Australian Government
Department of Health
Office of the Gene Technology Regulator

December 2017

Risk Assessment and Risk Management Plan
(consultation version)
for

DIR 160

Limited and controlled release of perennial ryegrass genetically modified for fructan biosynthesis

Applicant - Department of Economic Development, Jobs, Transport and Resources

This RARMP is open for consultation until 18 January 2018.

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

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

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

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

for

Licence Application No. DIR 160

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed field trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed field trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application number	DIR 160
Applicant	Department of Economic Development, Jobs, Transport and Resources (DEDJTR)
Project title	Limited and controlled release of perennial ryegrass genetically modified for fructan biosynthesis
Parent organism	Perennial ryegrass (*Lolium perenne*)
Introduced genes and modified traits	• Two fructan biosynthesis genes (sucrose:sucrose 1-fructosyltransferase and fructan:fructan 6G-fructosyltransferase) from perennial ryegrass for increased plant nutritional quality and biomass production • *hph* selectable marker gene from *Escherichia coli*
Proposed location	One site in the Southern Grampians Shire in south-west Victoria.
Proposed release size	Up to 160 m^2 each year
Proposed release dates	May 2018 – June 2020
Primary purpose	To assess agronomic characteristics and to multiply seed for future trials

Risk assessment

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

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

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

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

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food or 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 not required for testing or further planting, and to conduct post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

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Abbreviations

Act	*Gene Technology Act 2000*
DEDJTR	Department of Economic Development, Jobs, Transport and Resources
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
6G-FFT	*fructan:fructan 6G-fructosyltransferase*
h	hours
ha	hectare
HGT	horizontal gene transfer
hph	*hygromycin phosphotransferase*
m	metres
mm	millimetres
NLRD	Notifiable Low Risk Dealing
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
1-SST	*sucrose:sucrose 1-fructosyltransferase*

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

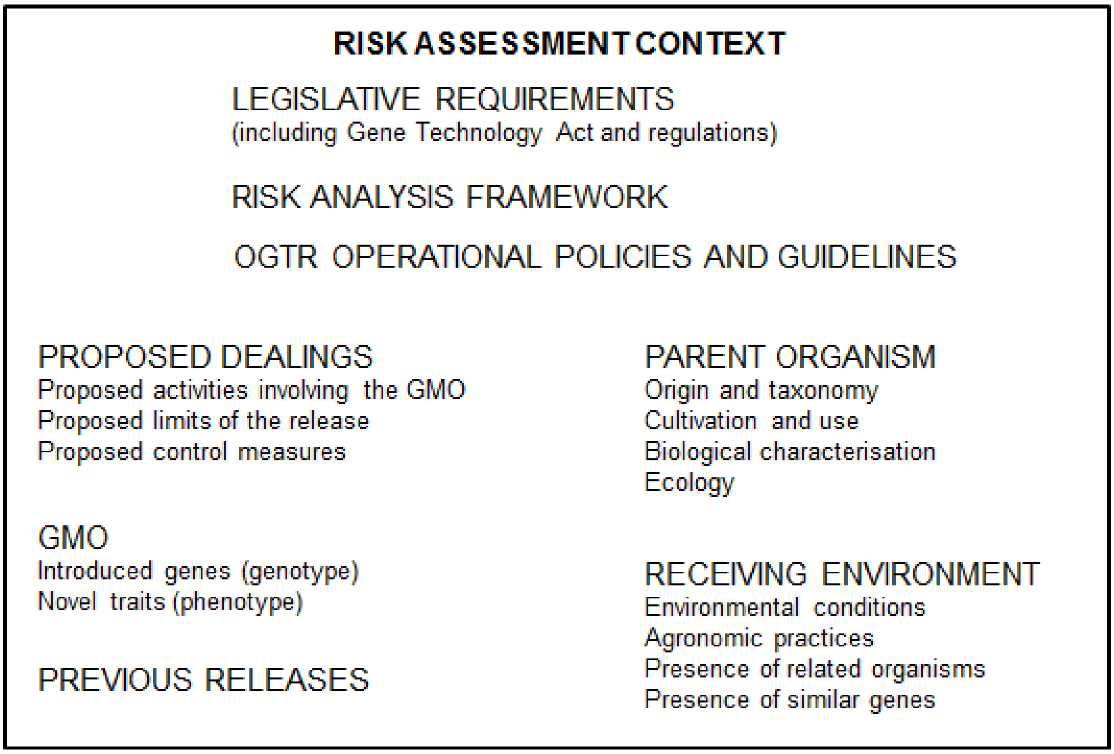

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

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

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

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

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

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

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed dealings

9. The Department of Economic Development, Jobs, Transport and Resources (DEDJTR) in Victoria propose to release one line of perennial ryegrass genetically modified for fructan biosynthesis into the environment under limited and controlled conditions. The purpose of the release is to assess agronomic characteristics of the GM perennial ryegrass and to multiply seed for future trials.

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

- conducting experiments with the GMOs
- propagating 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 at one site over two years, between May 2018 and June 2020. In both years, the proposed planting area is 160 m^2. The local government area where the field trial site is located is Southern Grampians Shire in south-west Victoria.

12. Only trained and authorised staff would be permitted to deal with the GM perennial ryegrass.

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

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

- locating the proposed field trial site at least 2 km away from the nearest natural or artificial waterway
- containing plants in a 180 micron meshed polyhouse enclosure that will reduce wind-mediated pollen dispersal
- surrounding the polyhouse enclosure with a monitoring zone of at least 40 m that is kept fallow.

While the GM perennial ryegrass is flowering, the monitoring zone would be inspected weekly for species that are sexually compatible with perennial ryegrass, and any plants found would be destroyed

- surrounding the monitoring zone with an isolation zone of at least 100 m that is maintained in a manner that prevents the flowering of grasses
- controlling rodents by baiting and surrounding the site with a fence to restrict access by larger animals such as livestock and rabbits
- cleaning equipment prior to use for other purposes or removal from the trial site
- transporting GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- destroying all GMOs not required for analysis or future trials
- post-harvest tillage and irrigation of the trial site to promote germination of volunteers
- post-harvest monitoring of the planting area and monitoring zone for at least 12 months and until the site is free of volunteer perennial ryegrass for at least 6 months, with any perennial ryegrass volunteers destroyed before flowering
- not allowing the GM plant material to be used in human food or animal feed.

Section 4 The parent organism

14. The parent organism is perennial ryegrass (*Lolium perenne* L.), which is exotic to Australia. Perennial ryegrass is used for both pasture and as turf in Australia. As pasture, it is generally used in combination with other pasture grass species, for dairy and sheep grazing predominately in the temperate areas of Australia (New South Wales, Victoria and Tasmania) (Blair, 1997; Lazenby, 1997; Callow et al., 2003). In addition, perennial ryegrass is used as turf, often in combination with other turf grass species and primarily in temperate regions (Lamp et al., 2001).

15. Detailed information about the parent organism is contained in the reference document *The Biology of* Lolium multiflorum *Lam. (Italian ryegrass),* Lolium perenne *L. (perennial ryegrass) and* Lolium arundinaceum *(Schreb.) Darbysh (tall fescue)* (OGTR, 2017) which was produced to inform the risk assessment process for licence applications involving GM perennial ryegrass and related species. Baseline information from this document will be used and referred to throughout the RARMP.

16. The GM perennial ryegrass line was derived from a single transformation event of breeding line FLP-418, a tissue culture responsive genotype. The GM line was pair-crossed to a range of different commercial ryegrass cultivars. Two different cultivar/endophyte combinations (chosen from Trojan/NEA6, Alto/NEA12 and Bronsyn/NEA6 based on seed yield) will be used in the two separate plantings of the proposed field trial. Endophytes are fungi that live between the plant cells of many forage grasses (Kemp et al., 2007). These fungi do not cause any disease in the grass, and under most circumstances are beneficial to the growth and survival of infected plants (Clay and Schardl, 2002).

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

5.1 Introduction to the GMOs

17. The applicant proposes to grow one line of GM perennial ryegrass. This line contains two introduced genes intended to increase fructan biosynthesis and an introduced selectable marker gene (Table 1). Both of the introduced genes conferring altered fructan biosynthesis are derived from perennial ryegrass (*Lolium perenne L.*). The two genes for altered fructan biosynthesis are expressed as a translational fusion both driven by the one promoter which allows the two enzymes to be in close proximity for improved efficiency of the fructan biosynthesis pathway.

Table 1.The introduced genes in the GM perennial ryegrass.

Gene	Protein produced	Protein function	Source
Lp1-SST	sucrose:sucrose 1-fructosyltransferase	Fructan biosynthesis	Perennial ryegrass
Lp6G-FFT	fructan:fructan 6G-fructosyltransferase	Fructan biosynthesis	Perennial ryegrass
hph	Hygromycin phosphotransferase	Hygromycin resistance (selectable marker)	*Escherichia coli*

18. The GM perennial ryegrass line contains the selectable marker gene *hph* (also known as *hpt* or *aph4*) derived from the bacterium *Escherichia coli*. This gene confers antibiotic resistance on GM plant cells and was used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes.

19. Short regulatory sequences that control gene expression are also present in the GM perennial ryegrass line. The introduced perennial ryegrass genes are controlled by the perennial ryegrass *LpRbcS* gene promoter which is light regulated and targets expression to photosynthetic cells (Kyozuka et al., 1993) and transcription is terminated by *LpFT4*. The *hph* gene is controlled by the *Actin 1* constitutive promoter from rice (*Oryza sativa*) and terminated by the *35S* polyadenylation signal sequence derived from the common plant pathogen, Cauliflower Mosaic Virus (CaMV).

20. The GM perennial ryegrass line was 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 Fructan biosynthesis

21. In tropical and subtropical grasses, sucrose and starch are the primary vegetative storage forms of carbohydrates, while in temperate and cool zone grasses fructans are the main carbohydrate stored (Hendry and Wallace, 2008). Fructans are polymers of fructose (Pavis et al., 2001a) which occur in 15% of flowering plant species (Hendry, 1987). Fructan may account for more than 30% of the dry weight of grass leaves, stems and ears depending on their state of development and on environmental conditions (Pollock and Jones, 1979). Fructans are synthesised in the vacuoles (Darwen and John, 1989) of many economically important orders of Asterales (chicory, Jerusalem artichoke), Liliales (onion, tulip) and Poales (barley, wheat) (Weyens et al., 2004).

22. Fructans have been implicated in drought and cold tolerance (Pilon-Smits et al., 1995; Konstantinova et al., 2002; Livingston et al., 2009). Fructans accumulate in wheat (Bancal and Gaudillère, 1989; Jeong and Housley, 1990), *Lolium temulentum* (Pollock, 1984) and perennial ryegrass (Abeynayake et al., 2015) in response to cold stress and in perennial ryegrass (Amiard et al., 2003) and chicory plants (De Roover et al., 2000) following drought stress. Fructan accumulation has also been observed following salt stress in wheat (Kerepesi and Galiba, 2000; Sharbatkhari et al., 2016).

23. The Poaceae family contain fructans of a complex structure with β(2-6) and β(2-1) fructose linkages (Pavis et al., 2001b). Fructans from *Lolium* belong to the:

- inulin series with a terminal glucose residue and β(2-1) linked fructose residues
- inulin neoseries with an internal glucose residue and β(2-1) linked fructose residues
- levan neoseries with an internal glucose residue and β(2-6) linked fructose residues (Pavis et al., 2001b)(see Figure 2).

