Australian Government
Department of Health
Office of the Gene Technology Regulator

April 2018

Risk Assessment and Risk Management Plan

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

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 156

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concluded that the field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Application number	DIR 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 are taken into account in the risk assessment.

Pathways which may result in harm that were considered included exposure of people or animals to the GM plant material, likelihood of 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 imposed limits and controls effectively contain the GMOs and minimise exposure to the GMOs and their genetic material.

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 the conditions of the licence.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in 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.

Table of Contents

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).

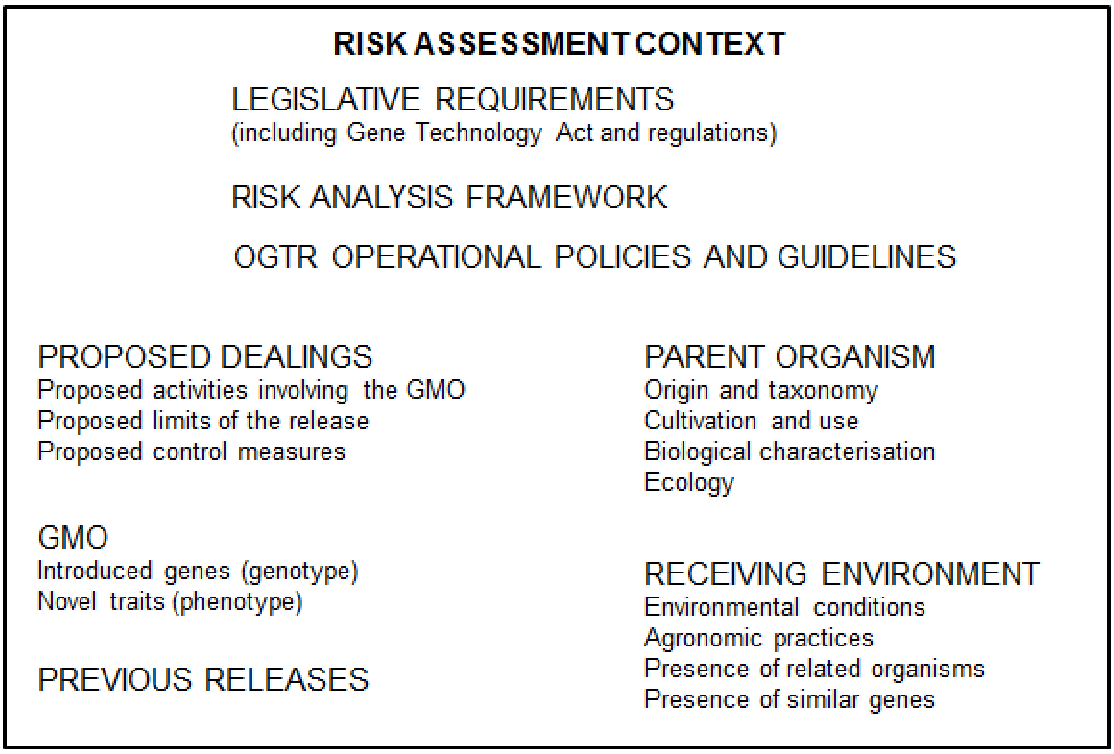

Figure 1. Summary of parameters used to establish the risk assessment context

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 the Regulator is satisfied that: (a) its principal purpose is to enable the applicant to conduct experiments; (b) 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; and (c) the proposed limits and controls were of a kind that it was appropriate for the Regulator not to seek advice from 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. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. One public submission was received and it is summarised and addressed in Appendix B.

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 (APVMA), the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. For example, application of glyphosate on the GM buffalo grass may be subject to regulation by the APVMA. 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 will be grown on one trial site with an area of up to 200 m^2. The trial site will be located in Bundoora, in the local government area of Whittlesea in Victoria[1].

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

13. The field trial will be located at the RMIT University East Bundoora Campus, in a suburban area of Melbourne. The field trial will be conducted in a 15 x 15 m fenced area, with the fence being more than 2.4 m high. The field trial will be located in an asphalted area close to the boundaries of the campus with the two outward-facing sides surrounded by an embankment which is at least 2 m high. Beyond the embankment are private houses (Figure 2). The closest private houses are approximately 40 m away from the field trial.

[1] During the consultation period, the applicant supplied details of the trial site. The RARMP and licence were amended to reflect this change in risk assessment context.

14. Approximately 200 m away from the field trial there is a large area (868.6 hectares) of parkland. This parkland contains waterholes. Two of these are between 250 m and 575 m away from the field trial.. They are connected to a river which is situated at least 700 m away from the field trial site.

15. Inside the RMIT campus there are five storm drains within 60 m of the field trial. The suburbs surrounding RMIT have storm water drainage systems for the roads. The field trial is surrounded on its north and east sides by elevated terrain of at least 2 m high (Figure 2). This suggests that any water run-off will flow from these elevated areas into the RMIT campus asphalted area and water will not flow out from the RMIT campus.

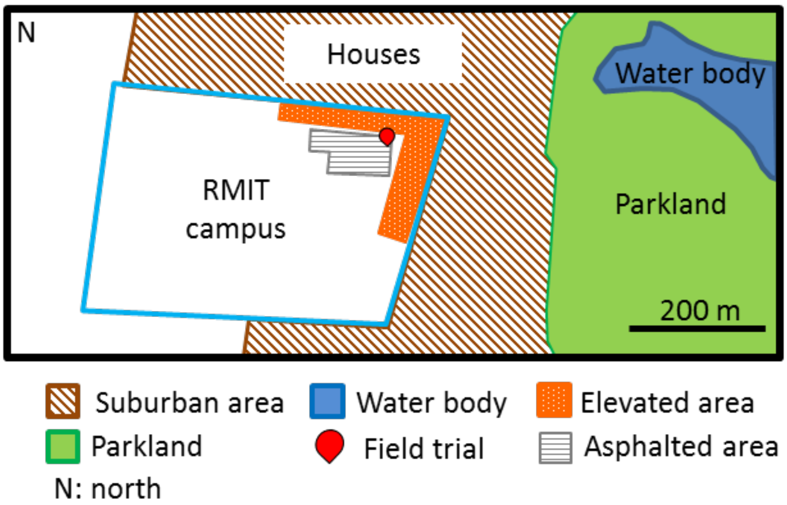

Figure 2. Schematic representation of the surroundings of the field trial site.

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

16. 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

17. The parent organism is buffalo grass (*Stenotaphrum secundatum* (Walt.) *Kuntze*) which is an introduced species in 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).

18. 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

19. 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*)
SpH3’	Transcriptional terminator	*Histone H1*	Wild tomato species (*Solanum pennellii*)

20. 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.

21. 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)

22. 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.

23. 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 3). 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).

