



**Australian Government**

**Department of Health**

Office of the Gene Technology Regulator

March 2018

# **Risk Assessment and Risk Management Plan**

for

## **DIR 160**

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

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

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# Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 160

## Decision

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

## The application

Application number	DIR 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 ( <i>Lolium perenne</i> )
Introduced genes and modified traits	<ul style="list-style-type: none"> <li>Two fructan biosynthesis genes (sucrose:sucrose 1-fructosyltransferase and fructan:fructan 6G-fructosyltransferase) from perennial ryegrass for increased plant nutritional quality and biomass production</li> <li><i>hph</i> selectable marker gene from <i>Escherichia coli</i></li> </ul>
Proposed location	One site in the Southern Grampians Shire in south-west Victoria.
Proposed release size	Up to 160 m <sup>2</sup> 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 are taken into account in the risk assessment.

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 and the imposed 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.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food 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	<i>Gene Technology Act 2000</i>
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
<i>6G-FFT</i>	<i>fructan:fructan 6G-fructosyltransferase</i>
h	hours
ha	hectare
HGT	horizontal gene transfer
<i>hph</i>	<i>hygromycin phosphotransferase</i>
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
<i>1-SST</i>	<i>sucrose:sucrose 1-fructosyltransferase</i>

# Chapter 1 Risk assessment context

## Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

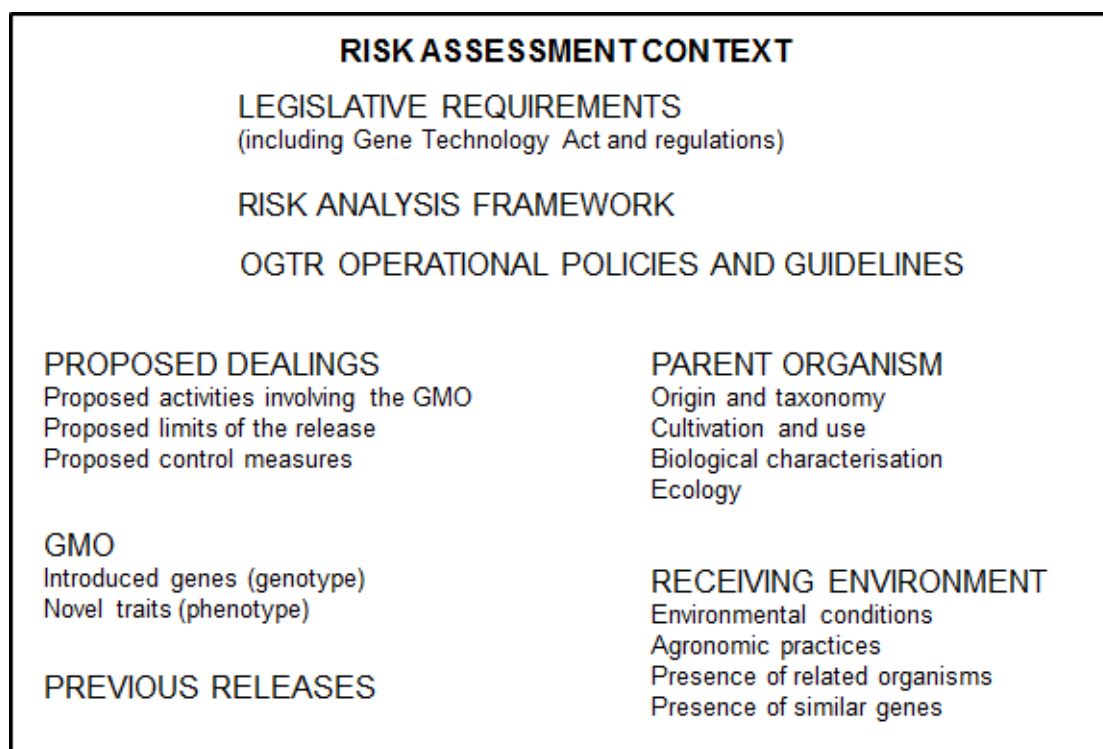


Figure 1. Summary of parameters used to establish the risk assessment context

## Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.
5. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that: its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed appropriate 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. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. No public submissions were received.

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 proposes 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<sup>2</sup>. 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 154 micron (aperture) steel mesh polyhouse enclosure that will reduce



wind-mediated pollen dispersal<sup>1</sup>

- 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 weekly 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 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
- tilling and irrigating the trial site post-harvest 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) (studies cited in 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 (Event 10) 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.

17. 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).

<sup>1</sup> During the consultation period, the applicant amended their DIR licence application with details of the polyhouse structure. The RARMP and licence were updated to reflect these amendments.

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

### 5.1 Introduction to the GMOs

18. 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
<i>Lp1-SST</i>	sucrose:sucrose 1-fructosyltransferase	Fructan biosynthesis	Perennial ryegrass
<i>Lp6G-FFT</i>	fructan:fructan 6G-fructosyltransferase	Fructan biosynthesis	Perennial ryegrass
<i>hph</i>	Hygromycin phosphotransferase	Hygromycin resistance (selectable marker)	<i>Escherichia coli</i>

19. The GM perennial ryegrass 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.

20. Short regulatory sequences that control gene expression are also present in the GM perennial ryegrass. The introduced perennial ryegrass genes are controlled by the perennial ryegrass *LpRbcS* gene promoter which is light regulated and targets expression to photosynthetic cells (Kyojuka 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).