24. Many enzymes are involved in carbon allocation towards fructan biosynthesis. The enzyme sucrose:sucrose 1-fructosyltransferase (1-SST) catalyses the first step in fructan biosynthesis and other enzymes control the fructan structural diversity including fructan:fructan 1-fructosyltransferase (1-FFT), sucrose:fructan 6-fructosyltransferase (6-SFT), and fructan:fructan 6G-fructosyltransferase (6G-FFT) as shown in Figure 2.

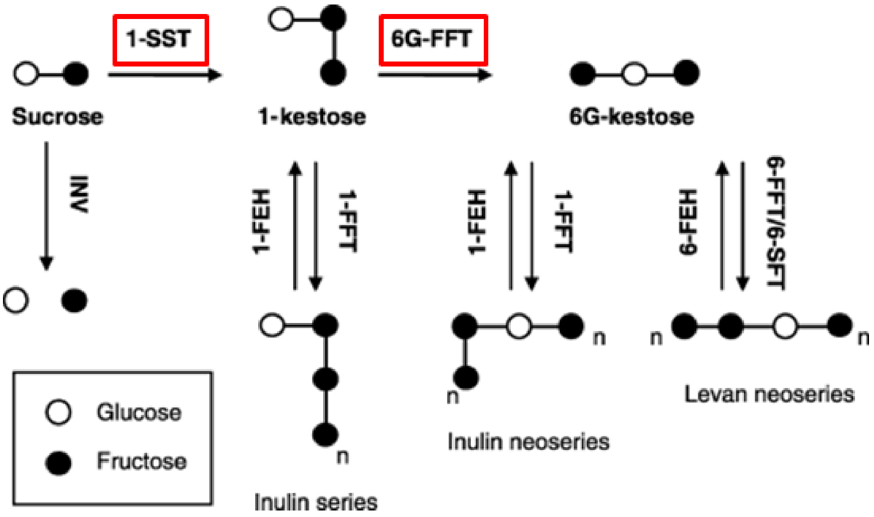

Figure 2. Proposed fructan metabolism pathway in *Lolium perenne* (Chalmers et al., 2005). GM perennial ryegrass will contain additional copies of 1-SST and 6G-FFT, highlighted by red boxes. Abbreviations used: INV (invertase); 1-SST (sucrose:sucrose 1-fructosyltransferase); 6G-FFT (fructan:fructan 6-glucose fructosyltransferase); 1-FEH (fructan 1-exohydrolase); 6-FEH (fructan 6-exohydrolase); 1-FFT (fructan:fructan 1-fructosyltransferase); 6-FFT (fructan:fructan 6-fructosyltransferase); 6-SFT (sucrose:fructan 6-fructosyltransferase).

5.2.2 The sucrose:sucrose 1-fructosyltransferase (1-SST) gene

25. The 1-SST enzyme catalyses the first step in fructan biosynthesis producing the inulin trisaccharide 1-kestose. In perennial ryegrass 1-SST is expressed in young leaf bases and mature leaf sheaths (Chalmers et al., 2003; Lasseur et al., 2006).

26. GM plants have been modified to produce inulins by expressing 1-SST and 1-FFT from artichoke in potato (Hellwege et al., 1997; Hellwege et al., 2000), petunia (van der Meer et al., 1998) and sugar beet (Sévenier et al., 1998). GM perennial ryegrass (Hisano et al., 2004) and GM rice (Kawakami et al., 2008) overexpressing wheat 1-SST and 1-FFT showed increased fructan content and increased tolerance to freezing, similar responses were seen in GM tobacco expressing 1-SST from lettuce (Li et al., 2007).

27. Under drought stress, wheat had reduced levels of 1-SST and increased levels of 1-FEH, resulting in depolymerisation of fructans (Yang et al., 2004).

28. Fructans have also been implicated in disease resistance. More fructans were seen to accumulate in wheat cultivars which were resistant to snow mold, than in susceptible cultivars, and levels of 1-SST were higher in the resistant cultivars (Kawakami and Yoshida, 2002).

5.2.3 The fructan:fructan 6G- fructosyltransferase (6G-FFT) gene

29. 6G-FFT is a key enzyme in the formation of the inulin neoseries of fructans. 6G-FFT activity catalyses the transfer of a fructose unit from a fructan (e.g. 1-kestose) on to carbon 6 of the glucose unit of another fructan or sucrose (Vijn et al., 1997), which is further elongated with β(2-1) or β(2-6) linkages to produce the inulin neoseries or levan neoseries fructans, respectively (Figure 2). 6G-FFT activity from onion and asparagus has been shown to produce inulin series and inulin neoseries

fructans (Vijn et al., 1997; Ueno et al., 2005). In perennial ryegrass, 6G-FFT activity is highest in the basal segment of elongating leaves and mature leaf sheaths (Pavis et al., 2001a).

30. GM sugar beet plants expressing onion 1-SST and 6G-FFT produced inulin neoseries fructans and showed no abnormal phenotype (Weyens et al., 2004). Perennial ryegrass plants expressing the same genes produced 3-fold higher fructan levels with a higher degree of polymerisation than non-GM plants but no abnormal phenotype (Gadegaard et al., 2008).

5.2.4 The hph gene

31. The *hph* gene was isolated from the common gut bacterium *E. coli* and encodes the enzyme hygromycin phosphotransferase (HPT), which inactivates aminoglycoside antibiotics such as hygromycin. The *hph* gene is used extensively as a selectable marker in the production of GM plants. Regulatory agencies in Australia and in other countries have assessed the use of the *hph* gene in GM plants as not posing a risk to human health and safety or to the environment. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website.

32. Internationally, hygromycin B is used in animal production as a feed additive for swine and chickens to kill parasitic worms, e.g. in Hygromix® products registered by the U.S. Food & Drug Administration (US FDA website, accessed 15 September 2017). Hygromycin B is currently not registered for use as a veterinary medicine in Australia (APVMA PubCRIS database, accessed 15 September 2017) and is not on the international *OIE List of Antimicrobial Agents of Veterinary Importance* (OIE, 2015).

33. Hygromycin B is not used in human medicine in Australia and is currently not listed in the Australian Register of Therapeutic Goods (TGA website, accessed 15 September 2017). Furthermore, the antibiotic is not considered high priority for managing the development of antibiotic resistance: it is not listed in the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance's *Importance Ratings and Summary of Antibacterial Uses in Humans in Australia* (ANSTAG, 2015) or the *World Health Organization list of Critically Important Antimicrobials for Human Medicine* (WHO, 2017).

34. In addition to hygromycin B, the HPT protein phosphorylates the closely related compounds hygromycin B_2, destomycin A and destomycin B (Rao et al., 1983; FSANZ, 2004). These compounds are not generally used in human or veterinary medicine.

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

35. The introduced fructan biosynthesis genes are perennial ryegrass genes that are naturally expressed in perennial ryegrass. Perennial ryegrass is an established forage grass with a long history of use as pasture for grazing (OGTR, 2017). The proteins in perennial ryegrass are regularly consumed by livestock without adverse effects.

36. The introduced fructan biosynthesis enzymes increase production of fructans. Fructans are present in a wide variety of plants and are present in a number of foods and feedstuff eaten by people and animals with generally no ill-effects (Ritsema and Smeekens, 2003; Weyens et al., 2004). They potentially have favourable effects in the prevention of cardiovascular diseases, colon cancer and osteoporosis and can be used as a low calorie food ingredient to replace sugar or fat as they are not digested by humans (Weyens et al., 2004).

37. In horses, excess fructan consumption has been implicated in the illness laminitis which causes inflammation particularly of the feet (Watts and Pollitt, 2010). This occurs if horses eat excess carbohydrates (including sugars, starch or fructan) which they are unable to digest in the foregut (Watts and Chatterton, 2004).

38. Ryegrasses (*Lolium* spp.) are the dominant source of allergenic pollen in cool, temperate climates due to their wide distribution and abundant production of airborne pollen during flowering (Smart et al., 1979; Spangenberg et al., 2005). Perennial ryegrass is considered the main contributor to

grass pollen in the Australian cities of Canberra, Adelaide, Melbourne and Perth (Davies et al., 2015). Estimates suggest that 84% of USA residents are exposed to perennial ryegrass pollen (Lankow et al., 2015) and as many as 37% of individuals are immunoreactive to perennial ryegrass pollen in some populations (Scala et al., 2010).

39. The main allergenic determinants in ryegrass pollen are two proteins designated Lol p 1 and Lol p 2 (Spangenberg et al 2005). Lol p 1 is the major ryegrass pollen allergen to which 95% of patients showed increased levels of IgE antibodies (Kahn and Marsh, 1986), while 45% of grass pollen allergic patients are reactive to Lol p 2 (Freidhoff et al., 1986).

40. The introduced perennial ryegrass regulatory sequences and fructan biosynthesis genes are not associated with the known allergenic determinants of ryegrass pollen. Additionally, given the promoter driving their expression targets photosynthetically active tissue, the fructan biosynthesis genes would not be expressed at significant levels in the pollen.

41. Perennial ryegrass has a mutualistic symbiotic relationship with the endophyte, *Neotyphodium lolli* (Hettiarachchige et al., 2015), which deters insect attack. Endophytes produce a range of alkaloid metabolites which vary among endophyte species and can have detrimental effects on the health of grazing animals depending on the level of associated alkaloid toxicity (Schardl et al., 2004). The GM perennial ryegrass plants will carry a commercial endophyte strain, either NEA6 or NEA12, which have lower production of alkaloids than endophytes that are naturally found in the environment.

42. The GM perennial ryegrass line contains the *hph* selectable marker gene. Regulatory agencies in Australia and other countries have found no evidence that the HPT protein is toxic or allergenic (FSANZ, 2004; EFSA, 2009).

5.4 Characterisation of the GMOs

43. GM perennial ryegrass plants modified for fructan biosynthesis were previously analysed in a combination of field trials (DIR 082 - seasonal growth and forage quality) and glasshouse experiments (leaf cutting rotations and nitrogen response). A single GM line, Event 10, was selected for further field analysis.

44. When grown under glasshouse conditions Event 10 resulted in a significantly higher biomass, with up to 13 fold higher biomass than the non-GM parental breeding line FLp418-20. Similarly, in field trials, fresh weights of Event 10 plants at harvest ranged between 350 – 500 g compared to 30 – 130 g in the isogenic control. The higher biomass was observed across multiple harvests in varying seasons.

45. In glasshouse experiments, the GM perennial ryegrass line gained biomass more quickly than its isogenic control or two commercial perennial ryegrass cultivars (Figure 3).