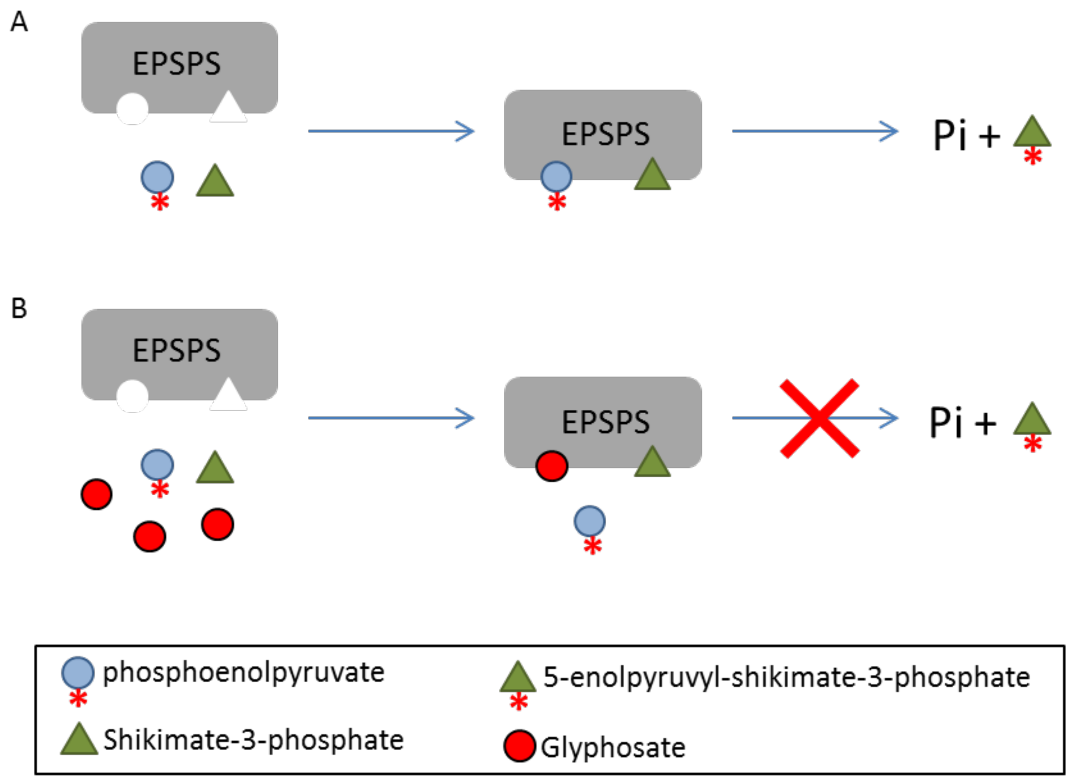

Figure 3. Glyphosate inhibition of EPSPS. (A) EPSPS catalyses the transfer of the enolpyruvyl moiety from phosphoenol pyruvate to shikimate-3-phosphate forming the products 5-enolpyruvyl-shikimate-3-phosphate and inorganic phosphate (Pi). (B) Glyphosate inhibits this reaction by binding EPSPS and precluding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

24. 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). One report found that overexpressing exogenous EPSPS protein in GM thale cress increases their fecundity and auxin content (Fang et al., 2018).

25. 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).

5.2.2 The GA 2-oxidase 3 gene from spinach

26. 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

27. 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 family (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.

28. 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.

29. 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).

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

31. 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

32. 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.

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. The applicant has not provided information that would demonstrate an increase in fecundity and/or auxin content from the over-expressed EPSPS.

Section 6 The receiving environment

33. 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).

34. 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

35. The release will take place in Bundoora, 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).

36. 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 as long as water supply is adequate, the plant can endure high temperatures (OGTR, 2018).

37. 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

38. 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).

39. 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).

40. 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).

41. 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). 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).

42. 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

43. 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, and the plants irrigated as necessary. 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. Mowing, irrigation and fertilizer application promote vegetative growth in buffalo grass. 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

44. 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 genetically very 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).

45. There are seven species of *Stenotaphrum* (Sauer, 1972). Only *S. micrathum* has been described in Australia. This is found on 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.

46. 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

47. 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

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

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

7.1.2 Approvals by other government agencies

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

7.2 International approvals

GM buffalo grass with the same introduced genes was to be released in a field trial in the USA in July 2012. However, GMO legislation in the USA is different to that in Australia, and USDA determined that the GM buffalo grass plants were not considered regulated. This decision was made because the parent plant was not considered a pest, the introduced genetic material was not sourced from pest organisms and the method used to genetically modify the plant did not involve plant pests (USDA letter re transgenic *Stenotaphrum*). Since it is not regulated as a GMO in the USA, it is unknown whether the GM buffalo grass is grown there.

Chapter 2 Risk assessment

Section 1 Introduction

51. 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 4). 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.

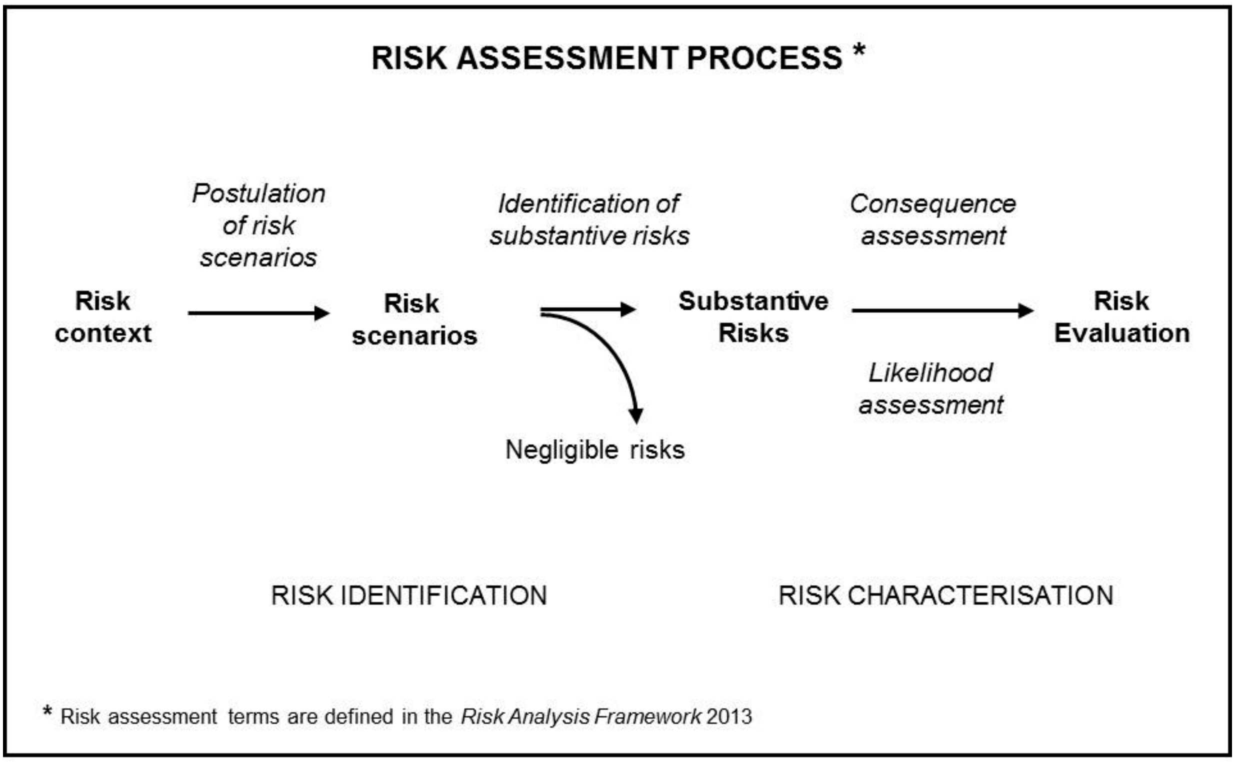

Figure 4. The risk assessment process

52. 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.

53. 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.

54. 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.

55. 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

56. 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.

57. 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

58. 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.

59. 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.

60. 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.

61. 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

62. 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.

63. 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.

64. 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.

65. 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

66. 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).

67. 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

68. 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.

69. 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

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

Causal pathway

71. 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.

72. 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; and through inhalation when mowing the grass. It is expected that research staff will follow standard Occupational Health and Safety guidelines to minimise exposure to GMOs, e.g. wearing protective clothing when working in the trial site or wearing dust masks when mowing the GM buffalo grass. Following these guidelines will reduce the exposure of the research staff to the GMOs.