21. The GM perennial ryegrass 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

22. 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).

23. 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 Gaudillière, 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).

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

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

25. 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.

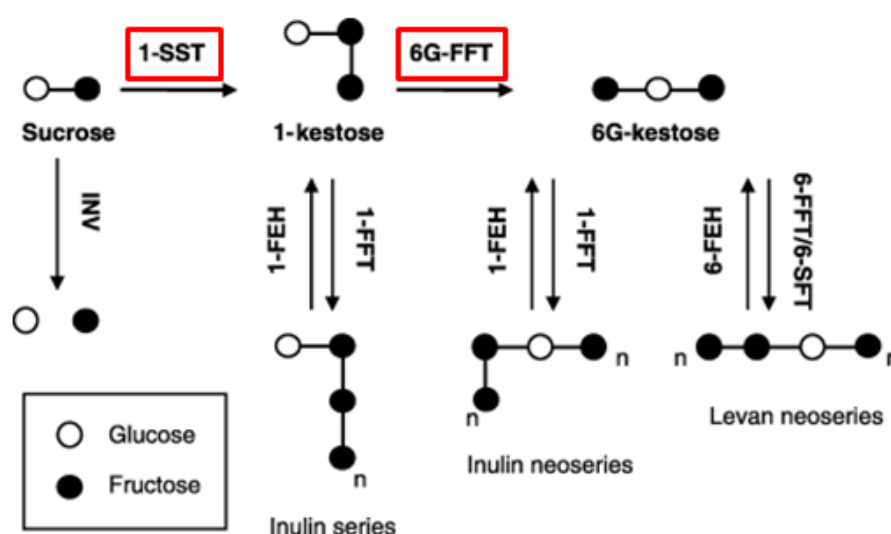


Figure 2. Proposed fructan metabolism pathway in *Lolium perenne* (Chalmers et al., 2005). GM perennial ryegrass will contain additional copies of 1-SST and 6G-FFT, highlighted by red boxes. Abbreviations used: INV (invertase); 1-SST (sucrose:sucrose 1-fructosyltransferase); 6G-FFT (fructan:fructan 6-glucose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

26. The expression of fructan biosynthesis genes in photosynthetic cells, and the resultant accumulation of fructans in these cells, is hypothesised to prevent the inhibition mechanisms of photosynthesis. This is thought to delay leaf senescence and increase CO<sub>2</sub> fixation which results in increased plant biomass production (see patent WO2010124324 A1). Additionally, by targeting fructan biosynthesis gene expression to the photosynthetic cells it is thought the fructans preferentially accumulate in the leaves and provide more energy for grazing livestock.

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

27. 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).

28. 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).

29. 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).

30. 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

31. 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  $\beta(2-1)$  or  $\beta(2-6)$  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).

32. 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

33. 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.

34. 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).

35. 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).

36. In addition to hygromycin B, the HPT protein phosphorylates the closely related compounds hygromycin B<sub>2</sub>, 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

37. 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.

38. 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).

39. 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).

40. 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).

41. 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).

42. 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.

43. Perennial ryegrass has a mutualistic symbiotic relationship with the endophyte, *Neotyphodium lolii* (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.

44. 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

45. 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). Two cultivars containing the same transformation event were selected for further field analysis.

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

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

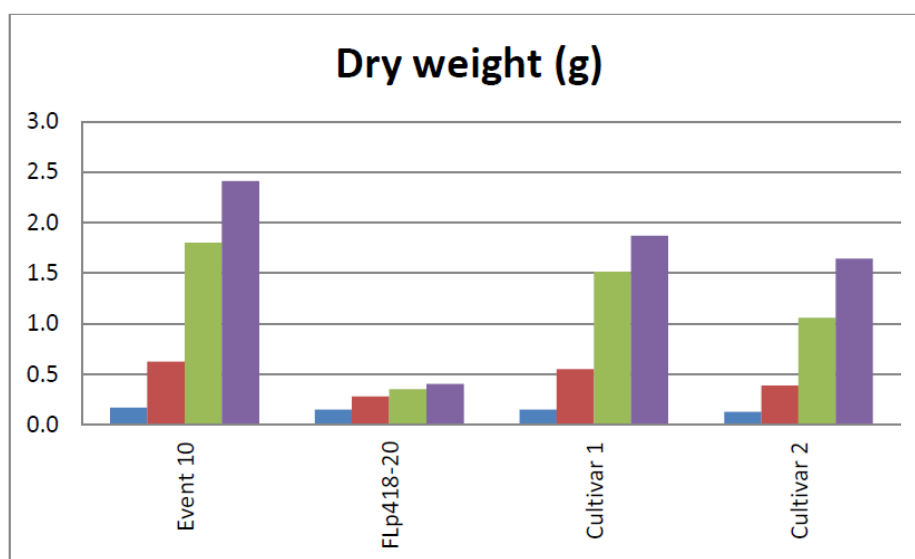


Figure 3. Dry weight measurements in a glasshouse study examining biomass gain over time in GM perennial ryegrass (Event 10) compared to its near-isogenic control (FLp418-20) and two commercial cultivars. All plants started as 3-tillers, cut to 5cm residual height. The plants were harvested at a residual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

48. In the glasshouse, the GM perennial ryegrass had a higher nutritional quality than its near-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 the GM perennial ryegrass and control non-GM commercial cultivars, however due to the higher yield of the GM plants their total fructan yield was 2.1-2.2 fold higher than the commercial cultivars.