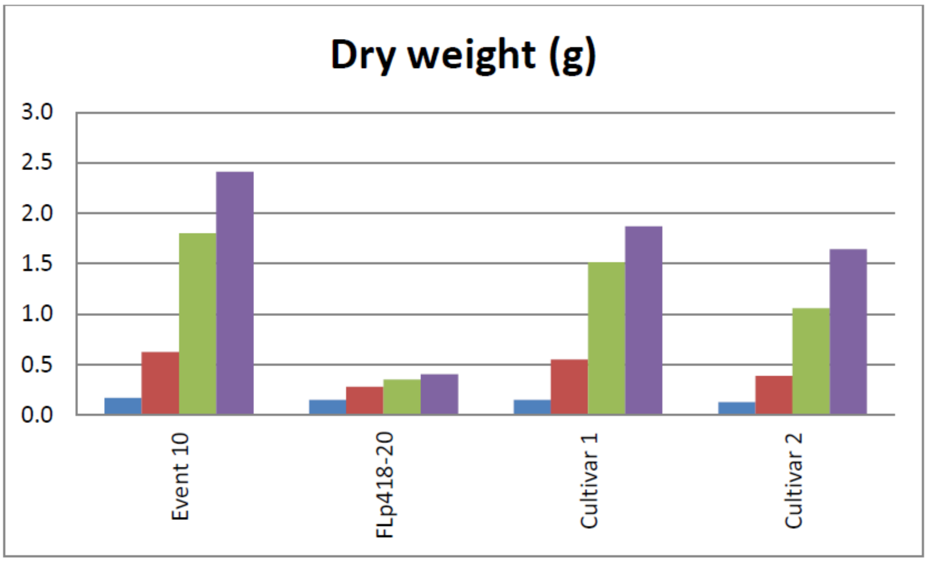

Figure 3. Dry weight measurements in a glasshouse study examining biomass gain over time in GM perennial ryegrass (Event 10) compared to its isogenic control (FLp418-20) and two commercial cultivars. All plants started as 3-tillers, cut to 5cm residual height. The plants were harvested at a residual height of 5cm for biomass when they reached the 3 leaf stage. Data is shown for harvest 1 (blue), harvest 2 (red), harvest 3 (green) and harvest 4 (purple). Information supplied by the applicant.

46. In the glasshouse, the GM perennial ryegrass line had a higher nutritional quality than its isogenic control, with an increase of up to 1.7 MJ/kg dry matter of metabolisable energy. In field trials the GM perennial ryegrass had higher metabolisable energy than commercial cultivars, with an increase of 0.5 – 0.8 MJ/kg dry matter. Dry matter digestibility was improved in spring and summer harvests. There were no significant differences in leaf blade fructan concentration between Event 10 and control commercial cultivars, however due to the higher yield of Event 10 its total fructan yield was 2.1-2.2 fold higher than the commercial cultivars.

47. No secondary genetic effects, other than the increased biomass and nutritional quality, have been observed for the GM perennial ryegrass plants that have been evaluated when growing under glasshouse conditions or during field trials under DIR 082.

48. Both the expression and selectable marker cassettes are single copy in the GM perennial ryegrass line, Event 10, as determined by Southern hybridisation.

49. The GM perennial ryegrass line, Event 10, underwent polycrosses to produce homozygous seeds.

Section 6 The receiving environment

50. 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).

51. Information relevant to the growth and distribution of perennial ryegrass in Australia is discussed in document *The Biology of* Lolium multiflorum *Lam. (Italian ryegrass),* Lolium perenne *L. (perennial ryegrass) and* Lolium arundinaceum *(Schreb.) Darbysh (tall fescue)* (OGTR, 2017).

6.1 Relevant abiotic factors

52. The release is proposed to take place on the Agriculture Victoria Research Division research farm situated 11 km south of Hamilton in south-west Victoria. This region is in the temperate climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). The average temperatures in Hamilton range between 9 – 26°C in summer and 4 – 13°C in winter (Bureau of Meteorology website).

53. There are no natural streams, creeks or springs, or artificial dams or irrigation channels running through or near the trial site.

6.2 Relevant biotic factors

54. Common pests of perennial ryegrass in Australia include the black field cricket, black headed pasture cockchafer, red headed pasture cockchafer, common army worm, common cutworm, pasture tunnel moth, red legged earth mite, lucerne flea and cereal rust mite (OGTR, 2017).

55. The major disease of ryegrass in Australia is rust, both crown rust and stem rust which are caused by fungus and can reduce dry matter and seed yield significantly (VicGov Pasture diseases in ryegrass). Other fungal pathogens include blind seed disease which reduces seed quality and yield and has cost the Victorian seed industry up to $2.5 million in some years, especially when it is humid during seed harvest (Cunningham et al., 1994).

6.3 Relevant agricultural practices

56. The applicant proposes to plant GM perennial ryegrass seed by hand. The GM perennial ryegrass plants will be grown under a 180 micron mesh polyhouse cover with irrigation. Herbicides may be used in the GM field trial in the early post emergence stage for the purpose of weed suppression, if required. As the GM plants develop the manual removal of weed species will be implemented. Additionally, a commercially available plant growth regulator may be used on the GM perennial ryegrass at reproductive stage 2 to reduce lodging, increase seed yield and prevent seed loss.

57. Fertiliser will be applied during sowing of the trial and in early spring.

58. The applicant proposes that upon flowering and pollen production no access to plants will be allowed unless in an emergency to assist with containing the pollen in the polyhouse structure. After seed set, all seed will be hand harvested.

6.4 Presence of related plants in the receiving environment

59. As discussed in Section 4, perennial ryegrass (*Lolium perenne* L.) is widely cultivated in Australia for grazing and as turf. Perennial ryegrass is able to hybridise with other grass species present in Victoria including Italian ryegrass (*Lolium multiflorum* Lam.), annual ryegrass (*L. rigidum* Gaud.), rigid ryegrass (*L. loliaceae*), hardy ryegrass (*L. remotum*), meadow fescue (*Festuca pratensis*), red fescue (*F. rubra* L.) and tall fescue (*F. arundinaceum*). However some hybrids are sterile including *L. perenne* x *L. loliaceum* and *L. perenne* × *L. remotum* (OGTR, 2017).

60. Within one km of the proposed site there are sexually compatible plant species including *L. perenne*, *L. arundinaceum* and *L. multiflorum* which are sown and some *L. rigidum* which is naturalised. In the further surrounding regions these species are grown in controlled grazing and crop production environments, and non-agricultural production environments such as roadside verges and as turf (information supplied by applicant).

61. Annual ryegrass (*L. rigidum* Gaud.) is a serious and costly weed of cropping systems in southern Australia (Steadman et al., 2004).

6.5 Presence of similar genes and encoded proteins in the environment

62. Both introduced genes were isolated from perennial ryegrass. As discussed in Section 4, perennial ryegrass is widely cultivated in Australia for both pasture and turf.

63. The *hph* gene is derived from *E. coli*, which is a common gut bacterium.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Approvals by the Regulator

64. The GM perennial ryegrass plants proposed for release have been approved previously for limited and controlled release into the Australian environment under licence DIR 082. There have been no adverse effects reported from the DIR 082 field trial.

7.1.2 Approvals by other government agencies

65. There are no approvals of GM perennial ryegrass, or applications for GM perennial ryegrass under consideration, by other Australian authorities.

7.2 International approvals

66. None of the GM perennial ryegrass lines covered in this application have been approved for release in any other country.

67. Other GM perennial ryegrass lines have been approved for field trials in both Denmark (EU GMO register, accessed 15 September 2017) and the USA (USDA GMO register, accessed 15 September 2017). The traits which have been modified include altered fructan levels, fungal resistance, drought tolerance and reduced pollen allergens.

Chapter 2 Risk assessment

Section 1 Introduction

68. 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.

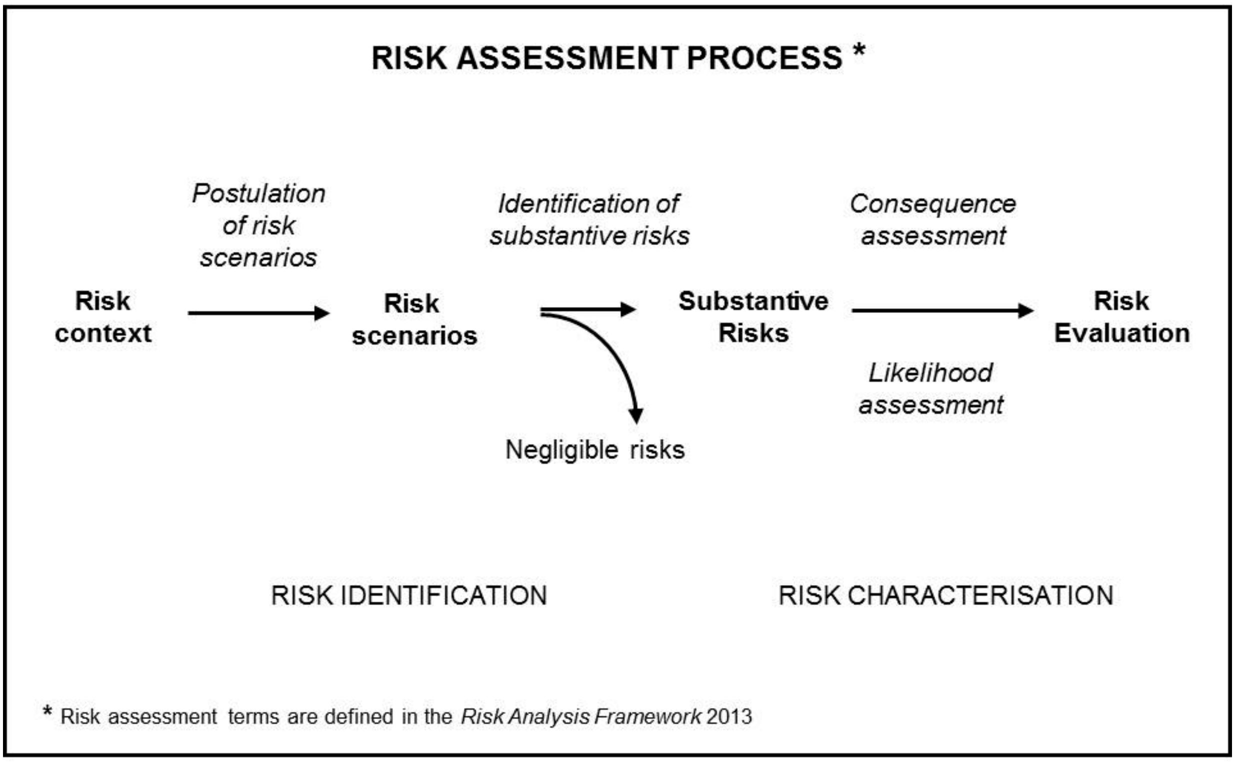

Figure 4. The risk assessment process

69. 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.

70. 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, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

71. Postulated risk scenarios are screened to identify those that are considered to have some 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.

72. 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

73. Postulated 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).

iii. Potential harm to people or the environment.

74. 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

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

76. As discussed in Chapter 1, the GM perennial ryegrass plants have been modified by the introduction of two genes derived from perennial ryegrass and intended to alter nutritional quality and biomass production. These introduced genes are considered further as potential sources of risk.

77. All of the GM perennial ryegrass plants also contain the *hph* gene which confers antibiotic resistance and was used as a selectable marker gene. This gene and its product have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. As the gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application.

78. The introduced genes are controlled by introduced regulatory sequences. These were derived from perennial ryegrass, rice and the common plant pathogen, Cauliflower Mosaic virus. 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, potential harms from the regulatory sequences will not be further assessed for this application.

79. 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

80. 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. pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities.

81. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs.

82. 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: HGT events rarely occur and the wild-type gene sequences 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.

83. 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

84. 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
- 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).