73. The applicant proposes a fence with lockable gates surrounding the trial site and monitoring zone, which would restrict unauthorised access to the trial. Although the field trial is situated within the RMIT University campus not many people are expected to pass near trial site as it is located on the outer fringes of the campus. Passers-by could be exposed to GM buffalo grass pollen, and residents of the houses in the vicinity might be exposed to some pollen of the GM buffalo grass in severe winds.

74. As in most grasses (Whitehead, 1969), outcrossing of buffalo grass is most probably mediated by wind (OGTR, 2018). 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 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.

75. The applicant has proposed measures to minimise the number of flowers that could be produced in the trial site. The plants will be mown frequently and fertiliser will be added to the plants when required. These measures encourage vegetative growth of the GM buffalo grass over reproductive growth (see section 3.1.1, chapter 3 for discussion).

76. These measures together with the small size of the trial and the low density of plants at planting are expected to result in a small number of flowers present in the trial and consequently, in low concentrations of GM buffalo grass pollen. 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). 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

77. 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).

78. 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).

79. 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).

80. 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* and/or of *GA 2-oxidase 3* may 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.

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

82. ***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, 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

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

Causal pathway

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

85. 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.

86. 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, would restrict the numbers of native animals, including birds that could be exposed to the GM plants.

87. 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.

88. 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

89. 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.

90. 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* and/or of *GA 2-oxidase 3* may lead to changes in oxalic acid accumulation. This is an area of uncertainty for the risk assessment.

91. ***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

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

Causal pathway

93. 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.

Considerations regarding the propagules of buffalo grass

94. 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. If fragments of stolons remained on the site at the end of the trial, these could give rise to buffalo grass volunteers.

95. Some varieties of buffalo grass are also able to reproduce sexually (see Chapter 1, section 6.4). Publications 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. The overexpression of *GA 2-oxidase* genes in GM plants has been found to affect flowering time and reduce seed set, while the overexpression of EPSPS has been found to lead to increases in the number of seeds produced by GM plants (see section 5.2, chapter 1). No information was provided by the applicant regarding the fecundity of the GM buffalo grass. This is an area of uncertainty in the risk assessment.

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

97. Therefore, buffalo grass volunteers could arise from seeds shed from the plants grown in the trial. However, the number of seeds remaining at the trial site is expected to be low (see discussion in risk scenario 1 and chapter 3). The trial would be small and only over one season, plants would be planted at a low density, maintained well and mown frequently. These limits and controls will ensure that few flowers will be produced during the trial.

Considerations regarding establishment and persistence of GM buffalo grass

98. 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.

99. Commercial propagation of buffalo grass is vegetative, and, therefore, the available cultivars in the market are clones (Busey, 2003b). Self-pollination events or cross-pollination between plants of 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 the seed produced during the trial would be inbred and show inbreeding depression.

100. Even if volunteer plants survived initially after the trial, 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). This may mean that GM buffalo grass volunteers in unirrigated areas would die during dry years.

101. 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). Non-GM 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 in endogenous levels of GA 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.

102. 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, and the requirement for a volunteer-free period of at least 9 months (see discussion in chapter 3 section 3.1.1) will minimise the likelihood of persistence of GM buffalo grass at the trial site.

Management of GM buffalo grass

103. Should the GM buffalo grass persist at the trial site leading to dispersal into the local environment, volunteers can be controlled by a range of other herbicides as well as physical weed management techniques (Duff et al., 2009; HerbiGuide, 2014b). Because buffalo grass does not produce rhizomes, the removal of above ground stolons and vegetative parts is sufficient to kill the plant since the grass cannot regenerate itself (The Buffalo Lawn Care). For small invaded areas, digging up the grass followed by disposal is a recommend practice, although for big areas it creates a disposal problem (Auckland Council). Other recommended practices to manage weedy buffalo grass are solarisation, the use of weed mats and repeated cultivation (HerbiGuide, 2014; Weedbusters; Auckland Council). These physical methods would be also effective in managing the GM buffalo grass. Herbicides recommended to kill buffalo grass, apart from glyphosate, are haloxyfop, quizalofop and fluazifop (HerbiGuide, 2014). These could also be used to manage the GM buffalo grass. Buffalo grass can spread to wetlands or other aquatic environments which are sensitive to herbicide contamination (Water and Rivers Commission, 2001). Seven different herbicides, including glyphosate, are registered for use in aquatic environments (APVMA PubCRIS database). These could be potentially used to manage the GM buffalo grass near aquatic environments. Currently, the only herbicide registered to manage buffalo grass growing near aquatic environments is glyphosate.

Potential harm

104. A potential harm from volunteer GM buffalo grass populations at the trial site 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.

Reduced establishment or yield of desirable plants

106. 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).

107. 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. If the GM buffalo grass persisted at the trial site, then was able to establish and persist in an area where glyphosate is applied to manage weeds, then the glyphosate tolerance of the GM buffalo grass would be an advantage. However, the glyphosate tolerance trait can be easily detected upon glyphosate application and alternative destruction methods employed, e.g. other herbicides could be used or the plants could be removed mechanically (see pathway considerations for details).

108. If the introduced glyphosate tolerance trait by overexpression of EPSPS was to be inherited separately from the dwarfing modification in offspring, this could lead to greater fitness of the GM

buffalo grass. GM thale cress plants overexpressing EPSPS have been found to produce more seeds than the non-GM counterparts (Fang et al., 2018). GM buffalo grass overexpressing only EPSPS may have normal growth and may produce more seeds and, therefore, may have a greater potential for spreading. However, the *EPSPS* and *GA 2-oxidase 3* genes were introduced on a single expression cassette, i.e. they are adjacent in the genome which means it is highly unlikely that they would be inherited separately in the offspring.

109. ***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

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

Causal pathway

111. 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.

112. 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.

113. 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.

114. The GM buffalo grass might be fertile to some extent. As discussed in risk scenario 1, the proposed limits, controls and management would minimise the number of flowers on the GM buffalo grass plants, and the removal of developing inflorescences by mowing will further reduce the potential for seed set. Therefore, the number of seeds available for dispersal is expected to be low.

115. 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 or wind. 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).

116. 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

117. 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 applicant proposed to follow the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. The trial plants would be regularly mown to simulate normal cultivation practices, stimulate vegetative growth and remove developing 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. This includes the lawn mower and the protective equipment worn by research staff when handling the GM buffalo grass. The licence imposes inspecting the trial site for stolon fragments after mowing and destroying any stolons found. The combination of all these measures would minimise:

- the amount of reproductive material available for dispersal by any means and
- the likelihood of dispersal of GM buffalo grass stolons and seeds by human activity.

Potential for spread by animal activity

118. The fence surrounding the trial will restrict access by grazing animals; however, birds and burrowing animals such as rabbits, rodents and seed-eating ants could enter the trial site to feed on buffalo grass.

119. For animal dispersal of stolon fragments, animals would have to detach a fragment big enough to contain a node capable of producing roots, carry it off the trial site and drop the fragment in an area where buffalo grass can establish and persist.

120. The possibility of animals feeding on buffalo grass and dispersing stolons is unlikely as the grass would be digested by the animals and stolons do not have structures for animal dispersal. Birds will rip up 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 be dispersed by other means. The applicant proposed to monitor the trial for pests and weeds and to carry out management as needed.

121. No scientific literature was found about the dispersal of buffalo grass seed by animals, and the seed is not known to be attractive to animals.

122. Ants may transport seeds to nest sites over distances that are typically between tens of centimetres and a few metres (Gómez & Espadaler 1998) and, therefore, will not likely disperse the GM seed beyond the trial site. 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 or stolon fragments 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, animals are not expected to be attracted to buffalo grass seed because the seeds are very small (OGTR, 2018). This is in contrast to crop plants such as wheat and sorghum. In addition, the limits, controls and management will reduce the amount of available viable seeds and stolon fragments (see risk scenarios 1 and 3).