49. 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.

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

51. The GM perennial ryegrass underwent polycrosses to produce seeds homozygous for the genetic modification.

## Section 6 The receiving environment

52. 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).

53. 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

54. 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](#)).

55. 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

56. 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).

57. 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

58. The applicant proposes to plant GM perennial ryegrass seed by hand. The GM perennial ryegrass plants will be grown with irrigation in a metal structure enclosed on the roof and outer ends by 180 micron thick greenhouse film. The sides of the structure, the anteroom and the vent boxes will be covered with stainless steel mesh cloth in 0.1mm wire with an aperture size of 154 microns. 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.

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

60. 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

61. 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. loliaceum*), 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* x *L. remotum* (OGTR, 2017).

62. 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).

63. 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**

64. 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.

65. 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**

66. 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 (licence surrendered in 2016). There have been no adverse effects reported from the DIR 082 field trial.

#### **7.1.2 Approvals by other government agencies**

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

### **7.2 International approvals**

68. The GM perennial ryegrass in this application has not been approved for release in any other country.

69. 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 modifications include altered fructan levels, increased fungal resistance, increased drought tolerance and reduced pollen allergens.



## Chapter 2 Risk assessment

### Section 1 Introduction

70. 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.

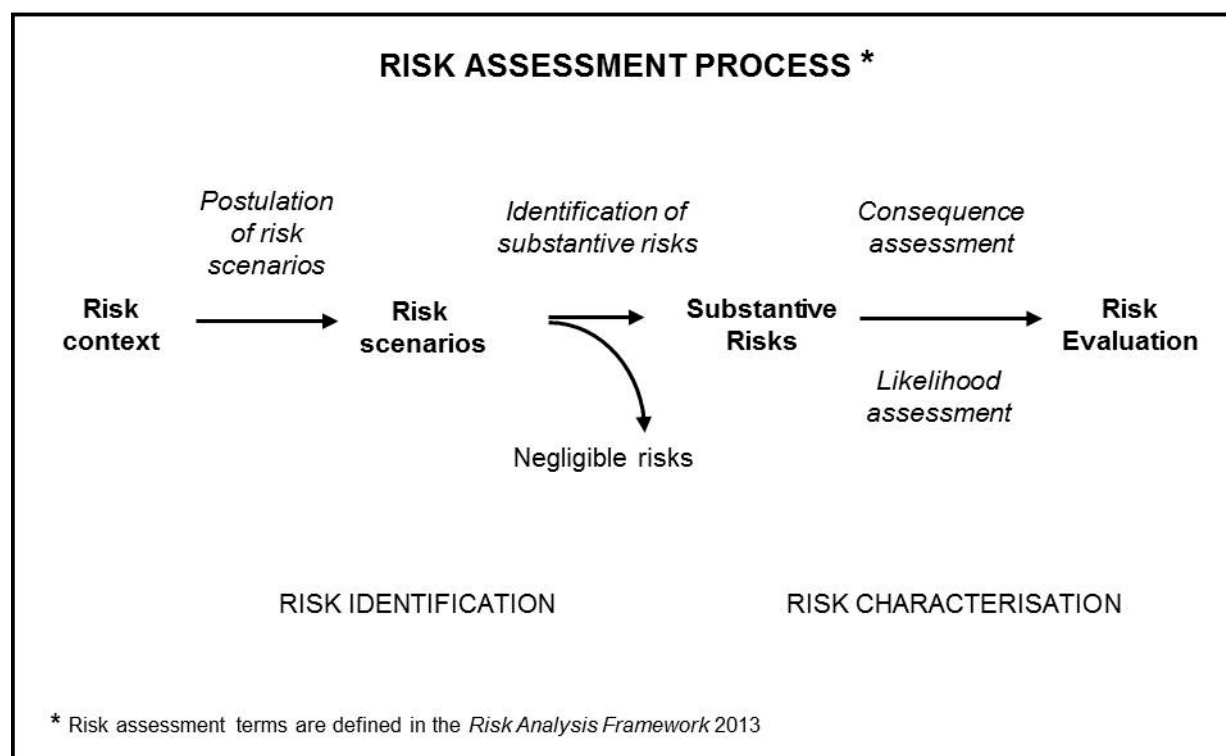


Figure 4. The risk assessment process

71. 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.

72. 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.

73. 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.

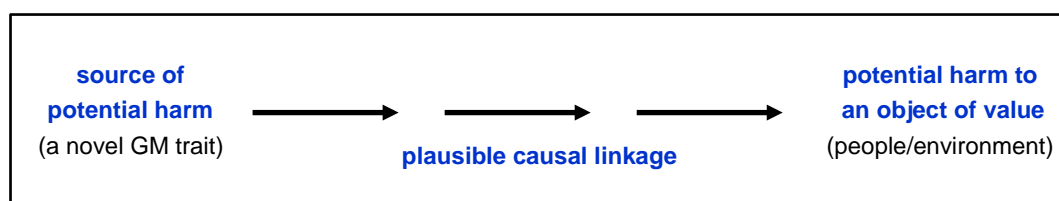
74. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood

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

75. 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.



