has issued a Sign-off for the area.	At least once every 35 days	Buffalo grass Volunteers	Destroy

43. Details of any inspection activity must be recorded in a Logbook and must include:

(a) date of the inspections;

(b) name of the person(s) conducting the inspections;

(c) details of the experience, training or qualification that enables the person(s) to recognise Buffalo grass, if not already recorded in the Logbook;

(d) details of areas inspected including current land use;

(e) details of the developmental stage of the GMOs while they are being grown;

(f) details of any post-harvest rainfall events, including measurements at or near the area, or any irrigation events including the amount of water applied;

(g) for post-harvest areas, details of any recent management practices applied (including herbicide applications)

Note: this may include spraying or maintenance measures used to facilitate inspections for Volunteers

(h) details of any Volunteers observed during post-harvest inspections or land-management activities, including number, developmental stage and approximate position of the Volunteers within each area inspected ⌘;

(i) date(s) and method(s) of Destruction of any Buffalo grass Volunteers;

(j) details of any damage and any repairs to the fence surrounding the Monitoring Zone.

⌘ *Examples of acceptable ways to record the positional information for Volunteers in the Logbook include:*

- descriptive text

- marking on a diagram or

- indicating grid references on corresponding map/sketch.

Note: Details of Inspection activities must be provided to the Regulator (Condition 48(f)).

44. While post-harvest inspection requirements apply to the Planting Area, Monitoring Zone and any associated areas:

(a) the area must be maintained in a manner appropriate to allow identification of Volunteers;

(b) the area must not be grazed by livestock; and

(c) no plants may intentionally be grown in the area unless the plants are agreed to in writing by the Regulator.

45. After Harvest of the Planting Area and prior to an application for Sign-off, the Planting Area and Monitoring Zone must be irrigated at least once every 35 days between October and February each year.

Note: A period of natural rainfall may be taken as irrigation only with the agreement of the Regulator. Evidence (such as rainfall measurements, photos etc.) that the rainfall has been sufficient to promote germination should be provided.

Contingency Plan

46. If any unintentional presence of the GMOs is detected outside the areas requiring inspection, the Contingency Plan must be implemented.

Section 4 Sign off

47. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and associated areas if:

(a) all post-harvest inspection activities have been conducted for at least 12 months on these areas;

(b) conditions have been conducive for germination and detection;

(c) the Planting Area and Monitoring Zone have been irrigated as required under condition 45; and

(d) no Volunteers that are GMOs have been detected on these areas in the most recent nine month inspection period

Note: The Regulator will take into account the management and inspection history for the Planting Area and associated areas, including post-harvest irrigation, rainfall, application of herbicide and occurrence of Volunteers, in deciding whether or not further inspections are required to manage persistence of the GMOs.

Section 5 Reporting and documentation

The following licence conditions are imposed to demonstrate compliance with other conditions, facilitate monitoring of compliance by staff of the OGTR, and emphasise appropriate selection of the Planting Area.

48. Notifications must be sent to the Regulator as follows:

Notice	Content of notice	Timeframe
(a) Before the trial	i. Names of all organisations and persons who will be covered by the licence, with a description of their responsibilities, ii. Detail of how the persons covered by the licence will be informed of licence conditions, iii. Detail of how the licence holder will access and control the Planting Area, Monitoring Zone, Isolation Zone and approved facilities (if any) for the duration of the licence, iv. Written methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism, and v. A Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected.	Prior to planting (to be updated immediately if the notified details change)
(b) Intention to Plant	i. Details of the Planting Area including size, GPS coordinates, a street address or other directions and a diagrammatical representation of the site (e.g. Google Maps), ii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details), iii. Date on which the GMOs will be planted, iv. Period when the GMOs are expected to Flower, v. Date of the Harvest, vi. Details on how all areas requiring post-harvest inspections are intended to be used until Sign-off, vii. Details on how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers or Buffalo grass plants growing in the Isolation Zone, and viii. History of how the site has been used for the previous two years.	At least 7 days prior to planting (to be updated immediately if the notified details change)
(c) Planting	i. Actual date(s) of planting the GMOs, and ii. Any changes to the details provided under part (b) of this condition.	Within 7 days of any planting
(d) Destruction	Actual date(s) of Destroying the GMOs.	Within 7 days of commencement of any Destruction activities.

Notice	Content of notice	Timeframe
(e) Cleaning	i. Actual date(s) on which any areas needing Cleaning were Cleaned, and ii. Method of Cleaning	Within 7 days of completion of any Cleaning
(f) Inspection activities	Information recorded in a Logbook as per the inspection requirements (Conditions 35, 43 and 48).	Within 35 days of inspection

Note: Other reports and documents that may need to be sent to the Regulator are described under Conditions 8, 10, 12, 17 and 26.

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