123. The small size and duration of the trial will also limit the number of animals that could come into contact with the GM buffalo grass. In particular, since the trial would be used to establish the GM buffalo grass, the GM buffalo grass would not fully cover the trial site for some time, especially 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.

Potential for spread by wind or water

124. 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 and mature seed was present on plants or the soil surface. Stolon fragments and seeds could be also transported by water during heavy run-off or flooding after a storm. The proposed trial site is located more than 100 m away from a natural waterway (see section 3.1, chapter 1). However, there are artificial waterways in the vicinity of the trial site (see section 3.1, chapter 1). Inside the RMIT campus there are five storm drains within 60 m of the field trial. In addition, the suburbs surrounding RMIT have storm water drainage systems. The field trial is separated from the surrounding suburban areas by an embankment which is at least 2 m high (Figure 2). Therefore, in the event of a storm, water run-off will flow from these elevated areas into the RMIT university campus. A management control has been imposed to reduce the likelihood of water dispersal of GM buffalo grass (see section 3.1.1, chapter 3), i.e. the trial site must be surrounded by a mesh barrier which must leave no gap between the soil surface and the mesh. This mesh barrier will act as a sieve in the event of water running through the planting area, preventing the escape of stolons and rachis segments. It will also reduce the likelihood of wind dispersal of rachis segments by decreasing the speed of wind inside the planting area (see section 3.1.1, chapter 3).

125. The installation of a mesh barrier around the trial site in combination with the controls that would reduce the number of GM buffalo grass propagules present in the trial (inspection of the monitoring zone, mowing of the plants and inspection for and disposal of the grass clippings) will make the dispersal of GM buffalo grass by wind or water unlikely. In addition, standard licence conditions would require the applicant to report any severe weather events to evaluate the need for managing any risks and to present an acceptable contingency plan which must be enacted in case the GM buffalo grass is found outside trial limits.

Potential for establishment and persistence of GM buffalo grass volunteers after dispersal

126. The GM buffalo grass is expected to have limited ability to establish ongoing volunteer populations in the environment near the trial site due to the high water requirements of buffalo grass and the slow growth trait introduced into the GM buffalo grass (see risk scenario 3).

Potential harm

127. 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 limits, controls and management would reduce the amount of GM buffalo grass stolons and seeds available for dispersal and minimise their likelihood of dispersal. Buffalo grass also 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

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

Causal Pathway

129. 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).

130. 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.

131. 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).

132. 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.

133. As discussed in risk scenario 1, the applicant proposes to add fertiliser and frequently mow the GM buffalo grass plants to promote vegetative growth and mechanically remove many of the flowers. These measures together with the small size and limited duration of the trial will ensure that only small quantities of GM pollen are produced.

134. 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 close to the ground and at the same height as the leaves and stems 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. In addition, a mesh barrier will be installed around the monitoring zone to minimise the potential for dispersal of GM buffalo grass

propagules by water. This mesh barrier will also act as a windbreak, reducing the speed of wind inside the trial site and hence decreasing the travel distance of pollen. The applicant has stated that the closest grasses to the field trial are located 30 m away.

135. 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 the small amount of GM pollen that might be available to the plants in the lawn. Furthermore, 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. Only volunteer buffalo grass in urban areas may not be managed and may flower. If these volunteer plants are derived from Australian turf cultivars, they may be somewhat fertile.

136. In summary, gene flow to non-GM buffalo grass would be highly unlikely because: (1) the number of flowers in the trial would be reduced as the grass would be frequently mown and fertiliser would be applied routinely; (2) the travel distance of GM pollen is expected to be short because pollen will be shed close to the ground and wind speeds will be decreased by the installation of a mesh barrier around the monitoring zone that would act as a windbreak; (3) any receptive non-GM buffalo grass will be separated from the GM buffalo grass by an isolation distance of at least 30 m and is expected to bear a small amount of flowers due to the routine mowing of the grass.

137. Even if GM pollen reached other buffalo grass plants and fertilised some flowers, GM hybrid buffalo grass would have limited ability to establish ongoing volunteer populations in the environment near the trial site, as discussed in risk scenario 3.

138. 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. Any GM volunteers could be identified easily by their glyphosate tolerance. Alternative management of glyphosate tolerant buffalo grass is available for the removal of any volunteer plants (see risk scenario 3).

Potential harm

139. 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 limits and 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

140. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis[2].

141. 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

[2] 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.

- 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.

142. 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.

143. 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.

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

- 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.

145. 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. Information on the tolerance to abiotic stresses is particularly relevant, since greater tolerance in the GM buffalo grass may enable it to occupy a greater area in Australia compared to its already weedy non-GM parent.

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

Section 4 Risk evaluation

147. 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.

148. 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.

149. 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.

150. 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

151. 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.

152. 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.

153. 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.

154. 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

155. 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

156. 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 imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the licence.

3.1 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

157. 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

158. The applicant proposed 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), as well as the potential for dispersal, persistence and outcrossing of the GMOs (risk scenarios 3-5).

159. The applicant proposed 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).

160. The applicant proposed 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

161. The applicant proposed 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).

162. The applicant proposed 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).

163. 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. Note that the GM buffalo grass has been modified for reduced growth rate.

164. The field trial site is located away from natural waterways but near several storm drains (section 3.1, chapter 1). To minimise the potential for dispersal of GM buffalo grass stolons and seeds by flooding (risk scenario 4), the applicant proposed to cover all storm drains within 100 m of the trial site with mesh, of the same specification as prescribed for PC2 glasshouse level specification to ensure no plant material enters the waterways draining from the trial site. This would prevent the dispersal of stolons and rachis segments through these waterways. However, the mesh may obstruct the storm drains and lead to flooding of the area. Instead, a licence condition has been imposed to surround the monitoring zone with a mesh barrier that would prevent the escape of stolons or rachis segments due to water run-off. Since the seeds are contained within squarish rachis segments (OGTR 2018) that are 5-10 mm wide (Sauer 1972) a mesh barrier with a pore diameter not exceeding 2 mm would be enough to stop the dispersal of seeds and stolons. This mesh barrier will have to reach from ground level to a height of at least 1.5 m (see below in controls for pollen dispersal).

165. A standard licence condition also requires notification of any extreme weather event affecting the trial site while GMOs are growing and until the site is signed off to allow assessment and management of any risks.

166. The applicant proposed to mow the buffalo grass when it reached a height of 3-5 cm to reduce the number of flowers present in the trial. Mowing is required to be conducted in a manner to minimise the potential for dispersal of reproductive propagules. This could be achieved by using a closed catcher at the back of the lawn mower and/or avoiding mowing in high winds. The mowing

residues or leaf clippings may contain GM buffalo grass stolons and seeds that could be dispersed into the area around the trial site (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 licence condition has been imposed that requires leaf clippings to be collected and disposed of in a way that ensures the destruction of stolons and seeds. Other licence conditions require the planting area to be surrounded by a monitoring zone and an isolation zone, and the planting area to be inspected after each mowing to detect, remove and destroy any stolon fragments.

167. The applicant proposed that all equipment used with the GMOs would be cleaned before use for other purposes. This includes the lawn mower, shoes or boots and clothing. The applicant proposed to use a cleaning area of 4 square metres located 15 metres away from the trial site, on the asphalt area to the west of the fenced space. This cleaning area would be clearly marked and demarcated from the trial area, and be a minimum of 10 metres away from the storm drains. However, since the storm drains will not be covered with mesh, a licence condition has been imposed instead that the cleaning of the equipment must occur within the area surrounded by the mesh barrier, so propagules will be contained and will not disperse by water run-off.

168. 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).

169. Buffalo grass is a perennial plant that can reproduce vegetatively. The applicant proposed 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 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 at the trial site (risk scenario 3).