76. 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

77. 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.

78. 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.

79. 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.

80. 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.

81. 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

82. 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.

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

84. 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.

85. 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

86. 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).

87. 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

88. 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.

89. 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	<ul style="list-style-type: none"> <li>• The GM plant material would not be used as human food.</li> <li>• The proposed limits and controls would restrict exposure of people to the GM plant material through skin contact or inhalation of pollen.</li> <li>• There were no adverse health effects on people handling the GM plants in glasshouse trials or previous non-flowering field trials.</li> </ul>
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	<ul style="list-style-type: none"> <li>• GM plant material from the trial would not be used as livestock feed.</li> <li>• 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.</li> <li>• The proposed limits and controls would minimise</li> </ul>

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
					exposure of native animals, birds or desirable insects to the GM plant material.
3	Introduced genes conferring altered fructan biosynthesis	<p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Persistence of GM plants after completion of the trial</p> <p>↓</p> <p>Establishment of volunteer GM plants in the environment</p> <p>↓</p> <p>Expression of introduced genes in the volunteer plants</p>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment or yield of desirable plants</p> <p>OR</p> <p>Increased levels of pests or pathogens</p>	No	<ul style="list-style-type: none"> <li>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.</li> <li>The proposed controls would minimise persistence of GMOs after completion of the trial.</li> </ul>
4	Introduced genes conferring altered fructan biosynthesis	<p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Dispersal of GM perennial ryegrass seeds outside the trial site</p> <p>↓</p> <p>Establishment of volunteer GM plants in the environment</p> <p>↓</p> <p>Expression of introduced genes in the volunteer plants</p>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment or yield of desirable plants</p> <p>OR</p> <p>Increased levels of pests or pathogens</p>	No	<ul style="list-style-type: none"> <li>The proposed controls would minimise dispersal of GM seed.</li> <li>Risk scenarios 1 and 2 did not identify any increased risk of toxicity or allergenicity in the GM plants.</li> </ul>
5	Introduced genes conferring altered fructan biosynthesis	<p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Pollen flow to non-GM perennial ryegrass outside the trial site</p> <p>↓</p> <p>Production of hybrid seed with GM traits</p>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment or yield of desirable plants</p> <p>OR</p> <p>Increased levels of pests or pathogens</p>	No	<ul style="list-style-type: none"> <li>The proposed controls would minimise pollen flow to non-GM perennial ryegrass outside the trial site.</li> <li>Consumption of perennial ryegrass containing low levels of GM plants by livestock is not expected to cause adverse health effects.</li> </ul>
6	Introduced genes conferring altered fructan biosynthesis	<p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Outcrossing with plants that are sexually compatible with perennial</p>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p>	No	<ul style="list-style-type: none"> <li>The proposed controls would minimise outcrossing with sexually compatible plants.</li> <li>The introduced genes are commonly found in the environment and are not known to be toxic.</li> </ul>

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
		ryegrass ↓ Introgression of GM traits into populations of related species	Reduced establishment or yield of desirable plants OR Increased levels of pests or pathogens		

#### 2.4.1 Risk scenario 1

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Growing GM perennial ryegrass plants at the trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of introduced genes in GM plants</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of people who deal with the GM plant material or of people in the vicinity of the trial site</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Toxicity or allergenicity to people

##### **Risk source**

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

##### **Causal pathway**

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

92. 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.

93. 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.

94. 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 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 14.00-18.00 and a minor peak between 06.00-10.00 (Smart and Knox, 1979).

95. The GM perennial ryegrass grown at the trial site will be enclosed within a 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 (see further discussion in Chapter 3 Section 3.1).

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

### Potential harm

97. 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).

98. 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.

99. 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. Thus pollen from this GM perennial ryegrass would not be expected to be more allergenic than pollen from non-GM ryegrass.

100. 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 non-flowering 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

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	↓
	Growing GM perennial ryegrass plants at the trial site
	↓
	Expression of introduced genes in GM plants
<i>Potential harm</i>	↓
	Exposure of animals eating GM plant material
	↓
	Toxicity to desirable animals

#### Risk source

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

#### Causal pathway

102. GM perennial ryegrass expressing the introduced genes would be grown at the trial site. Animals entering the trial site could consume GM plant material.

103. 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.

104. 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.

105. The proposed trial site is enclosed in polyhouse structure, constructed from 180 micron thick polymer with 0.1 mm wire steel mesh sides. This is expected to exclude birds and other 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 154 micron aperture mesh in the polyhouse structure would also exclude many insects.

106. 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**

107. 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.

108. 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.

109. 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**

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Growing GM perennial ryegrass plants at the trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Persistence of GM plants after completion of the trial</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Establishment of volunteer GM plants in the environment</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of introduced genes in the volunteer plants</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	<p style="text-align: center;">Toxicity or allergenicity to people</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Toxicity to desirable animals</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced establishment or yield of desirable plants</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Increased levels of pests or pathogens</p>



**Risk source**

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

**Causal pathway**

111. 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.

112. 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 material would persist.