85. These harms are based on those used to assess risk from weeds (Virtue, 2004; Keese et al., 2014). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

86. Six 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 – 2.4.6. Postulation of risk scenarios considers impacts of the GM perennial ryegrass or its products 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.

87. In the context of the activities proposed by the applicant and considering both the short and long term, none of the six risk scenarios gave rise to any 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 altered fructan biosynthesis	Growing GM perennial ryegrass 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	• The GM plant material would not be used as human food. • 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 or previous field trials.
2	Introduced genes conferring altered fructan biosynthesis	Growing GM perennial ryegrass 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 from the trial would not be used as livestock feed. • The source organism is routinely used for animal feed and the introduced genes are commonly found in the environment and are not known to be toxic. • The proposed limits and controls would minimise exposure of native animals, birds or desirable insects to the GM plant material.

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
3	Introduced genes conferring altered fructan biosynthesis	Growing GM perennial ryegrass 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 OR Increased levels of pests or pathogens	No	• The source organism is routinely used for animal feed and the introduced genes are commonly found in the environment and are not known to be toxic. • The proposed controls would minimise persistence of GMOs after completion of the trial.
4	Introduced genes conferring altered fructan biosynthesis	Growing GM perennial ryegrass plants at the trial site ⬇ Dispersal of GM perennial ryegrass 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 OR Increased levels of pests or pathogens	No	• The proposed controls would minimise dispersal of GM seed. • Risk scenarios 1 and 2 did not identify any increased risk of toxicity or allergenicity in the GM plants.
5	Introduced genes conferring altered fructan biosynthesis	Growing GM perennial ryegrass plants at the trial site ⬇ Pollen flow to non-GM perennial ryegrass outside the trial site ⬇ Production of hybrid seed with GM traits	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased levels of pests or pathogens	No	• The proposed controls would minimise pollen flow to non-GM perennial ryegrass outside the trial site. • Consumption of perennial ryegrass containing low levels of GM plants by livestock is not expected to cause adverse health effects.

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
6	Introduced genes conferring altered fructan biosynthesis	Growing GM perennial ryegrass plants at the trial site ⬇ Outcrossing with plants that are sexually compatible with perennial ryegrass ⬇ Introgression of GM traits into populations of related species	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased levels of pests or pathogens	No	• The proposed controls would minimise outcrossing with sexually compatible plants. • The introduced genes are commonly found in the environment and are not known to be toxic.

2.4.1 Risk scenario 1

Risk source	Introduced genes conferring altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass 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

88. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal pathway

89. GM perennial ryegrass expressing the introduced genetic elements would be grown at the trial site. People could potentially be exposed to the GM plant material through skin contact or inhalation.

90. The licence application proposes that the GM plant material will not be used for human food. In addition, perennial ryegrass is not used as food in Australia, so there is little potential for accidental ingestion. Thus, it is not expected that people would be exposed to the GM perennial ryegrass by consumption.

91. The licence application proposes that only trained and authorised staff would be permitted to deal with the GM perennial ryegrass. Due to the small scale of the proposed trial, only a few people would be expected to handle the GM perennial ryegrass. These people could be exposed to plant material through skin contact or inhalation during cultivation, transportation or analysis of the GM perennial ryegrass.

92. As perennial ryegrass is wind pollinated, people working on the trial site or passing in the vicinity of the trial site could inhale airborne pollen during flowering of the GM perennial ryegrass. Perennial ryegrass produces copious amounts of pollen (Smart et al., 1979) which has been reported to travel distances of at least 36 m (Cunliffe et al., 2004) and at least 80 m (Giddings et al., 1997a), with pollen deposition reducing with distance. Perennial ryegrass anthesis occurs on average for 17 days (Elgersma, 1990), once daily around midday and is more profuse on warm, bright days (Thorogood et

al., 2002). In Melbourne, a bimodal release of perennial ryegrass pollen was also seen with a major peak between 1400-1800h and a minor peak between 0600-1000h (Smart and Knox, 1979).

93. The GM perennial ryegrass grown at the trial site will be enclosed within a 180 micron mesh polyhouse structure. Although the mesh size is larger than perennial ryegrass pollen, the polyhouse structure is expected to reduce wind flow and air turbulence which will limit the spread of perennial ryegrass pollen, and thus also limit human exposure to pollen.

94. The potential for pollen dispersal from the GM perennial ryegrass is discussed in more detail in Risk Scenario 5.

Potential harm

95. 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).

96. Although no toxicity or allergenicity studies have been performed on the GM plant material, the introduced genes were isolated from perennial ryegrass that is already widespread and prevalent in the environment. As discussed in Section 5.3 of Chapter 1, the proteins in perennial ryegrass are regularly consumed by livestock without adverse effects, so are not expected to be toxic.

97. As discussed in Section 5.3 of Chapter 1, non-GM perennial ryegrass pollen is a common source of airborne allergens and is a major cause of hay fever and seasonal allergic asthma. It is not expected that the introduced genes for fructan biosynthesis, which are controlled by a promoter which is specific to photosynthetic cells such as leaf tissue, would affect the expression of proteins in the pollen allergen production pathway.

98. As discussed in Section 5.4 of Chapter 1, the licence applicant has grown the GM perennial ryegrass line proposed for release in glasshouse trials to flowering, and in field trials under licence DIR 082 which did not permit the GM perennial ryegrass to flower. No adverse health effects were reported by people dealing with the GM plants in the glasshouse or field trials.

Conclusion: Risk scenario 1 is not identified as a substantive risk because the GM plant material would not be used as human food and the introduced genes are widespread in the environment and not expected to be toxic. Additionally, the proposed limits and controls would restrict exposure of people to the GM plant material through skin contact or inhalation of pollen, and there were no adverse health effects on people handling the GM plants in glasshouse and previous field 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 altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass 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

99. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal pathway

100. GM perennial ryegrass expressing the introduced genetic elements would be grown at the trial site. Animals entering the trial site could consume GM plant material.

101. The GM perennial ryegrass plants have increased biomass and metabolic energy compared to non-GM perennial ryegrass (as discussed in Section 5.4 of Chapter 1). This may make them more palatable and attractive to animals than non-GM perennial ryegrass.

102. The licence application proposes that the GM plant material will not be used for animal feed. Thus, agricultural livestock are not expected to be exposed to the GM perennial ryegrass.

103. The proposed trial site is enclosed in a 180 micron mesh polyhouse structure which is expected to exclude birds and animals, unless they are large enough to break through the mesh or able to burrow under the mesh. The applicant proposes that the polyhouse structure will be surrounded by a 1.2 m fence which would be rabbit proof, which indicates it will exclude smaller native animals, and prevent animals such as livestock from accessing the GM plants. The 180 micron mesh polyhouse structure would also exclude many insects, including desirable species such as pollinators.

104. The small size and short duration of the proposed trial would also restrict the numbers of animals, birds or invertebrates that could be exposed to the GM plants.

Potential harm

105. The introduced proteins involved in fructan biosynthesis are based on proteins present in non-GM perennial ryegrass. These proteins in perennial ryegrass are regularly consumed by livestock, wild animals and birds without adverse effects, so are not expected to be toxic to animals.

106. The introduced proteins involved in fructan biosynthesis could alter fructan composition in the GM perennial ryegrass plants. As discussed in Chapter 1, Section 5.3, fructans are normally present in a number of feed products eaten by animals and generally have no adverse effects. As discussed in Chapter 1, Section 5.4, there is no significant difference in total fructan concentrations in leaf blades between the GM plants and commercial perennial ryegrass cultivars.

107. Non-GM perennial ryegrass is susceptible to fungal diseases which cause toxicity to animals. Ryegrass staggers occurs in animals grazing pastures containing perennial ryegrass infected with certain endophytes that produce toxins, it is not usually fatal, and animals usually recover unaided (Reed, 1999). Ergot is another fungal disease associated with pasture grasses which is toxic to animals on consumption (Clarke, 1999). The susceptibility of GM perennial ryegrass to diseases is expected to be the same as for non-GM plants.

Conclusion: Risk scenario 2 is not identified as a substantive risk because the GM plant material would not be used as livestock feed, proteins from perennial ryegrass are regularly consumed and are not toxic, and the proposed limits and controls of the trial would minimise exposure of native animals, birds or desirable insects 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 altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass 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 OR Increased levels of pests or pathogens

Risk source

108. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal pathway

109. GM perennial ryegrass would be grown at the trial site and would bear seed. If either live GM plants or viable seed persisted at the trial site after completion of the trial, this could lead to establishment of volunteer GM perennial ryegrass populations in the environment.

110. The main method of reproduction of perennial ryegrass is by seed, however it can reproduce vegetatively. Perennial ryegrass is a bunchgrass (Thorogood, 2003) which can spread laterally in pastures by producing tillers and stolons up to a length of 15.5 cm (Sawada, 1991). The species has also been described as producing short rhizomes from which plants can resprout quickly following fire (Sullivan, 1992). The applicant has proposed to treat plants with a non-selective knockdown herbicide to facilitate decomposition after harvest to ensure no vegetative persistence would occur.

111. Seed yield of perennial ryegrass is variable depending on cultivars (Elgersma, 1990) and environmental conditions. Estimated perennial ryegrass seed production was 14,040 seed m^{-2} in a NSW study (Lodge, 2004) and 35,000 – 160,000 seed m^{-2} in a UK field study (Hampton and Hebblethwaite, 1983). Specifically, seed yield of perennial ryegrass cultivars of interest for the applicant have been reported as 74,300 seed m^{-2} for Trojan, 101,700 seed m^{-2} for Alto and 109,100 seed m^{-2} for Bronsyn (Foundation for Arable Research, 2011), calculated using an average seed weight of 2.3 mg (Naylor, 1980).

112. Some GM perennial ryegrass seeds may remain in the soil at the trial site after harvest, due for instance, to seed losses during harvest. These seeds could germinate and grow into volunteer GM perennial ryegrass plants. Germination would likely occur soon after the harvest as perennial ryegrass seed has a short dormancy period. Lush and Birkenhead (1987) showed in a study in Australia that it takes 2.8 days (in spring) to 6 days (in winter) for 50% of seeds to germinate in the field. Perennial ryegrass has also been reported to have high germination rates at a broad range of temperatures (Lodge, 2004).

113. The applicant proposes to hand harvest the GM perennial ryegrass seed to minimise the loss of seed and to till and irrigate the trial site following harvest to promote germination of residual seed. In addition, the applicant has proposed to monitor the planting area and monitoring zone for perennial ryegrass volunteers for at least 12 months after harvest, and until the site is free of volunteers for at least six consecutive months, and to destroy any volunteers found before they flower. These measures are expected to minimise persistence of GM plants or seeds at the trial site.

114. Non-GM perennial ryegrass is naturalised and widespread in Victoria (Atlas of Living Australia, 2017). Thus, it is plausible that if GM perennial ryegrass persisted at the trial site, it could spread and establish volunteer populations. As discussed in Chapter 1, Section 5.2.1, fructan accumulation is associated with tolerance to abiotic stresses such as drought or cold temperatures. Therefore there is uncertainty over whether the altered trait involving fructan biosynthesis could increase the survival or range of the GM plants in the environment. However, it is noted that, as discussed in Chapter 1,

Section 5.4, there is no significant difference in total fructan concentrations in leaf blades between the GM plants and commercial perennial ryegrass cultivars.