170. Buffalo grass reproduces asexually through the generation of stolons and also sexually through the production of seeds. The applicant proposed 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 Qld 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 during post-harvest monitoring.

171. However, buffalo grass also produces seed and there is uncertainty about the dormancy and longevity of the seed in soil. Therefore, a 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 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. It is important to note that the number of viable seeds produced in the trial will be low due to the controls to minimise flowering and seed set of the GM buffalo grass (see controls for pollen dispersal). The proposed period of post-harvest monitoring, combined with the irrigation requirement, is considered appropriate to minimise persistence of GM buffalo grass at the trial site (risk scenario 3).

172. The applicant proposed to perform monthly post-harvest inspections. 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 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

173. The applicant proposed to manage pollen flow from the GM buffalo grass by mowing the buffalo grass frequently. The purpose of mowing is two-fold. Firstly, mowing encourages vegetative growth and inhibits the production of inflorescences (Busey, 2003b; The Buffalo Lawn Care). Secondly, mowing removes any developing inflorescences from the grass. Buffalo grass inflorescences are visible when the grass is not mown for two weeks (Duff et al., 2009). Once inflorescences are mature and pollination occurs, published data indicates that more than 3 weeks are needed for the embryo to develop into a mature seed (Genovesi et al., 2009). So if the grass is mown frequently, at least once a week in spring and summer which is the flowering period, inflorescences will not have time to develop, shed pollen and set seed before being removed from the trial site.

174. 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). The mature inflorescences are 50-100 mm long and 5-10 mm wide and develop on top of a peduncule (Sauer, 1972). The total length of the buffalo grass inflorescences ranges from 140 – 200 mm (Duff et al., 2009). GM buffalo grass inflorescences are expected to be shorter, similar to *Nicotiana* plants overexpressing *GA 2-oxidase 3* (Lee and Zeevaart, 2005). In this study, a severely dwarfed GM plant showed a reduction in stem elongation of approximately 60%. If the GM buffalo grass inflorescences were shortened by 60% their length would range from 56 – 80 mm.

175. To remove most flowers, the GM buffalo grass should be mown and maintained below a height that is shorter than the length of its inflorescences. 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). Therefore, keeping the height of the GM buffalo grass plants below 50 mm will remove most flowers (even if the inflorescences of the GM buffalo grass are shortened by 60 %) while allowing good management of the grass. Mowing will be 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 licence condition has been imposed that the buffalo grass has to be monitored weekly during flowering and kept to a height of less than 50 mm. This is in agreement with the management practices proposed by the applicant that included mowing every time the grass grows to a height of 30 - 50 mm (section 6.3, chapter 1). This would promote vegetative growth, reduce the number of flowers and thus keep pollen levels low, which will prevent allergic reactions to pollen (risk scenario 1), outcrossing (risk scenario 5) and seed set (risk scenarios 3 and 4).

176. 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 irrigate and apply fertiliser to the buffalo grass plants frequently to avoid stress due to low water and nutrient levels. A licence condition requires the GM buffalo grass plants to be irrigated and fertilised as necessary to minimise the induction of flowering. This crop management measures will promote the vegetative growth of the buffalo grass, keep the number of flowers produced by the plants to normal physiological levels and in combination with mowing, decrease the likelihood of pollen allergies (risk scenario 1) and outcrossing (risk scenario 5), and reduce the number of seeds produced in the trial site (risk scenarios 3 and 4).

177. A licence condition has been imposed to require that the GM buffalo grass plants must be monitored weekly for the presence of intact, fully expanded inflorescences and an estimate of the numbers be provided to the Regulator. This allows the Regulator to keep track of the efficiency of the

measures imposed to minimise flowering. Another licence conditions imposes a contingency plan that must be implemented in the event of failure of mowing, irrigation and fertilisation to limit flowering. The measures in this contingency plan should be designed to prevent gene flow and submitted to the Regulator prior to planting.

178. 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 licence condition imposes 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, and the mesh barrier (discussed further below), the distance is considered adequate to reduce pollen flow to buffalo grass plants outside the trial.

179. Another management control imposed to decrease the travel distance of pollen is installing a mesh barrier around the monitoring zone that would act as a windbreak. Windbreaks decrease the wind speed on their lee side. For a windbreak to be effective it needs to have a wind permeability between 45% to 55% (Owen-Turner and Hardy, 2006). Another consideration is the height of the windbreak. The effective height of the windbreak is the height above the crop. The higher the effective height the greater area protected. The greatest wind speed protection is in the quiet zone which extends for a distance of 6-10 times the effective height of the windbreak. In areas past this quiet zone wind speed will increase until all the benefit from the windbreak ceases (Owen-Turner and Hardy, 2006). In a study of maize pollen dispersal and cross-pollination, a mesh windbreak of 1 mm diameter and an effective height of 4 m suppressed average cross-pollination rate by 60% within 100 m to the pollen donor (Ushiyama et al., 2009). In the same study a model predicted that mesh windbreaks of 1 or 2 mm diameter and an effective height of 1 m would reduce cross-pollination rates by 50 – 60% (Ushiyama et al., 2009). For the GM buffalo grass trial, installing a mesh barrier 150 cm in height surrounding the Monitoring Zone will offer suitable protection since the quiet zone will extend 8.70 – 14.50 m which will cover the 2 m wide monitoring zone and a large part of the 15 m x 15 m planting area (section 6.6, chapter 1). The mesh barrier could have a pore diameter of 1 or 2 mm. A variety of polythene mesh products can be used as artificial windbreaks (Owen-Turner and Hardy, 2006). These are durable and are available with known porosities which facilitates the selection of the appropriate permeability (Owen-Turner and Hardy, 2006). In summary, the mesh barrier will act as a windbreak, decreasing the speed of wind inside the trial site, which would avoid the dispersal of rachis segments by wind (risk scenario 4) and decrease the travel distance of GM pollen and therefore the likelihood of gene flow to non-GM buffalo grass (risk scenario 5).

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

180. A number of licence conditions have been imposed 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 the RMIT East Bundoora campus in the local government area of Whittlesea, Victoria,
- locate the proposed field trial site at least 100 m away from the nearest natural waterway,
- surround the planting area with a monitoring zone of 2 m that is kept bare of vegetation. During the trial, 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,
- surround the trial site with a fence capable of excluding livestock and limiting unauthorised human access,
- install a mesh barrier around the monitoring zone with a height of 1.5 m and a pore diameter not exceeding 2 mm which will prevent water dispersal of GM buffalo grass propagules and will act as a windbreak,
- monitor the buffalo grass weekly once established and mow before the grass grows taller than 50 mm,
- inspect the area after mowing to detect any stolon fragments on the ground, collect all leaf clippings and dispose of them in ways which prevent stolon and seed dispersal,
- clean the equipment prior to use for other purposes or removal from the trial site,
- irrigate and fertilise the GM buffalo grass as necessary to minimise the induction of flowering,
- monitor the GM buffalo grass plants weekly for the presence of intact, fully expanded inflorescences and provide an estimate of the numbers to the Regulator,
- 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

181. 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

182. 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.

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

184. 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

185. RMIT University is 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.

186. RMIT University is also 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 is required before planting the GMOs.

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

187. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, RMIT University is required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

188. The licence requires the licence holder to immediately report any of the following to the Regulator:

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

189. A number of written notices are also required under the licence to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices 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

190. 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.

191. 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.

192. 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

193. 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 dispersal of buffalo grass by animals,
- 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 RARMP

194. The RARMP concludes that this 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.

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

References

Aldous, D.E., and Chivers, I.H. (2002). Sports turf and amenity grasses: a manual for use and identification (Landlinks Press).