113. 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<sup>-2</sup> in a NSW study (Lodge, 2004) and 35,000 – 160,000 seed m<sup>-2</sup> 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<sup>-2</sup> for Trojan, 101,700 seed m<sup>-2</sup> for Alto and 109,100 seed m<sup>-2</sup> for Bronsyn ([Foundation for Arable Research, 2011](#)), calculated using an average seed weight of 2.3 mg (Naylor, 1980).

114. 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).

115. 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.

116. 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**

117. 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.

118. 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.

119. 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. There is uncertainty regarding whether the increased biomass also means there will be increased number of tillers produced per plant and, therefore, higher seed yield. 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.

120. 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). Therefore, there is uncertainty over whether the altered trait could reduce the effectiveness of herbicide on the GM perennial ryegrass plants.

121. 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

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	<p>↓</p> <p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Dispersal of GM perennial ryegrass seeds outside the trial site</p> <p>↓</p> <p>Establishment of volunteer GM plants in the environment</p> <p>↓</p> <p>Expression of introduced genes in the volunteer plants</p> <p>↓</p>
<i>Potential harm</i>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment or yield of desirable plants</p> <p>OR</p> <p>Increased levels of pests or pathogens</p>

**Risk source**

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

**Causal pathway**

123. 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.

124. 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.

125. Perennial ryegrass seeds are spread by wind and have shattering seed heads to aid windborne dispersal (Elgersma et al., 1988). The mesh that forms the sides of the proposed polyhouse structure surrounding the trial site has an aperture size of 156 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.

126. 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).

127. 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 3, the applicant has proposed to treat plants with a non-selective knockdown herbicide to ensure no vegetative material would persist after harvest.

128. 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.

129. 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.

130. 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**

131. 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**

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	<p>↓</p> <p>Growing GM perennial ryegrass plants at the trial site</p> <p>↓</p> <p>Pollen flow to non-GM perennial ryegrass outside the trial site</p> <p>↓</p> <p>Production of hybrid seed with GM traits</p> <p>↓</p>
<i>Potential harms</i>	<p>Toxicity or allergenicity to people</p> <p>OR</p> <p>Toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment or yield of desirable plants</p> <p>OR</p> <p>Increased levels of pests or pathogens</p>

### **Risk source**

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

### **Causal Pathway**

133. 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.

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

135. 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 and there is also some uncertainty around pollen flow distances for perennial ryegrass (see discussion in Chapter 3 Section 3.1).

136. The applicant proposes a combination of measures to manage pollen flow. They will enclose the trial site in a polyhouse structure. Perennial ryegrass pollen is 23 to 60 micron in size (Jansen and Den Nijs, 1993) which is smaller than the polyhouse mesh size. However, as discussed in Chapter 3, Section 3.1.1, 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. The combination of these controls is expected to minimise pollen flow to non-GM perennial ryegrass.

137. 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**

138. 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.

139. 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.

140. 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 reduce 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

<i>Risk source</i>	Introduced genes conferring altered fructan biosynthesis
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Growing GM perennial ryegrass plants at the trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Outcrossing with plants that are sexually compatible with perennial ryegrass</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Introgression of GM traits into populations of related species</p> <p style="text-align: center;">↓</p>
<i>Potential harms</i>	<p style="text-align: center;">Toxicity or allergenicity to people</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Toxicity to desirable animals</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced establishment or yield of desirable plants</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Increased levels of pests or pathogens</p>

#### Risk source

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

#### Causal Pathway

142. 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.

143. 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. loliaceum*), hardy ryegrass (*L. remotum*), meadow fescue (*Festuca 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).

144. 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](#)).

145. *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* x *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](#)).

146. 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).

147. 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.

148. 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**

149. 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).

150. 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).

151. 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.

152. 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.

153. The introduced fructan biosynthesis genes are derived from the parent plant, *Lolium perenne* and are commonly found in the environment. Therefore, introgression of these genes from non-GM perennial ryegrass into populations of related species may occur. This is considered much more likely than the introgression of the genes from GM perennial ryegrass due to the proposed limits and controls on the trial.

**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

154. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis<sup>2</sup>.

155. 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.

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

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

158. For DIR 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 competitiveness and survival, particularly relating to increased tolerance to abiotic and biotic stresses
- potential for reduced herbicide effectiveness on the GM perennial ryegrass
- potential for long distance pollen flow of perennial ryegrass.

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

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

## Section 4 Risk evaluation

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

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

- risk criteria

<sup>2</sup> 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.



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

163. 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.

164. 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.

## Chapter 3 Risk management plan

### Section 1 Background

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

166. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

167. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

168. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

### Section 2 Risk treatment measures for substantive risks

169. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people 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 controls (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

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

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

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

171. 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**

172. The applicant proposed that the duration of the field trial would be limited to two years. In both years, the proposed planting area is 160 m<sup>2</sup>. 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).

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

174. The applicant proposed 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**

175. The applicant proposed 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).

176. Perennial ryegrass is a perennial plant that can reproduce vegetatively and is not killed by harvesting. The applicant proposed to destroy all GMOs not required for analysis or future trials. A 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 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).

177. The applicant proposed 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. Given the fast germination rate of perennial ryegrass seeds, the monitoring period of 12 months after harvest with at least six months volunteer free will minimise the likelihood of persistence of GM perennial ryegrass at the trial site.

178. The applicant proposed 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 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). In addition, any area onto which seed may have been dispersed, if any, e.g. during cleaning of equipment, must be tilled and irrigated. 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 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).