Potential harm

115. A potential harm from volunteer GM perennial ryegrass populations would be toxicity or allergenicity to people. People do not consume perennial ryegrass plants, but they could be exposed to the GM perennial ryegrass grown in this trial through inhalation of pollen. As discussed in risk scenario 1, the potential allergenicity of the GM perennial ryegrass pollen is not expected to be different from non-GM perennial ryegrass pollen.

116. Volunteer GM perennial ryegrass plants could be eaten by desirable animals, including livestock, native animals and birds. As discussed in risk scenario 2, the GM perennial ryegrass plants are not expected to have increased toxicity compared to non-GM perennial ryegrass.

117. Volunteer GM perennial ryegrass plants could potentially compete with and reduce establishment or yield of desirable plants, such as agricultural crops in farms or turf, or native plants in nature reserves. Perennial ryegrass is considered a significant environmental and agricultural weed in Australia (Groves et al., 2005; Randall, 2017). As discussed in Chapter 1, Section 5.4, the GM perennial ryegrass has increased biomass and a faster growth rate than non-GM commercial cultivars. This may mean that the GM plants are more competitive than non-GM perennial ryegrass. However, the GM perennial ryegrass planted in previous glasshouse experiments and field trials were grown under irrigation and fertilisation; no data has been provided about the performance of the GMOs in non-ideal conditions including under different abiotic stresses.

118. Non-GM perennial ryegrass volunteers can be effectively controlled by a range of herbicides (Dear et al., 2006), although some herbicide resistance in perennial ryegrass has been observed overseas in recent years (Ghanizadeh et al., 2015; Heap, 2017). GM perennial ryegrass with increased biomass may have lower susceptibility to herbicide, as increased weed size has been linked with reduced herbicide control for some grasses (Koger et al., 2005; Soltani et al., 2016).

119. The GM plants may have increased biomass and potentially increased palatability, so may provide better food for pests. Perennial ryegrass is widespread in the environment currently, therefore volunteer populations of GM perennial ryegrass plants are not expected to significantly increase the presence of pests and diseases that use ryegrass as a host in the environment.

Conclusion: Risk scenario 3 is not identified as a substantive risk because the GM plant material is not expected to be toxic and the proposed controls would minimise persistence of GMOs after completion 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 altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass plants at the trial site ⬇ Dispersal of GM perennial ryegrass 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 OR Increased levels of pests or pathogens

Risk source

120. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal pathway

121. GM perennial ryegrass would be grown at the trial site and would bear seed. GM seeds or GM vegetative parts could potentially be dispersed outside the trial site by wind or water, by human activity or by animal activity. This GM seed could germinate and the GM vegetative parts (e.g. stems or tillers) could propagate and give rise to plants expressing the introduced genes. These volunteer GM plants could spread and persist in the environment and establish populations of GM perennial ryegrass.

122. The applicant has proposed limits and controls to prevent the spread of seeds or plant material from the trial site due to human activity. The proposed field trials would occur on a research station with restricted access and it is expected that only people conducting dealings would enter the site. The applicant proposes that all equipment used in contact with the GMOs would be cleaned before removal from the trial site or use for other purposes and staff entering the polyhouse structure would wear single-use disposable coveralls and booties. Transport of GM perennial ryegrass seeds to and from the trial site would be conducted in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These controls would minimise the likelihood of dispersal of GM perennial ryegrass seeds or vegetative parts from the trial site by human activity.

123. Perennial ryegrass seeds are spread by wind and have shattering seed heads to aid windborne dispersal (Elgersma et al., 1988). The proposed polyhouse structure surrounding the trial site has a mesh size of 180 microns. This would contain perennial ryegrass seeds, which have a length of 5 to 8 mm and a diameter of 1 to 1.5 mm (Cool and Hannaway, 2004). GM perennial ryegrass seed would be hand harvested to minimise the loss of seed.

124. GM perennial ryegrass seeds on the soil surface could be transported by water during heavy runoff or flooding. The applicant has proposed that the field trial site would be located at least 2 km from any natural or artificial waterway, which would minimise the potential for seed dispersal through flooding. Perennial ryegrass is moderately tolerant to waterlogging or flooding (Razmjoo et al., 1993). It will tolerate extended periods of flooding (up to 25 days) when temperatures are below 27˚C. Seed dispersal in irrigation water has been observed for *Lolium* spp. in Chile, with germinable seeds recovered from the irrigation water (Tosso et al., 1986).

125. Perennial ryegrass is able to reproduce vegetatively by forming clones, with adventitious roots, from cut stem pieces kept in water (Uchida and Arasea, 2005). There is no literature available on the likelihood of vegetative dispersal occurring in this manner under field conditions. Vegetative dispersal of perennial ryegrass over short distances (on average 4 cm, maximum 15.5 cm) is possible from aerial tillers with propagules in pasture (Sawada, 1991). As discussed in risk scenario 2, the applicant has proposed to treat plants with a non-selective knockdown herbicide to ensure no vegetative material would persist after harvest.

126. Animals, such as native animals, birds, rabbits, rodents and seed-eating ants, could potentially enter the trial site in order to feed on GM perennial ryegrass. The genetic modifications for increased fructans could potentially increase the palatability of the GM perennial ryegrass. Ants (Campbell, 1966) and rodents (Hulme, 1994) have both been observed to transport perennial ryegrass seeds. In a study of seed dispersal by sheep, seeds of perennial ryegrass were transported in the wool of grazing sheep, and remained in the wool for 1-2 months (Fischer et al., 1996). Grass seeds are capable of germination after passing through the digestive systems of grazing animals such as cattle and sheep

(Chambers and MacMahon, 1994). A study of seed dispersal after ingestion by goats reported that 1.6% of perennial ryegrass seeds remained viable after digestion, and 0.4% were able to form seedlings (Harrington et al., 2011); the seeds were completely excreted by 48 h post ingestion. Some bird species have been shown to graze on *Lolium* spp. (Patton and Frame, 1981; Buckingham et al., 2011) and a preliminary study where birds were fed perennial ryegrass found less than 0.2% of perennial ryegrass seeds excreted were viable (Woodgate et al., 2011). However, no literature is available on the potential of perennial ryegrass seed dispersal by birds.

127. The applicant proposes to control rodents in the trial site by baiting and maintaining the monitoring zone in a manner as to not attract or harbour rodents. The applicant also proposes to exclude birds by enclosing the trial site in a polyhouse mesh structure and restrict access by larger animals by surrounding the polyhouse structure with a 1.2 m meshed fence. In addition, any GM seed that is transported a few metres from the parent plant would likely still be located within areas of the trial site where the applicant proposes to monitor and destroy volunteers. The proposed controls are expected to minimise dispersal of GM seed or vegetative parts outside the trial site.

128. As discussed in risk scenario 3, if GM perennial ryegrass plants did escape the trial site, it is plausible that they could establish as volunteer populations in the environment.

Potential harm

129. 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 perennial ryegrass populations in the environment.

Conclusion: Risk scenario 4 is not identified as a substantive risk because the GM perennial ryegrass is not expected to be toxic and the proposed controls would minimise dispersal of GM seed or vegetative parts. 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 altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass plants at the trial site ⬇ Pollen flow to non-GM perennial ryegrass outside the trial site ⬇ Production of hybrid seed with GM traits ⬇
Potential harms	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased levels of pests or pathogens

Risk source

130. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal Pathway

131. GM perennial ryegrass would be grown at the trial site and would produce pollen. If the GM pollen fertilised non-GM perennial ryegrass plants that flowered simultaneously the non-GM plants

would produce hybrid GM seed. The seed could enter grazing pastures for animal feed, turf, or grow into volunteer GM perennial ryegrass plants in the environment.

132. Perennial ryegrass is self-incompatible (Fearon et al., 1983), though it will set seed when manually selfed (Spoor, 1976).

133. As perennial ryegrass is a highly outcrossing, wind pollinated species, extensive gene flow can occur (Kloot, 1983). Pollen viability data is lacking in recent literature for perennial ryegrass; Gregor (1928) found 33% of perennial ryegrass flowers set seed when pollinated with 24 h old pollen, but not with 48 h old pollen which was stored in vials in the dark. Under natural conditions, pollen viability data available for the closely related species tall fescue found pollen viability reduced to 5% in 30 minutes in sunny conditions or 150 minutes in cloudy conditions (Wang et al., 2004). Gene flow is dependent on pollen viability and the distance pollen can travel. Perennial ryegrass pollen has been reported to travel distances of over 36 m (Cunliffe et al., 2004), over 80 m (Giddings et al., 1997a) and has been modelled to travel 1 km (Giddings, 2000). In both field studies pollen flow was observed to decline rapidly with distance (Giddings et al., 1997a; Cunliffe et al., 2004). Strong, turbulent winds can increase the distance pollen can travel (Giddings et al., 1997b).

134. A study on pollen flow found that little outcrossing occurred beyond 6 m in perennial ryegrass and no evidence of crossing was found beyond 12 m, when grown in a single space-planted row (Copeland and Hardin, 1970). A later study of perennial ryegrass gene flow from a donor plot to recipient plants isolated from other ryegrass showed that relative fertility of the recipient plants decreased from an average of 2.6% at 36 m to 1% at 144 m (Cunliffe et al., 2004). Gene flow was higher in the direction of prevailing winds but always produced less than 5% relative fertility at 36 m and less than 2% at 144 m. Commercial seed production standards in Australia require that perennial ryegrass grown as basic seed must have an isolation distance of 100 m from grass species for cultivation areas larger than 2 ha or 200 m for areas less than 2 ha (Seed Services Australia, 2013).

135. The applicant proposes to manage pollen flow by enclosing the trial site in a 180 micron mesh polyhouse structure, which will reduce wind flow and air turbulence to limit the spread of perennial ryegrass pollen. Perennial ryegrass pollen is 23 to 60 micron in size (Jansen and Den Nijs, 1993) which is smaller than the polyhouse mesh size. However, mesh enclosures have been shown to reduce dispersal of maize and grass pollen (Neal and Anderson, 2004; Watanabe et al., 2006a) and to reduce wind flow and air turbulence (Teitel, 2007). The polyhouse structure would be surrounded by a monitoring zone of at least 40 m maintained as bare fallow and an isolation zone of 100 m, surrounding the monitoring zone, would be maintained in a manner to prevent flowering of grasses. These controls are expected to minimise pollen flow to non-GM perennial ryegrass.

136. In the unlikely event that a GM perennial ryegrass plant fertilised a non-GM perennial ryegrass plant, the hybrid offspring would contain the introduced genes conferring increased fructan biosynthesis. These hybrids would be heterozygous and possibly have lower expression levels of the introduced genes. If GM hybrids occurred they would be a small proportion of the population as the GM trial site is small and a long distance from sexually compatible species, so GM pollen would be sparse compared with abundant pollen from nearby non-GM plants.

Potential harm

137. As discussed in risk scenario 1, allergenicity and toxicity to people is not expected to be increased in the GM perennial ryegrass. This will be the same if the introduced genes are expressed in hybrid GM perennial ryegrass plants.

138. If hybrid GM plants were produced in pastures the hybrid GM plants would only be a very small proportion of a non-GM perennial ryegrass crop and they could also only form a very small part of the daily diet of livestock or animals. As discussed in risk scenario 2, toxicity to desirable animals is not expected to be changed in the GM perennial ryegrass by the altered trait, consequently no increase in toxicity is expected if the introduced genes are expressed in hybrid perennial ryegrass plants.