Aldous, D.E., Hunter, A., Martin, P.M., Panayiotis, N.A., and McAuliffe, K.W. (2014). Management of Sports Turf and Amenity Grasslands. In Horticulture: Plants for People and Places, Volume 2, G. R. Dixon, and D. E. Aldous, eds. (Springer, Netherlands), pp. 731-761.

Amrhein, N., Deus, B., Gehrke, P., and Steinrucken, H.C. (1980). The Site of the inhibition of the shikimate pathway by glyphosate: II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. Plant Physiol *66*, 830-834.

Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. Critical Reviews in Toxicology *36*, 219-251.

Aryal, S.K., Crow, W.T., McSorley, R., Giblin-Davis, R.M., Rowland, D.L., Poudel, B., and Kenworthy, K.E. (2015). Effects of infection by *Belonolaimus longicaudatus* on rooting dynamics among St. Augustinegrass and Bermudagrass genotypes. Journal of Nematology *47*, 322-331.

Bammer, G., and Smithson, M. (2008). Uncertainty and risk: Multidisciplinary perspectives (London: Earthscan).

Beard, J.B. (2012). Origin, biogeographical migrations and diversifications of turfgrasses. Report No. SR132. (Michigan State University Press).

Borrell, J.S. (2012). Rapid assessment protocol for pollen settling velocity: implications for habitat fragmentation. Bioscience Horizons: The International Journal of Student Research *5*, hzs002-hzs002.

Brisbane City Council (2016). Buffalo grass - *Stenotaphrum secundatum*. Accessed: 22/6/2016. Available online at this link.

Burd, M., and Allen, T. (1988). Sexual allocation strategy in wind-pollinated plants. Evolution *42*, 403-407.

Busey, P. (1995). Genetic diversity and vulnerability of St. Augustinegrass. Crop Science *35*, 322-327.

Busey, P. (2003a). Cultural management of weeds in turfgrass: a review. Crop Science *43*, 1899-1911.

Busey, P. (2003b). St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In Turfgrass Biology, Genetics, and Breeding, M.D. Casler, and R.R. Duncan, eds. (Hoboken, New Jersey: John Wiley & Sons), pp. 309-330.

CABI (2014). *Stenotaphrum secundatum* (buffalo grass). (Wallingford, UK: CAB International) Accessed: 6/6/2017. Available online at this link.

Cabrera, O., and Scholthof, K.B.G. (1999). The complex viral etiology of St. Augustine decline. Plant Disease *83*, 902-904.

Chen, C., Lu, S., Chen, Y., Wang, Z., Niu, Y., and Guo, Z. (2009). A Gamma-ray–induced dwarf mutant from seeded Bermudagrass and its physiological responses to drought stress. Journal of the American Society for Horticultural Science *134*, 22-30.

Chen, J., Thammina, C., Li, W., Yu, H., Yer, H., El-Tanbouly, R., Marron, M.*, et al.* (2016). Isolation of prostrate turfgrass mutants via screening of dwarf phenotype and characterization of a perennial ryegrass prostrate mutant. Horticulture Research *3*, 16003.

Clark, A.J., and Brinkley, T. (2001). Risk management: for climate, agriculture and policy. (Canberra: Commonwealth of Australia).

Clayton, W.D., Vorontsova, M., Harman, K.T., and Williamson, H. (2006). *Stenotaphrum secundatum*. (Royal Botanic Gardens Kew) Accessed: 9/8/2017. Available online at this link.

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J.*, et al.* (2005). Factsheet - *Stenotaphrum secundatum*. (CSIRO Sustainable Ecosystems, Department of Primary Industries and Fisheries (Qld), Centro Internacional de Agricultura Tropical (CIAT) and International Livestock Research Institute (ILRI)) Accessed: 4/1/2017. Available online at this link.

CRC for Australian Weed Management (2007). Managing garden weeds-creeping plants (CRC for Australian Weed Management).

Daviere, J.M., and Achard, P. (2013). Gibberellin signaling in plants. Development *140*, 1147-1151.

Davies, J.M. (2014). Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. Clinical & Experimental Allergy *44*, 790-801.

Duble, R.L. (2004). St. Augustine decline. (Texas Cooperative Extension) Accessed: 5/1/2017. Available online at this link.

Duff, A.A., Loch, D.S., and Colmer, T. (2009). Adaptation and management of Australian buffalograss cultivars for shade and water conservation. Report No. TU04013. (Queensland Primary Industries and Fisheries, Department of Employment, Economic Development and Innovation: Horticulture Australia Limited).

Fang, J., Nan, P., Gu, Z., Ge, X., Feng, Y.-Q., and Lu, B. (2018). Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic Arabidopsis plants. Frontiers in Plant Science *9*, 233.

Felsot, A.S. (2000). Insecticidal genes part 2: Human health hoopla. Agrichemical & Environmental News *168*, 1-7.

Friedman, J., and Barrett, S.C. (2009). Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. Annals of botany *103*, 1515-1527.

Gaines, T.A., Zhang, W., Wang, D., Bukun, B., Chisholm, S.T., Shaner, D.L., Nissen, S.J.*, et al.* (2010). Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci U S A *107*, 1029-1034.

Garcia-Rivera, J., and Morris, M.P. (1955). Oxalate content of tropical forage grasses. Science *122*, 1089-1090.

Genovesi, A.D., Jessup, R.W., Engelke, M.C., and Burson, B.L. (2009). Interploid St Augustine [*Stenotaphrum secundatum* (Walt.) Kuntze] hybrids recovered by embryo rescue. In Vitro Cellular and Developmental Biology *45*, 659-666.

Gómez, C., Espadaler, X. (1998). Myrmecochorous dispersal distances: a world survey. Journal of Biogeography *25*, 573-580.

Groves, R.H., Hosking, J.R., Batianoff, G.N., Cooke, D.A., Cowie, I.D., Johnson, R.W., Keighery, G.J.*, et al.* (2003). Weed categories for natural and agricultural ecosystem management (Bureau of Rural Sciences, Canberra).

Hayes, K.R. (2004). Ecological implications of GMOs: robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for genetically modified organisms. (Tasmania: CSIRO Division of Marine Research).

HerbiGuide (2014). Buffalo grass - *Stenotaphrum secondatum* (Walter) Kuntze. (HerbiGuide Pty Ltd) Accessed: 19/7/2017. Available online at this link.

HIA (2016). Australian horticulture statistics handbook 2014/15 (Sydney, Australia: Horticulture Innovation Australia Limited).

ILSI (2016). A review of the food and feed safety of the EPSPS protein. (ILSI Research Foundation, Washington, USA).

Kim, S.H., Kim, H.M., Ye, Y.M., Kim, S.H., Nahm, D.H., Park, H.S., Ryu, S.R.*, et al.* (2006). Evaluating the allergic risk of genetically modified soybean. Yonsei Med J *47*, 505-512.

Kimball, J.A. (2015). Breeding for Cold Tolerance in St. Augustinegrass using Conventional and Molecular Breeding Methods. PhD Thesis, Noth Carolina State University (USA).

Kimball, J.A., Tuong, T.D., Arellano, C., Livingston III, D.P., and Milla-Lewis, S.R. (2017). Assessing freeze-tolerance in St. Augustinegrass: temperature response and evaluation methods. Euphytica *213*, 110.

Kiotseridis, H., Cilio, C.M., Bjermer, L., Tunsater, A., Jacobsson, H., and Dahl, A. (2013). Grass pollen allergy in children and adolescents-symptoms, health related quality of life and the value of pollen prognosis. Clin Transl Allergy *3*, 19.

Klee, H.J., Muskopf, Y.M., and Gasser, C.S. (1987). Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Mol Gen Genet *210*, 437-442.