179. 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 licence condition requires post-harvest inspections to occur at least every 35 days to ensure that volunteers are found and destroyed prior to flowering.

180. 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 proposed 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 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.

181. The applicant proposed 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 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 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. Additionally, the licence requires a contingency plan to be in place in the event of damage due, for instance, to extreme weather.

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

183. The applicant proposed that rodents in the planting area would be controlled by baiting. In addition, the applicant proposed 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 will restrict the potential for dispersal of GM seed by rodents (risk scenario 4). A licence condition requires implementation of measures including rodent baits and/or traps to control rodents within the planting area.

184. The applicant proposed that the planting area would be enclosed in a polyhouse structure constructed from 180 micron thick greenhouse film with 0.1 mm wire steel mesh sides. This 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 proposed 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 will 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).

185. 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 licence conditions regarding rodent, bird and animal controls require these measures (i.e. the polyhouse structure, fence and baiting/trapping) to be in place from planting until 60 days after harvest. The adjacent area and monitoring zone must be in place from planting and be maintained as fallow to be able to identify volunteers until post-harvest requirements have been completed. 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

186. Perennial ryegrass is highly outcrossing and the applicant proposed to manage pollen flow from the GM perennial ryegrass by four measures.

- Firstly, the planting area would be enclosed in a polyhouse structure. This will limit the spread of perennial ryegrass pollen.
- Secondly, the polyhouse enclosure will be equipped with an anteroom to prevent easy escape of pollen when doors are opened to gain access to the planting area.
- 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.

187. Commercial seed production standards for perennial ryegrass grown as basic seed in Australia require an isolation distance of 100 m from grass species where the recipient grass area is less than 2 ha and of 200 m where the recipient grass area is more than 2 ha (Seed Services Australia, 2013). Thus, the isolation distance of 140 m may not be sufficient, alone, to limit gene flow from the GM perennial ryegrass sufficiently. However, the isolation distance is combined with use of a 154 micron mesh polyhouse enclosure.

188. 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). Polyester mesh bags with a pore size of 185 x 839 microns were observed to reduce pollen flow of predominately pine pollen, with a pollen grain size of 40 x 70 microns, and some grass pollen by approximately 40%, whereas 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 roof and both ends of the polyhouse will be covered with 180 micron thick impermeable greenhouse film. The stainless steel sides will have a mesh aperture of 154 microns. The small aperture size, along with the thickness of the wire itself means that only 36% of the meshed area is permeable. The stainless steel mesh is expected to partially block pollen passage and also to cause friction on air movement, leading to a reduction in air velocity as the air moves through the screens. Mesh screens with porosities from 25% to 53% have been found to reduce the ventilation rate in comparison to a greenhouse without such screens (cited in López et al., 2016).

189. Gene flow is dependent on pollen viability and the distance pollen can travel. Regarding pollen viability, 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).

190. 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).

191. 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.

192. In the closely related species annual ryegrass (*L. rigidum*), pollen from a large-scale source has been found to travel up to 3 km in the presence of strong (46 km/h) southerly winds (Busi et al., 2008). In contrast to previous small plot or pot studies (e.g. Cunliffe et al., 2004), pollen mediated gene flow was observed using a large field source of herbicide resistant annual ryegrass plants as pollen donors, crossing with herbicide susceptible plants at various distances; the seeds recovered were tested for herbicide resistance. The frequency of herbicide resistant seeds decreased with distance from 33% at 0 m to 4% detected at 3 km. The percentage of herbicide resistance in seeds was observed when one or two herbicide susceptible donor plants were placed at 0, 100 and 200 m distance from the field edges with the herbicide resistant plants. Whereas single herbicide sensitive donor plants resulted in 19.4% herbicide resistant offspring, pollen competition from a second susceptible plant resulted in 0.29% herbicide resistant offspring at a distance of 200 m.

193. In a similar study with annual ryegrass (*L. rigidum*), Loureiro et al. (2016) measured pollen flow downwind to a maximum distance of 25 m and in all other directions to a maximum distance of 15 m. Cross-pollination of *L. rigidum* at 7 m was about 5.5%. In contrast, Maxwell (1992; as cited in Loureiro et al., 2016) found that cross-pollination of *L. multiflorum* at 7 m is about 1%. These and other studies suggest that there are large differences in pollen flow between *Lolium* species, and data for one species may not be very useful for another species. Thus, there is some uncertainty around pollen flow distances for perennial ryegrass.

194. Outcrossing between the GM perennial ryegrass and sexually compatible plants outside the trial site (risk scenarios 5 and 6), and exposure of people to the GM pollen (risk scenario 1) will be minimised by

- the combination of controls, i.e. use of a polyhouse, a monitoring zone and an isolation zone and
- the large amount of non-GM ryegrass pollen available from ryegrass plants in the surrounding areas compared to GM ryegrass pollen from the trial site which would make pollination of non-GM ryegrass with GM ryegrass pollen highly unlikely.

195. The applicant proposed to routinely inspect the mesh polyhouse structure and the fence surrounding the polyhouse for damage, and repair any damage found. A licence condition requires that the polyhouse structure be inspected for damage at least every two weeks and, during flowering, at least every three days. The applicant has reported that they have a number of structures containing the 180 micron thick greenhouse film. Most of these structures are more than 3 years old and have shown very limited damage in winds as high as 110km/h. The applicant further observed that the structure mentioned in the application has shown no signs of damage from high winds, and is protected by trees from the prevailing wind directions.