139. If hybrid GM perennial ryegrass seeds grew into volunteer plants in the environment, the potential harms of the introduced genes would be the same as discussed in risk scenario 3.

Conclusion: Risk scenario 5 is not identified as a substantive risk because the proposed controls would minimise pollen flow to non-GM perennial ryegrass outside the trial site and consumption of perennial ryegrass containing low levels of GM plants by livestock is not expected to cause adverse health effects. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.6 Risk scenario 6

Risk source	Introduced genes conferring altered fructan biosynthesis
Causal pathway	⬇ Growing GM perennial ryegrass plants at the trial site ⬇ Outcrossing with plants that are sexually compatible with perennial ryegrass ⬇ Introgression of GM traits into populations of related species ⬇
Potential harms	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased levels of pests or pathogens

Risk source

140. The source of potential harm for this postulated risk scenario is the introduced genes for fructan biosynthesis.

Causal Pathway

141. GM perennial ryegrass would be grown at the trial site and would produce fertile flowers. If the GM perennial ryegrass outcrossed with related species that flowered simultaneously, this could produce hybrid GM seed. GM hybrid plants could backcross with the related species leading to introgression of GM traits into the related species.

142. As described in Chapter 1, Section 6.4, the plant species that are sexually compatible with perennial ryegrass and present in Victoria are Italian ryegrass (*Lolium multiflorum* Lam.), annual ryegrass (*L. rigidum* Gaud.), rigid ryegrass (*L. loliaceae*), hardy ryegrass (*L. remotum*), meadow fescue (*Fesctuca pratensis*), red fescue (*F. rubra*) and tall fescue (*F. arundinaceum*). However, some hybrids are reported to be sterile (*L. perenne* x *L. loliaceum*, *L. perenne* x *L. remotum*). Perennial ryegrass flowers during the same time period as Italian ryegrass, annual ryegrass, meadow fescue, red fescue and tall fescue in spring/summer (OGTR, 2017) so these five species could potentially cross with GM perennial ryegrass and produce fertile offspring. All of these five species are naturalised in Australia and categorised as weedy to some extent (Groves et al., 2003).

143. Hybrids with *L. perenne* are often difficult to distinguish from one of their parents (Jessop et al., 2006). Hybrids of *L. perenne* x *L. multiflorum* are known as *Lolium* x *hybridum* or intermediate ryegrass and can arise spontaneously where the parent species grow together. *L.* x *hybridum* is higher-yielding than *L. perenne* and more resistant to adverse winter conditions than *L. multiflorum* (Kemešytė et al., 2013). *L.* x *hybridum* has been reported in Victoria (Atlas of Living Australia, 2017).

144. *Festulolium* grasses are defined as hybrids between any ryegrass (*Lolium*) and fescue (*Festuca*) species (Kopecký et al., 2016). They combine the higher yields of nutritious fodder from *Lolium*

together with added resilience to abiotic and biotic stress from *Festuca*. Hybrids of *L. perenne* × *L. pratense* (meadow fescue) are known as *Festulolium loliaceae* (Giddings et al., 1997a) and have only been reported in Western Australia (Atlas of Living Australia, 2017).

145. Outcrossing between the GM perennial ryegrass and related species could occur either by pollen from the GMOs fertilising related species within or outside the trial site, or by pollen from related species fertilising the GMOs. If pollen from the GM perennial ryegrass fertilised a related species, hybrid GM seeds growing on the plant could be widely dispersed. For instance, perennial ryegrass seeds shatter (Elgersma et al., 1988) and are transported by wind, water, externally on animals and internally through animal digestive tracts (OGTR, 2017). If pollen from a related species fertilised a GMO, hybrid seeds growing on the GM plant would have fewer dispersal routes, as the proposed control measures should restrict GM seed dispersal and persistence at the trial site (risk scenarios 3 and 4).

146. As discussed in risk scenario 5, the applicant has proposed a number of controls to restrict pollen flow, which would also minimise the potential for outcrossing with plants that are sexually compatible with perennial ryegrass.

147. As discussed in risk scenario 3, the altered trait involving fructan biosynthesis could potentially increase the tolerance of the GM perennial ryegrass plants to abiotic stresses such as drought or cold temperatures. There is uncertainty over whether the altered trait could increase the survival or range of hybrid plants in the natural environment.

Potential harms

148. The closely related grass, annual ryegrass (*L. rigidum* Gaud.) is a serious and costly weed of cropping systems in southern Australia (Steadman et al., 2004). It is highly competitive and can compete with crops as early as the two-leaf stage. Annual ryegrass was first recognised as resistant to herbicide in Australia in 1982 when it developed resistance to diclofop-methyl (Christopher et al., 1991). Currently, annual ryegrass has been identified as resistant to numerous classes of herbicides with different active ingredients in Australia including atrazine, chlorsulfuron, clethodim, glyphosate, haloxyfop-methyl, imazapyr, iodosulfuron-methylsodium, paraquat, amitrole and trifluralin (Heap, 2017). Other species that are closely related to perennial ryegrass vary in weediness however are less problematic than annual ryegrass (Groves et al., 2003).

149. As discussed in Chapter 1, Section 5.4, the GM perennial ryegrass has significantly higher biomass, rate of growth and nutritional quality than a non-GM control. If the introduced genes were present in an interspecies hybrid, the hybrid could also have these traits, though likely not to the same extent. As discussed in Risk Scenario 3, increased biomass and growth rate could cause a plant to be more competitive. Increased biomass could also reduce susceptibility of a plant to herbicides. Increased biomass and nutritional quality could potentially make a plant a better host for pests that are present across ryegrass-related species (Harris and Lowien, 2003; Clark, 2008).

150. Ryegrass pollen is a major source of allergenic reaction in sensitive people (Smart et al., 1979). However, as discussed in risk scenario 1, the genetic modification of increased fructan biosynthesis should not increase pollen allergenicity, including if this genetic element was introgressed into a sexually compatible species.

151. As discussed in risk scenario 2, toxicity to desirable organisms is not expected to be changed in the GM perennial ryegrass by the introduced genes. This will be the same if the introduced genes are expressed in other sexually compatible species.

Conclusion: Risk scenario 6 is not identified as a substantive risk because the introduced genes are commonly found in the environment and are not toxic and the proposed controls would minimise outcrossing with sexually compatible plants. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

153. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). 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
- 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.

154. 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.

155. 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.

156. For DIR 160, uncertainty is noted particularly in relation to:

- Potential for increased toxicity to livestock or increased allergenicity to people of the GM perennial ryegrass
- Potential for the genetic modifications to increase plant competiveness and survival
- Potential for reduced herbicide effectiveness on the GM perennial ryegrass
- Potential for long distance pollen flow of perennial ryegrass

157. 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.

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

Section 4 Risk evaluation

159. 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.

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

- risk criteria
- level of risk

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

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

161. Six 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 on people handling the GM plants in glasshouse and previous field trials
- limits on the size and duration of the proposed release
- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM perennial ryegrass plants and their genetic material.

162. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM perennial ryegrass 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[2].

[2] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities, and the public.

Chapter 3 Risk management plan

Section 1 Background

163. 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.

164. 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.

165. 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.

166. 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

167. 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 perennial ryegrass. 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

168. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been drafted to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in Chapter 4 (the draft licence).

3.1 Draft licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by the Department of Economic Development, Jobs, Transport and Resources (DEDJTR)

169. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by DEDJTR in the application. These are taken into account in the six 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

170. The applicant proposes that the duration of the field trial would be limited to two years. In both years, the proposed planting area is 160 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).

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

172. The applicant proposes that no GM plant material would be used for human food or animal feed. This would minimise exposure of people or desirable animals to the GM perennial ryegrass by consumption (risk scenarios 1 and 2).

Controls for persistence or disposal

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

174. Perennial ryegrass is a perennial plant that can reproduce vegetatively and is not killed by harvesting. The applicant proposes to destroy all GMOs not required for analysis or future trials. A draft licence condition requires all GM plants in the field to be destroyed (e.g. by herbicide application) within 14 days after completion of harvest. This will help to restrict persistence of GM perennial ryegrass on the trial site (risk scenario 3). In addition, a draft licence condition requires that GMOs must be harvested or destroyed within 10 months of planting to restrict the number of seeds released into the seed bank (risk scenario 3).

175. The applicant proposes to monitor the planting area for perennial ryegrass volunteers for at least 12 months after harvest, and until the site is free of volunteers for at least six consecutive months, and to destroy any volunteers found before they flower. Perennial ryegrass seed germinates quickly and under a wide range of temperatures (Lush and Birkenhead, 1987; Lodge, 2004). It takes 2.8 days (in spring) to 6 days (in winter) for 50% of seeds to germinate in the field (Lush and Birkenhead, 1987), and 70.5% of perennial ryegrass seeds germinated within 21 days following one month of storage after harvest (Lodge, 2004). A field experiment in NSW indicated that 14 months after seed production the seed bank contained 14% of the number of perennial ryegrass seeds released, and after 26 months no seed bank remained. However, this study stated that some seed was produced by volunteers growing during the experiment, and did not measure the viability of seed in the seed bank (Lodge, 2004). Thus, the seeds present in the seed bank after 14 months may have been second or third generation seeds and/or seeds not capable of germination.

176. The applicant proposes to till and irrigate the planting area to promote germination and reduce persistence of GM seed. Tillage is a commonly used practice to promote the germination of weeds (Liebman et al., 2001) and has been shown to stimulate seedling emergence in annual ryegrass (Peltzer and Matson, 2002). The draft licence conditions require tillage and irrigation of the planting area and the adjacent area, which is the land between the planting area and the inner edge of the monitoring zone, where seed may have been dispersed on the soil surface (risk scenario 4). Tillage and irrigation must occur at least once. If GM perennial ryegrass volunteers grow following the tillage and irrigation, these treatments must be repeated, until further tillage and irrigation no longer induce germination of volunteers. The proposed period of post-harvest monitoring, combined with the tillage and irrigation requirements, is considered appropriate to minimise persistence of GM perennial ryegrass seed (risk scenario 3).

177. The applicant did not propose a frequency for post-harvest inspections. In the Kangaroo Valley cultivar of perennial ryegrass, it takes on average 150 days from seedling emergence to anthesis, but individual plants from early-flowering biotypes were observed to flower as soon as 106 days after seedling emergence (Shah et al., 1990). Perennial ryegrass plants can also grow vegetatively from rhizomes and stolons (Sawada, 1991) and these plants may grow more rapidly than seedlings. There is also a possibility that recently emerged small perennial ryegrass volunteers could be missed during inspections. A draft licence condition requires post-harvest inspections to occur at least every 35 days to ensure that volunteers are found and destroyed prior to flowering.

178. GM perennial ryegrass seed lost during harvest activities could potentially fall a short distance outside the planting areas unobserved. There is also potential for short-distance dispersal of GM perennial ryegrass seeds by ants or rodents (risk scenario 4). The applicant proposes to surround the planting area with a 40 m monitoring zone which would be subject to inspection requirements to detect any volunteers arising from short-distance seed transport. A draft licence condition also requires that the adjacent area (land between the planting area and the inner edge of the monitoring zone) is subject to inspection requirements.