Koornneef, M., and Meinke, D. (2010). The development of Arabidopsis as a model plant. The Plant Journal *61*, 909-921.

Kuparinen, A. (2006). Mechanistic models for wind dispersal. Trends in plant science *11*, 296-301.

Lee, D.J., and Zeevaart, J.A. (2002). Differential regulation of RNA levels of gibberellin dioxygenases by photoperiod in spinach. Plant Physiol *130*, 2085-2094.

Lee, D.J., and Zeevaart, J.A. (2005). Molecular cloning of GA 2-oxidase3 from spinach and its ectopic expression in *Nicotiana sylvestris*. Plant Physiol *138*, 243-254.

Li, W., Katin-Grazzini, L., Krishnan, S., Thammina, C., El-Tanbouly, R., Yer, H., Merewitz, E.*, et al.* (2016). A novel two-step method for screening shade tolerant mutant plants via dwarfism. Front Plant Sci *7*, 1495.

Liebman, M., Mohler, C.L., and Staver, C.P. (2001). Ecological management of agricultural weeds (Cambridge, United Kingdom: Cambridge University Press).

Loch, D.S., Roche, M.B., Sun-Yue, J., Arief, V., Delacy, I.H., and Lambrides, C.J. (2009). Characterization of commercial cultivars and naturalized genotypes of *Stenotaphrum secundatum* (Walter) Kuntze in Australia. International Turfgrass Society Research Journal *11*, 549-561.

MacDonald, G. (1995). Armyworms (Department of Environment and Primary Industries).

Mullen, B.F., and Shelton, H.M. (1996). *Stenotaphrum secundatum*: a valuable forage species for shaded environments. Tropical Grasslands *30*, 289-297.

Muyt, A. (2001). Bush Invaders of South-East Australia (Meredith, VICTORIA: RG & FJ Richardson).

OGTR (2018). The biology of *Stenotaphrum secundatum* (Walter) Kuntze (buffalo grass). (Canberra , Australia: Office of the Gene Technology Regulator).

Okubo, A., and Levin, S.A. (1989). A theoretical framework for data analysis of wind dispersal of seeds and pollen. Ecology *70*, 329-338.

Owen-Turner, J., and Hardy, S. (2006). Windbreaks for Citrus. (CIITGroups Australia).

Ozbreed (2011). Lawn grub information sheet.

Padgette, S.R., Kolacz, K.H., Delannay, D.B., Re, D.B., LaVallee, B.J., Tinius, C.N., Rhodes, W.K.*, et al.* (1995). Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci *35*, 1451-1461.

Padgette, S.R., Taylor, N.B., Nida, D.L., Bailey, M.R., MacDonald, J., Holden, L.R., and Fuchs, R.L. (1996). The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. J Nutr *126*, 702-716.

Powles, S.B. (2010). Gene amplification delivers glyphosate-resistant weed evolution. Proc Natl Acad Sci U S A *107*, 955-956.

Radauer, C., and Breiteneder, H. (2006). Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. J Allergy Clin Immunol *117*, 141-147.

Rahman, M.M., Abdullah, R.B., and Wan Khadijan, W.E. (2013). A review of oxalate poisoning in domestic animals: tolerance and performance aspects. Journal of Animal Physiolopgy and Animal Nutrition *97*, 605-614.

Randall, R.P. (2017). A Global Compendium of Weeds, 3rd edn (Perth, Western Australia: R.P. Randall).

Rowe, R. (2013). Disposal of terrestrial invasive plants (Florida Exotic Pest Plant Council).

Sauer, J.D. (1972). Revision of *Stenotaphrum* (Gramineae: Paniceae) with attention to its historical geography. Brittonia *24*, 202-222.

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C.*, et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Research *24*, 1-17.

Schomburg, F.M., Bizzell, C.M., Lee, D.J., Zeevaart, J.A., and Amasino, R.M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell *15*, 151-163.

Schonbrunn, E., Eschenburg, S., Shuttleworth, W.A., Schloss, J.V., Amrhein, N., Evans, J.N., and Kabsch, W. (2001). Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci U S A *98*, 1376-1380.

Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. Toxicological Sciences *71*, 2-8.

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., *et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiology *161*, 1587-1594.

Takeno, K. (2016). Stress-induced flowering: the third category of flowering response. Journal of experimental botany *67*, 4925-4934.

Thomas, J.E., and Steele, V. (2011). First report of *Panicum mosaic virus* in buffalo grass (*Stenotaphrum secundatum*) from Australia. Australasian Plant Disease Notes, 16-17.

Ushiyama, T., Du, M., Inoue, S., Shibaike, H., Yonemura, S., Kawashima, S., and Amano, K. (2009). Three-dimensional prediction of maize pollen dispersal and cross-pollination, and the effects of windbreaks. Environmental biosafety research *8*, 183-202.

Water and Rivers Comission (2001). Herbicide Use in Wetlands (Government of Western Australia).

Whitehead, D.R. (1969). Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution *23*, 28-35.

Appendix A: Summary of submissions from prescribed experts, agencies and authorities[3]

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

Abbreviations **DIR**: Dealing involving Intentional Release; **GM**: genetically modified; **GMO**: genetically modified organism; **OGTR**: Office of the Gene Technology Regulator; **RARMP**: Risk Assessment and Risk Management Plan; **Regulator**: Gene Technology Regulator; **Sub. No.**: submission number

Sub. No.	Summary of issues raised	Comment
1	Concerned that glyphosate tolerance might make this buffalo grass a potent weed, but also cited the availability of alternative weed control agents and strategies, the nature of buffalo grass being less prone to becoming a weed than other grass species, and noting the controls proposed by the applicant.	Noted.
	Believes that licence should be amended to permit only non-GM buffalo grass of the same cultivar as the GM buffalo grass so in case of any outcrossing the resulting offspring would suffer from inbreeding depression.	The imposed limits and control measures will effectively control GM buffalo grass volunteers even if the plants do not show inbreeding depression.
2	No concerns with management of risks as detailed in RARMP and considers that these are appropriately managed for a field trial.	Noted.
	Expresses concerns should this herbicide tolerant GM buffalo grass be released commercially as it will spread to areas where it is not wanted and management will be more difficult and because lawn weeds might become glyphosate tolerant affecting management of these weeds in all sectors.	Noted.
	Asks for terminology to be harmonised in RARMP and to refer to buffalo grass as an introduced species or non-native species.	The RARMP has been amended to that effect.
3	Supports the conclusions of negligible risk for this proposed field trial.	Noted.

[3] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub. No.	Summary of issues raised	Comment
	Believes that in analogy to wheat DIR licences a 230 m isolation zone would be warranted.	In GM wheat DIR licences, the GM wheat plants are permitted to flower, whereas in DIR 156 the buffalo grass will be maintained a) to reduce flowering and b) to remove the majority of flowers that may develop by mowing (see risk scenarios 1, 3 and 4 in Chapter 2, and Chapter 3 of the RARMP). This will reduce the amount of GM pollen produced in the field trial and consequently the likelihood of gene flow. Installation of a windbreak around the monitoring zone has also been imposed (see Chapter 3 of the RARMP) which will further decrease the likelihood of gene flow occurring during the trial. Also, most receptive buffalo grass around the trial site is expected to be watered and mown which will prevent flowering and will further reduce the likelihood of gene flow.
	Provides expert comment that equipping weedy species with herbicide tolerance will lead to an increase in its weediness and advises all applicants to desist in pursuing the herbicide tolerance trait in environmental weeds as it is counterproductive to the tasks of weed risk managers. Therefore, opposes a possible commercial release of herbicide tolerant buffalo grass. Has no comment regarding the dwarf phenotype trait.	Noted.
	Suggests applicant consider monitoring pollen movement as part of their experiment. This data would help with the development of licence conditions for future dealings.	Information on long distance pollen dispersal has been identified as data that would be needed for a future commercial release or to justify a reduction in limits and controls for a future trial.
4	The Department agrees with the conclusions of the consultation RARMP.	Noted.
	Notes that in case of an unintentional escape or commercial release, the genetic modifications may increase the weediness of the buffalo grass and limit herbicide control options.	Noted.
	Believes the RARMP should include a longer discussion on the effectiveness of regular mowing.	The RARMP has been amended to better explain the effects of mowing, i.e. (1) encouraging vegetative growth over reproductive growth and (2) removing developing inflorescences (see chapter 3).
	The RARMP should include information on the distance from the trial site to any non-GM buffalo grass growing in the area and to natural environments.	Licence conditions state that no buffalo grass must grow within 30 m of the planting area. The RARMP now includes a detailed description of the location of the trial site, including the distance to natural environments (see section 3.1, chapter 1).