196. The fence must also be inspected at least every two weeks. This is consistent with previously issued licences and considered appropriate as the planting area is within a polyhouse enclosure.

197. The applicant proposed 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 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 licence condition requires fortnightly inspections. Due to the wide range of times when individual perennial ryegrass plants may commence flowering (Shah et al., 1990), licence conditions prescribe 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.

198. 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 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 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 licence conditions.

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

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

- limit the duration of the release to between May 2018 and June 2020
- limit the size of the release to 160 m<sup>2</sup> each year at one site in the shire of the Southern Grampians, Victoria
- locate the proposed field trial site at least 100 m away from the nearest natural or artificial waterway
- contain GM plants in a specified polyhouse enclosure
- inspect 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
- surround the polyhouse enclosure with a monitoring zone of at least 40 m that is kept fallow. While the GM perennial ryegrass is flowering, inspect the monitoring zone fortnightly for species that are sexually compatible with perennial ryegrass, and destroy any plants found
- surround the monitoring zone with an isolation zone of at least 100 m that is maintained in a manner that prevents the flowering of grasses
- control rodents by baiting and/or trapping
- surround the trial site with a fence capable of excluding rabbits and larger animals
- clean equipment prior to use for other purposes or removal from the trial site
- transport GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- destroy all GMOs not required for analysis or future trials

- till and irrigate the planting area and adjacent area post-harvest to promote germination of volunteers
- monitor the planting area, adjacent area and monitoring zone for at least 12 months post-harvest and until the site is free of volunteers for at least 6 months, with any perennial ryegrass volunteers destroyed before flowering
- not allow the GM plant material to be used in human food or animal feed.

### **3.2 Other risk management considerations**

200. 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**

201. 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.

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

203. 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**

204. DEDJTR is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM perennial ryegrass outside permitted areas.

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

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

206. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, DEDJTR are 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**

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



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

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

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

### **3.2.5 Monitoring for compliance**

209. 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.

210. 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.

211. 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**

212. 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, and specifically regarding the potential for increased tolerance to abiotic and biotic stresses
- 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 RARMP**

213. The RARMP concludes that this 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.

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

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## Appendix A Summary of submissions from prescribed experts, agencies and authorities<sup>3</sup>

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

Sub. No.	Summary of issues raised	Comment
1	Agrees with the overall conclusion of the RARMP.	Noted.
	The Regulator should further consider the potential for long distance pollen flow.	A literature search into long distance dispersal of perennial ryegrass pollen yielded no further relevant information.
2	No issues raised.	Noted.
3	Supports the conclusions of the RARMP.	Noted.
4	No serious concerns.	Noted.
	Notes that perennial ryegrass is an environmental weed and that it is difficult to predict whether genetic modifications will change its weed status. Also notes that enhancing the size of the species could make it more competitive however enhancing the nutrition may make it more prone to elimination by livestock. It is impossible to predict the significance of these changes.	Uncertainty is noted in the RARMP (Chapters 2 and 3) around potential for the genetic modifications to increase plant competitiveness and survival, particularly relating to increased tolerance to abiotic and biotic stresses.
	Control measures for risk management seem ok. There is no mention of pollen transfer by insects (e.g. thrips) but notes that exclusion zone should limit this transfer as well.	The mesh aperture of the polyhouse enclosure is 154 microns and will have an anteroom, which will reduce the likelihood of insects entering and exiting the structure. Perennial ryegrass, like most grasses does not have structures to attract insects as pollinators. Therefore pollen transfer by insects is not expected to be a pathway for pollen flow.
5	Agrees with the conclusions of the consultation RARMP. Also provided additional technical advice (summarised below) to strengthen consideration of potential environmental impacts.	Noted.
	The RARMP should include further detail on containment measures and controls on pollen dispersal: Notes the following in regard to the effectiveness of the 0.18 mm mesh polyhouse as a pollen containment measure: <ul style="list-style-type: none"> <li>Studies cited in the RARMP (paragraph 185) include studies on maize pollen containment (Watanabe 2006a, b) and pine pollen</li> </ul>	The literature on perennial ryegrass pollen dispersal and effectiveness of mesh as a containment measure is limited and literature on pollen dispersal in perennial ryegrass and related species is variable in quality: not all studies are methodologically sound and robust. Chapter 3 identified the potential for long distance pollen flow of perennial ryegrass as an area of uncertainty. Therefore, a number of measures in addition to the polyhouse have