179. The applicant proposes that the field trial site would be located at least 2 km away from natural or artificial waterways. This is expected to manage the possibility of dispersal of GM perennial ryegrass seeds by flooding (risk scenario 4). A standard licence condition requires that the outer edge of a planting area be at least 100 m away from waterways. Another consideration is that perennial ryegrass seed could be locally dispersed by high winds or heavy runoff in the event of a severe storm at seed maturity, particularly if there is storm damage to the polyhouse structure described below that would contain the small perennial ryegrass seeds under normal circumstances (risk scenario 4). A standard licence condition requires notification of any extreme weather condition affecting the trial site while GMOs are growing and until the site is signed off to allow assessment and management of any risks.

180. The applicant proposes that all equipment used with the GMOs would be cleaned before use for other purposes or removal from the trial site. It is proposed that people entering the planting area would wear dedicated clothing (e.g. coveralls or lab coats and booties) which would be removed when leaving the planting area to prevent transport of GM propagules or pollen on clothing. The applicant also proposes to transport and store GMOs in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These controls would restrict the potential for dispersal of GMOs by people (risk scenario 4).

181. The applicant proposes that rodents in the planting area would be controlled by baiting. In addition, the applicant proposes that the 40 m monitoring zone surrounding the planting area would be maintained as fallow, which is expected to deter rodents from entering the planting area, as has been shown in rice fields, maintaining a field as fallow has been shown to limit rat populations (Leung and Singleton, 1999). These measures would restrict the potential for dispersal of GM seed by rodents (risk scenario 4). A draft licence condition requires implementation of measures including rodent baits and/or traps to control rodents within the planting area.

182. The applicant proposes that the planting area would be enclosed in a 180 micron mesh polyhouse structure that is expected to exclude birds and animals unless they are large enough to break through the mesh or able to burrow under the mesh. In addition, the applicant proposes that the polyhouse structure would be enclosed in a fence capable of excluding livestock and rabbits, which indicates that the fence would also exclude small native animals. This would minimise the potential for dispersal of GM seeds or vegetative parts from the planting areas by birds or animals (risk scenario 4) as well as the exposure of animals or birds to the GMOs by consumption (risk scenario 2).

183. Dispersal of viable seeds by rodents, birds or large animals could occur at planting, while mature seeds are present on the GM plants, or while seeds lost during harvest are present on the soil surface but have not yet germinated or decomposed. Therefore, the draft licence conditions regarding rodent, bird and animal controls require these measures (i.e. the polyhouse structure, fence, baiting/trapping

and maintenance of the monitoring zone as fallow) to be in place from before planting until 60 days after harvest. As a study found that 70.5% of perennial ryegrass seeds germinated within 21 days (Lodge, 2004), a period of 60 days would allow almost all seeds on the soil surface to germinate or decompose.

Controls for pollen dispersal

184. The applicant proposes to manage pollen flow from the GM perennial ryegrass by four measures. Firstly, the planting area would be enclosed in a 180 micron mesh polyhouse structure, which is expected to partially block pollen passage and also to reduce wind flow and air turbulence to limit the spread of perennial ryegrass pollen. Secondly, the polyhouse enclosure will be equipped with an anteroom to prevent easy escape of pollen through open doors. Thirdly, a monitoring zone of at least 40 m surrounding the polyhouse structure will be maintained as fallow and inspected for related species during flowering of the GM perennial ryegrass. Fourthly, an isolation zone of at least 100 m, surrounding the monitoring zone, will be maintained in a manner to prevent flowering of grasses (e.g. mown, grazed or treated with selective grass herbicides) while the GM perennial ryegrass is flowering.

185. A study of gene flow from perennial ryegrass (Cunliffe et al., 2004) found that relative fertility of the recipient plants decreased from an average of 2.6% at 36 m (comparable to a 40 m monitoring zone) to 1% at 144 m (comparable to a 140 m combined monitoring zone plus isolation zone). Commercial seed production standards for perennial ryegrass grown as basic seed in Australia require an isolation distance of 200 m from grass species where the recipient grass area is less than 2 ha (Seed Services Australia, 2013). Thus, the proposed isolation distance of 140 m would not be sufficient, alone, to prevent gene flow from the GM perennial ryegrass. However, the proposed isolation distance is combined with use of a 180 micron polyhouse enclosure. Perennial ryegrass pollen is 23 to 60 microns in size (Jansen and Den Nijs, 1993) which is smaller than the polyhouse mesh size. However, in maize with a pollen grain size of 78 x 95 microns (Watanabe et al., 2006b), a 1000 micron mesh enclosure was shown to reduce pollen flow and outcrossing by 76% (Watanabe et al., 2006a). Cotton fabric exclusion bags with a pore size of 223 microns were observed to reduce pollen flow of grasses and pine by approximately 90% (Neal and Anderson, 2004). The proposed polyhouse enclosure has a pore size of 180 microns which is expected to be similarly effective. Thus, the combination of proposed control measures would minimise outcrossing between the GM perennial ryegrass and sexually compatible plants outside the trial site (risk scenarios 5 and 6), as well as minimising exposure of people to the GM pollen (risk scenario 1).

186. The applicant proposes to routinely inspect the 180 micron mesh polyhouse structure and the fence surrounding the polyhouse for damage, and repair any damage found. A draft licence condition requires that the polyhouse structure be inspected for damage at least every two weeks and during flowering be inspected for damage at least every three days. The fence will also be inspected at least every two weeks.

187. The applicant proposes to inspect the monitoring zone weekly while the GM perennial ryegrass is flowering to destroy any plants that are sexually compatible with perennial ryegrass. A draft licence condition requires similar inspection of the adjacent area, which is the land between the planting area and the inner edge of the monitoring zone, for sexually compatible plants and inspection of the planting area for sexually compatible plants (other than trial plants). Although the applicant proposed weekly monitoring, it takes 30 – 35 days from anthesis to development of viable seeds (Shah et al., 1990) so a draft licence condition requires fortnightly inspections. Due to the wide range of times when individual perennial ryegrass plants may commence flowering (Shah et al., 1990), draft licence conditions require that inspections of the monitoring zone, and other measures that are required while the GMOs are flowering, must commence at least two weeks before the expected start of flowering.

188. The applicant proposed two additional measures to restrict pollen flow. The first was that a tree lined wind break to the north-west and south-west of the trial site will reduce wind flow across the location, however, considering the existence of the polyhouse a wind break would not be expected to

have significant additional effects, and thus no conditions in the draft licence require a wind break. The applicant also proposed that while the GM perennial ryegrass plants are flowering, people would only access the polyhouse enclosure in case of emergency. However, considering the existence of an anteroom, and the draft licence condition requiring that people entering the polyhouse enclosure wear dedicated clothing which is removed when exiting, it is not considered that people entering the polyhouse during flowering are likely to transport pollen. Therefore, the proposed access restriction is not included in the draft licence conditions.

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

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

- limit the duration of the release to between May 2018 and June 2020
- limit the size of the release to 160 m^2 each year at one site in the shire of the Southern Grampians, Victoria
- locating the proposed field trial site at least 100 m away from the nearest natural or artificial waterway
- containing GM plants in a 180 micron meshed polyhouse enclosure
- inspecting the polyhouse enclosure for damage at least every two weeks and while the GM perennial ryegrass is flowering inspecting for damage at least every three days
- surrounding the polyhouse enclosure with a monitoring zone of at least 40 m that is kept fallow. While the GM perennial ryegrass is flowering, inspecting the monitoring zone fortnightly for species that are sexually compatible with perennial ryegrass, and destroying any plants found
- surrounding the monitoring zone with an isolation zone of at least 100 m that is maintained in a manner that prevents the flowering of grasses
- controlling rodents by baiting and/or trapping
- surrounding the trial site with a fence capable of excluding rabbits and larger animals
- cleaning equipment prior to use for other purposes or removal from the trial site
- transporting GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- destroying all GMOs not required for analysis or future trials
- post-harvest tillage and irrigation of the planting area and adjacent area to promote germination of volunteers
- post-harvest monitoring of the planting area, adjacent area and monitoring zone for at least 12 months and until the site is free of volunteers for at least 6 months, with any perennial ryegrass volunteers destroyed before flowering
- not allowing the GM plant material to be used in human food or animal feed.

3.2 Other risk management considerations

190. 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

191. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

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

193. 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

194. If a licence were issued, DEDJTR would be required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM perennial ryegrass outside permitted areas.

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

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

196. If a licence were issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, DEDJTR would be required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

197. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

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

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

- expected and actual dates of planting
- details of areas planted to the GMOs
- expected dates of flowering
- expected and actual dates of harvest
- dates of cleaning the planting areas

- details of inspection activities.

3.2.5 Monitoring for compliance

199. 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.

200. 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.

201. 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

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

- additional molecular and biochemical characterisation of the GM perennial ryegrass plants, particularly with respect to potential for increased toxicity or allergenicity
- additional phenotypic characterisation of the GM perennial ryegrass plants, particularly with respect to potential for increased competitiveness and survival
- information regarding potential change to herbicide susceptibility of the GM plants
- information regarding potential for long distance pollen flow from perennial ryegrass.

Section 5 Conclusions of the consultation RARMP

203. The RARMP concludes that the proposed limited and controlled release of GM perennial ryegrass 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.

204. If a licence were issued, conditions would be imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Draft licence conditions

Section 1 Interpretations and definitions

1. In this licence:

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

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

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

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

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

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

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

2. In this licence:

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

'Adjacent Area' means land other than the Planting Area which is inside the Polyhouse Enclosure, as indicated in Figure 1. Land defined as an Adjacent Area while a Polyhouse Enclosure is in place continues to be defined as an Adjacent Area if the Polyhouse Enclosure is removed after harvest of the GMOs.

'**Bare Fallow**' means land where no plants are intentionally grown, and plants that are unintentionally grown cover no more than 20% of the soil surface.

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

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

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

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

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

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

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

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

(a) manual uprooting;

(b) treatment with herbicide;

(c) burning/incineration;

(d) crushing or grinding of seed;

(e) autoclaving; or

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

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

'Equipment' includes, but is not limited to, seeders, harvesters, transport equipment (e.g. bags, containers, trucks) and tools.

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

'GM' means genetically modified.

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

'Isolation Zone' means an area of land extending outwards at least 100 m in all directions from the outer edge of the Monitoring Zone, as indicated in Figure 1.

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

'Monitoring Zone' means an area of land extending outwards at least 40 m from the edge of the Polyhouse Enclosure, as indicated in Figure 1. Land defined as a Monitoring Zone while a Polyhouse Enclosure is in place continues to be defined as a Monitoring Zone if the Polyhouse Enclosure is removed after harvest of the GMOs.

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

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

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

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

'Perennial Ryegrass' means plants of the species *Lolium perenne.*

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

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

'**Polyhouse Enclosure**' means a structure with walls and roof composed of mesh with a maximum pore diameter of 180 microns.

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

'Regulator' means the Gene Technology Regulator.

'Related Species' means plants from the genus *Lolium* or the genus *Festuca* that are sexually compatible with Perennial Ryegrass, excluding Perennial Ryegrass.

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

'Tillage' means the use of any technique to disturb the soil.

'Volunteers' means GM or non-GM Perennial Ryegrass plants, or hybrid plants of Perennial Ryegrass and a Related Species, that have not been intentionally grown.