Sub. No.	Summary of issues raised	Comment
	RARMP states incorrectly fence will keep birds out of the trial. Asks for additional information on likelihood of dispersal of seed or stolons by birds and rodents.	The RARMP has been amended to avoid the misinterpretation that a fence will stop birds from accessing the trial. Additional information has been added to the RARMP on the likelihood of dispersal of viable material by birds or rodents (see Chapter 2, risk scenario 4).
	Asks for WRA of biology document to be changed to distinguish between fertile and sterile cultivar of buffalo grass and to have further reasoning for the ratings of 'unlikely' or 'very low' for risk dispersal by birds, negative impacts on biodiversity and potential fire risk.	The biology document will be amended as appropriate when a review is conducted.
	RARMP may need to mention that this is the first time the site will be used for a field trial and the first time the applicant will conduct a field trial of a GM plant.	The previous use of the site and the experience of the applicant do not affect the level of risk of this limited and controlled release. The Regulator is experienced in working with new licence holders and is proactive to ensure compliance with the licence conditions.
	RARMP should mention that control options may be limited in case of the GM buffalo grass escaping. States that in Australia glyphosate is the only herbicide permitted for use near water and that this information should be included in the RARMP along with other management options available for use, especially near aquatic environments.	The RARMP has been amended to include a list of herbicides that can be used to manage the GM buffalo grass (see section 2.4.3, chapter 2). There are several herbicides registered to be used near aquatic environments that could be potentially used to manage the GM buffalo grass (APVMA); however, the only herbicide currently registered to manage buffalo grass growing near aquatic environments is glyphosate. Use of other herbicides for this purpose would require authorisation by the APVMA.
	RARMP should mention that GM turf grasses are not regulated in the USA, that one GM turf grass species escaped from a field trial in the USA and there are no commercially available GM turf grasses in the USA.	Depending on the modification, GM turf grasses may or may not be regulated in the USA. Buffalo grass modified in the same way as the GMOs in this trial are not regulated in the USA due to differences in the legislation compared to Australia. The RARMP has been clarified to this effect. The GM turf grass that escaped from a field trial was a different species with different reproductive and dispersal characteristics and, therefore, was not relevant for this RARMP.
5	Asks if the OGTR is aware of any data to indicate that there is a difference in the production of pollen in GM buffalo grass from non-GM buffalo grass or similar cultivars?	The OGTR is not aware of any data about pollen numbers produced by buffalo grass. This is noted as an area of uncertainty in the RARMP (Chapter 2, paragraph 144).
	Has data on adverse health impacts been collected in previous trials undertaken by the applicant? Is there a current requirement to collect this data?	The GM buffalo grass plants have been grown in the glasshouse for five years and no adverse health effects have been observed by the research staff. Information on the potential for increased toxicity or allergenicity has been identified as data that would be needed for a future commercial release or to justify a reduction in limits and controls for a future trial.

Sub. No.	Summary of issues raised	Comment
	The grass products, seeds and grass clippings should be handled and managed as GMO waste products.	Noted. The Regulator considers any live and viable plant materials which could give rise to a GMO as the GMO in all DIR licences (see licence condition 34).
6	The RARMP should consider aerosol exposure when mowing GM buffalo grass as a pathway to harm.	Aerosol exposure has been added to the pathway that considers exposure of people who deal with the GM plant material in the RARMP (see chapter2, risk scenario 1).
	Additional control measures to mitigate against unintended spread of GM buffalo grass beyond the trial site should be considered.	The requirement to install a mesh barrier around the monitoring zone has been added to the RARMP (see Chapter 3). This mesh barrier will minimise water and wind dispersal of GM buffalo grass propagules.
	Additional control measures aimed at minimising flowering of the GM buffalo grass should be considered.	The risk of this field trial has been assessed as negligible. The Regulator has further considered the licence conditions relating to flowering and gene flow. Following consultation an additional licence condition was added to install a mesh barrier around the monitoring zone. This will act as a windbreak to further reduce the likelihood of pollen dispersal out of the trial site. A reporting condition has also been added to the licence so the Regulator must be informed weekly of the number of inflorescences present in the trial. A contingency plan designed to prevent gene flow in the event of the GM buffalo plants showing excessive numbers of flowers must be submitted to the Regulator before any dealings are permitted under the licence. The combination of limits and controls, including the added requirement to install a mesh barrier around the monitoring zone, will reduce the level of risk from this small scale field trial adequately.
7	Agrees that this field trial presents a negligible level of risk to the health and safety of people and animals but believes the level of risk for the environment is low rather than negligible. If the GM buffalo grass escapes from the field trial this would have negative effects on the environment and management of this weedy GM buffalo grass will be more difficult. Recommends that further controls should be imposed given that the risk to the environment is low rather than negligible.	The risk of this field trial has been assessed as negligible. Risk scenario 4 in chapter 2 of the RARMP considered the risk of escape from the trial. Following consultation an additional licence condition was added to install a mesh barrier around the monitoring zone. This will further reduce the likelihood of plant material being dispersed by wind or water. The combination of limits and controls, including the mesh barrier will reduce the level of risk from this small scale field trial to negligible. A contingency plan must be provided before any dealings are permitted under the licence. In case of GM buffalo grass being dispersed into the wider environment, the Regulator must be notified and the contingency plan enacted.
	Scope of research should be expanded to address pathways for environmental contamination via animals.	The RARMP has been amended to include dispersal of buffalo grass by animals as additional information that may be required to assess an application for future releases (Chapter 3).

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

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

Submission	Summary of issues raised	Comment
1	Is opposed to GMOs and questions the safety and benefit. Lawns are not essential for humans.	The Regulator is required to prepare a RARMP for a DIR licence application before deciding whether or not to issue a licence and cannot consider the benefits of gene technology. Risks are considered in the context of currently accepted practices.
	Asks that a member from Bio-dynamics or Organics is consulted by the OGTR to be educated on the negative effects of GM.	Before the Regulator decides whether to issue a licence for release of GMOs, a RARMP is prepared. The RARMP was finalised following an extensive consultation process that involved the public. Members of Bio-dynamics or Organics could have submitted their advice about this trial and their submission would have been considered.
	Questions that the GA 2-oxidase gene from spinach has a history of safe use. States that when isolated from spinach and introduced to a different organism it may lead to harm.	The potential for the introduced genes to increase the toxicity and allergenicity of buffalo grass is addressed in Chapter 2, Sections 2.4.1 and 2.4.2 (Risk scenarios 1 and 2).
	Asks about the effect of GM lawn on insects.	The potential for the introduced genes to cause detrimental effects on insects is addressed in Chapter 2, Section 2.4.2 (Risk scenario 2).
	Is against GMOs with herbicide tolerance because this trait only promotes herbicide application, and herbicides are poisons.	The APVMA has regulatory responsibility for agricultural chemicals, including pesticides and herbicides.