<sup>3</sup> Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub. No.	Summary of issues raised	Comment
	<p>containment (Neal and Anderson, 2004); these may not be comparable to ryegrass pollen containment.</p> <ul style="list-style-type: none"> <li>A more recent study demonstrated that lighter ryegrass pollen escaped more readily than heavier maize pollen (van Hengstum et al. 2012). This study also showed that insect netting of 0.4 mm was ineffective at containing both maize and ryegrass pollen in glasshouses.</li> <li>Recommends inclusion of relevant information (paragraph 185) from the Neal and Anderson study on the effect of different materials and pore sizes on pollen containment.</li> <li>The RARMP should include details of the containment structure and the material used in the trial.</li> </ul>	<p>been imposed to minimise pollen dispersal (Chapter 2, Risk scenario 5 and licence).</p> <p>Further text has also been added to Risk scenario 5 reflecting the point that data for one species may not necessarily be useful for another species. The Regulator is aware of the studies referred to and they have been cited in the RARMP where relevant.</p> <p>Chapter 3 subsection 3.1 has been amended to include discussion of Neal and Anderson (2014) in regard to different materials and pore sizes.</p> <p>The applicant has provided additional details of the containment structure and material. The information has been added to Chapter 1 (Section 6.3) and relevant sections throughout the RARMP</p>
	<ul style="list-style-type: none"> <li>Current GM ryegrass field trials in the United States and European Union require individual plant bagging to prevent the escape of pollen into the environment. In addition, the previous Australian GM ryegrass trial (DIR 082/2007) required plants to be removed from the field prior to flowering.</li> </ul>	<p>Measures to minimise pollen dispersal are detailed in Chapter 2, Risk scenario 5. The polyhouse structure in combination with other controls is considered suitable to minimise pollen dispersal into the environment.</p> <p>Removal of plants prior to flowering was proposed by the applicant for DIR 082/2007 and formed part of the risk assessment context. In order to maintain the risk context for that application, the requirement was thus included as a licence condition.</p>
	<p>The RARMP should include additional information on ryegrass pollen flow distances and how this relates to the 140m isolation zone.</p>	<p>There is limited data on perennial ryegrass pollen dispersal distances. This was identified as an area of uncertainty in Chapter 3 of the RARMP. Available studies have been discussed in the RARMP (Risk Scenario 5) and a combination of measures imposed to minimise pollen dispersal and gene flow to sexually compatible relatives for this field trial (detailed in Chapter 2 and the Licence).</p>
	<p>The RARMP should include more discussion to support the 12 month post-harvest monitoring period.</p>	<p>Additional summary text has been included in the RARMP.</p>
	<p>The RARMP should include further discussion of the risk in the unlikely event of unintended release of the GM ryegrass through events such as flooding, windstorms or mesh damage.</p> <p>The RARMP should include discussion of processes that would be followed in the case of unexpected events.</p>	<p>The RARMP has considered pathways by which GM ryegrass could be dispersed into the environment and concluded that it poses negligible risk to people and the environment. 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. Additionally, the licence holder is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any</p>

Sub. No.	Summary of issues raised	Comment
		unintended presence of the GM perennial ryegrass outside permitted areas.
	<p>The RARMP should note the uncertainty regarding environmental factors that limit survival and weediness.</p> <ul style="list-style-type: none"> <li>Perennial ryegrass was identified as having extreme weediness potential in a recent weed risk assessment from Western Australia (OGTR 2017). It has many of the characteristics of a weed.</li> <li>Para 156 notes uncertainty in a number of areas but would benefit from also noting uncertainty regarding the environmental factors that most affect plant survival and thus potential spread and weediness. These environmental factors should also be noted in paragraph 202 on issues to be considered for future commercial releases.</li> </ul>	<p>The weediness status of perennial ryegrass is discussed in the baseline biology document (OGTR 2017), produced to inform the risk assessment, and referred to in Chapter 2 risk scenario 3.</p> <p>The sections on uncertainty in Chapters 2 and 3 have been amended to specifically note uncertainty around abiotic and biotic stresses.</p>
6	<p>Is the OGTR aware of any data to indicate there is a difference in allergic potential, biomass or volume of pollen between GM and non-GM ryegrass?</p>	<p>The introduced genes are from perennial ryegrass. These perennial ryegrass regulatory sequences and fructan biosynthesis genes are not associated with the known allergenic determinants of ryegrass pollen (see Chapter 1 Section 5.3).</p> <p>As discussed in Chapter 1 Section 5.4, when grown under glasshouse conditions the GM ryegrass had a significantly higher biomass, being up to 13 fold than the non-GM parental breeding line. Similarly, in field trials, fresh weights of the GM plants at harvest ranged between 350-500 g compared to 30-130 g in the near-isogenic control.</p> <p>Perennial ryegrass produces copious amounts of pollen. The applicant has not reported any change in pollen volume for the GM ryegrass.</p>
	<p>Does the applicant have an option to enclose the polyhouse with a mesh that can capture 23-60 micron sized pollen?</p>	<p>The applicant has proposed a polyhouse with 154 micron mesh, which is larger than perennial ryegrass pollen. However, the mesh is expected to reduce wind flow and air turbulence and, when combined with other measures, should be effective in minimising pollen dispersal and pollination of non-GM perennial ryegrass (Chapter 3, Section 3.1.1). Such measures include a monitoring zone of at least 40m maintained as bare fallow and an isolation zone of 100m.</p>
	<p>Has data on adverse health impacts been collected in previous trials undertaken by the applicant?</p>	<p>The introduced 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. As stated above, the introduced perennial ryegrass regulatory sequences and fructan biosynthesis genes are</p>

Sub. No.	Summary of issues raised	Comment
		<p>not associated with the known allergenic determinants of ryegrass pollen.</p> <p>Further, as noted in the RARMP, effects other than the expected increased biomass and nutritional quality were not observed for the GM perennial ryegrass plants grown in the field under earlier licence DIR 082.</p>

No submissions have been received from members of the public.