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

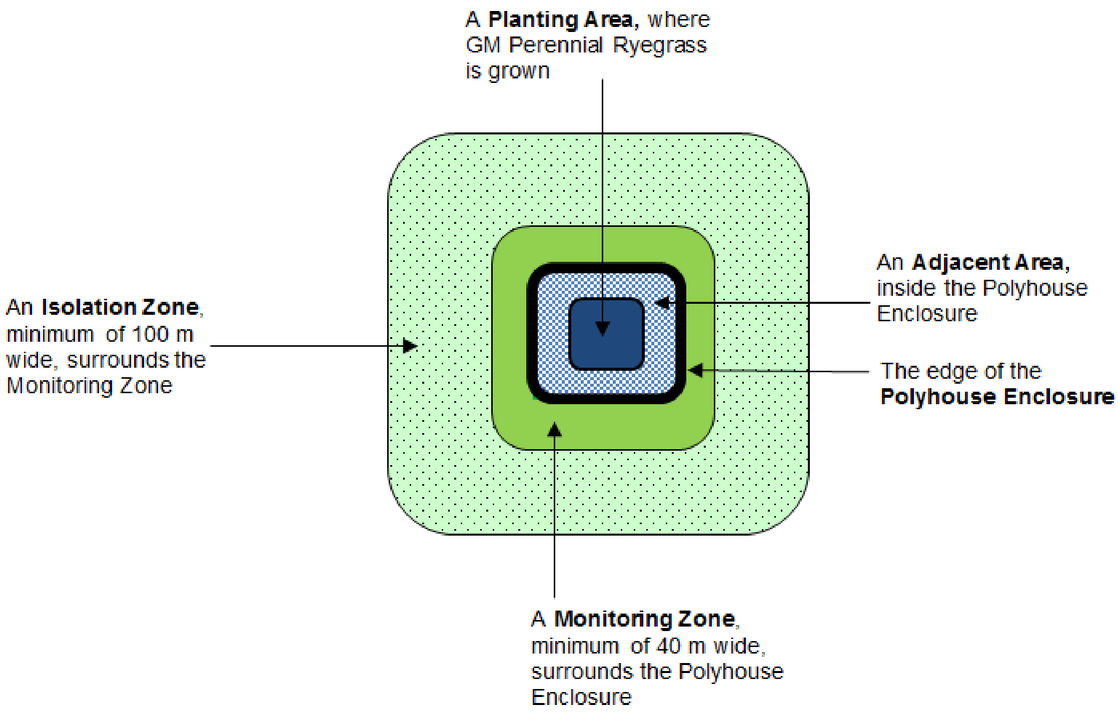

Figure 1. Diagram (not to scale) showing the relationships between the Planting Area, Adjacent Area, Polyhouse Enclosure, Monitoring Zone and Isolation Zone.

Section 2 General conditions and obligations

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

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

5. The holder of this licence ('the licence holder') is the Department of Economic Development, Jobs, Transport and Resources (DEDJTR).

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

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

Obligations of the Licence Holder

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

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

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

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

10. The licence holder must:

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

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

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

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

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

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

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

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

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

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

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

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

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

(d) written methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism, and to distinguish between categories of GMOs approved for release; and

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

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

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

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

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

(c) the surrender of the licence.

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

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

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

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

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

16. The licence holder must:

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

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

Provision of new information to the Regulator

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

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

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

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

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

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

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

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

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

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

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

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

Obligations of persons covered by the licence

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

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

Section 3 Limits and control measures

Limits on the release

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

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

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

(b) non-GM Perennial Ryegrass plants; and

(c) plants approved in writing by the Regulator.

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

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

Period	Maximum number of Planting Areas per year	Maximum size of Planting Area	Local Government Area in which Planting Area may be located
May 2018 – June 2020	1	160 m^2	Southern Grampians Shire

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

Containment measures

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

Pollen and seed dispersal during cultivation

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

26. Any extreme weather event that is expected to affect or has already affected a Planting Area or associated areas, while the GMOs are growing or while the Planting Area is subject to inspection

requirements, must be notified in writing to the Regulator as soon as practically and reasonably possible.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 48).

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

28. Rodents within the Planting Area and Adjacent Area must be controlled by trapping and/or baiting from prior to planting the GMOs until at least 60 days after the GMOs are harvested or Destroyed.

29. The Planting Area must be enclosed within a Polyhouse Enclosure from prior to planting the GMOs until at least 60 days after the GMOs are harvested or Destroyed.

30. Entry and exit to the Polyhouse Enclosure must be through an anteroom with an inner and an outer door.

31. People entering the Polyhouse Enclosure must wear dedicated outer clothing and footwear, and must remove this clothing and footwear in the anteroom when exiting the Polyhouse Enclosure. The used clothing and footwear must be Cleaned before use for any other purpose.

32. Equipment used in connection with the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

33. The Polyhouse Enclosure must be enclosed within a fence capable of excluding livestock and rabbits from prior to planting the GMOs until at least 60 days after the GMOs are harvested or Destroyed.

34. The Adjacent Area must be maintained as Bare Fallow from prior to planting the GMOs until at least 60 days after the GMOs are harvested or Destroyed.

35. The Polyhouse Enclosure must be surrounded by a Monitoring Zone that is maintained as Bare Fallow from prior to planting the GMOs until at least 60 days after the GMOs are harvested or Destroyed.

36. The Monitoring Zone must be surrounded by an Isolation Zone that is maintained in a manner that does not permit Flowering of Perennial Ryegrass or Related Species, from 14 days prior to the expected Flowering of any GMOs until all GMOs in the Planting Area have finished Flowering.

Note: Examples of methods to prevent Flowering of Perennial Ryegrass or Related Species include mowing, heavy grazing, or application of herbicide effective in killing these grass species.

37. While the GMOs are growing in a Planting Area, and for at least 60 days after harvest, inspections must be conducted by people trained to recognise Perennial Ryegrass and Related Species, and actions taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
(a) Planting Area	First inspection must occur at least 14 days prior to the expected commencement of Flowering of any GMOs*, and inspections must continue until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Related Species	Destroy before Flowering or prevent from Flowering simultaneously with the GMOs
(b) Adjacent Area and Monitoring Zone	First inspection must occur at least 14 days prior to the expected commencement of Flowering of any GMOs*, and inspections must continue until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Perennial Ryegrass and Related Species	Destroy before Flowering or prevent from Flowering simultaneously with the GMOs
(c) Polyhouse Enclosure	i. From 14 days prior to the expected commencement of Flowering of any GMOs*, and until all GMOs in the Planting Area have finished Flowering ii. At all other times while Polyhouse Enclosure is required under Condition 29.	i. At least once every three days ii. At least once every 14 days.	Damage that may reduce containment	Repair as soon as practicable
(d) Fence	While a fence is required under Condition 33.	At least once every 14 days	Damage that may permit animals to enter	Repair as soon as practicable

**Condition 51(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.*

Note: Details of any inspection activity must be recorded in a Logbook as detailed in Condition 51(e).

38. GMOs planted in the Planting Area must be harvested or Destroyed within 10 months of planting.

39. If the GMOs planted in the Planting Area are Destroyed, they are taken to have been harvested for the purposes of this licence and all conditions applying to post-harvest apply equally to post-destruction.

Processing or experimentation with GMOs

40. If processing of GM seed is not conducted under a Notifiable Low Risk Dealings (NLRD) authorisation, such activities may only be undertaken within:

(a) a Planting Area before Cleaning; or

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

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

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

(a) a Planting Area before Cleaning; or

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

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

Transport or storage of the GMOs

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

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

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

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

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

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

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 49).

Persistence of the GMOs

45. The Planting Area and Adjacent Area must be Cleaned within 14 days after completion of harvest of the GMOs.

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

Area of land	Period of inspection	Inspection frequency	Inspect for	Action
(a) Planting Area (b) Adjacent Area (c) Monitoring Zone (d) Any other area where GMOs have dispersed during planting, growing or harvesting (e) Any other area used to Clean Equipment used in connection with the GMOs (f) Any other area used to Destroy GMOs	From the day of completion of Cleaning of the Planting Area and Adjacent Area, until: i. an area becomes a new Planting Area under this licence*; or ii. the Regulator has issued a Sign-off for the area.	At least once every 35 days	Volunteers	Destroy before Flowering

** If an area subject to post-harvest inspection requirements under Condition 46 is replanted with GMOs, the new Planting Area no longer requires post-harvest inspections, but surrounding areas do still require post-harvest inspections. In this case, some areas would be subject to inspection requirements under both Condition 46 and Condition 37.*

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

(a) date of the inspections;

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

(c) details of the experience, training or qualification that enables the person(s) to recognise Perennial Ryegrass and/or Related Species, if not already recorded in the Logbook;

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

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

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

(g) for post-harvest areas, details of any post-harvest crops and any recent management practices applied (including Tillage events)

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

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

(i) date(s) and method(s) of Destruction or of preventing Flowering simultaneously with the GMOs of any Perennial Ryegrass, Related Species or Volunteers;

(j) details of any damage and any repairs to the Polyhouse Enclosure surrounding the Planting Area, while the Polyhouse Enclosure is required;

(k) details of any damage and any repairs to the fence surrounding the Polyhouse Enclosure, while the fence is required; and

(l) details of rodent control methods used and any evidence of rodent activity, while rodent control methods are required.

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

- descriptive text

- marking on a diagram

- indicating grid references on corresponding map/sketch

Note: Details of Inspection activities must be provided to the Regulator (Condition 51(e)).

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

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

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

(c) no plants may intentionally be grown in the area unless the plants are:

i. the GMOs or non-GM Perennial Ryegrass, planted in accordance with the conditions of this licence; or

ii. agreed to in writing by the Regulator.

Contingency plan

49. If any unintentional presence of the GMOs is detected outside the areas requiring inspection, the contingency plan must be implemented.

Section 4 Sign off

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

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

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

(c) the Planting Area and Adjacent Area have undergone at least one Tillage and irrigation event, where Tillage and irrigation both occur within a period of 14 days;

(d) no Volunteers that are GMOs have occurred after the most recent Tillage and irrigation event, as described in the previous clause; and

(e) no Volunteers that are GMOs have occurred in the most recent 6 month period.

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

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

Section 5 Reporting and documentation

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

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

Notice	Content of notice	Timeframe
(a) Intention to Plant	i. Details of the Planting Area including size, the Local Government Area, GPS coordinates, a street address or other directions and a diagrammatical representation of the site (e.g. Google Maps) ii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details) iii. Date on which the GMOs will be planted iv. Period when the GMOs are expected to Flower v. Period when harvesting is expected to commence vi. How all areas requiring post-harvest inspections are intended to be used until Sign-off, including the proposed post-harvest crop(s), if any vii. Details on how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers, Perennial Ryegrass or Related Species viii. History of how the site has been used for the previous two years	At least 7 days prior to each planting (to be updated immediately if the notified details change)

Notice	Content of notice	Timeframe
(b) Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition.	Within 7 days of any planting
(c) Harvest	Actual date(s) of harvesting or Destroying the GMOs.	Within 7 days of commencement of any harvesting.
(d) Cleaning	i. Actual date(s) on which any areas needing Cleaning were Cleaned. ii. Method of Cleaning	Within 7 days of completion of any Cleaning
(e) Inspection activities	Information recorded in a Logbook as per the inspection requirements (Conditions 37, 46 and 47).	Within 35 days of inspection

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

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