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Department of Health and Aged Care Office of the Gene Technology Regulator

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Risk Assessment and Risk Management Plan (consultation version) for

DIR 194

Limited and controlled release of perennial ryegrass genetically modified for increased metabolisable energy content

Applicant: Grasslanz Technology Australia Pty Limited

This F	RARMP is open for consultation until 17 January 2023.
Written comments this proposed rele	s on the risks to human health and safety and the environment posed by ase are invited. You may make your submission
via mail to:	The Office of the Gene Technology Regulator MDP 54, GPO Box 9848, Canberra ACT 2601 or
via email to:	<u>ogtr@health.gov.au</u> .
Please note that is and marketing and	sues regarding food safety and labelling, the use of agricultural chemicals, I trade implications do not fall within the scope of these evaluations as they

are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan

(consultation version) for Licence Application No. DIR 194

Introduction

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

The application

Project title	Limited and controlled release of perennial ryegrass genetically modified for increased metabolisable energy content ¹			
Parent organism	Perennial ryegrass (Lolium perenne L.)			
Genetic modifications				
Introduced genes	 Introduced genes conferring increased metabolisable energy content: <i>diacylglycerol o-transferase 1 (DGAT1)</i> gene from garden 			
	nasturtium (<i>Tropaeolum majus</i>) – encodes triacylglycerol synthesis enzyme			
	 cysteine oleosin gene from sesame (Sesamum indicum) – encodes oil body structural protein (oleosin) 			
	Introduced selectable marker gene:			
	 hygromycin phosphotransferase (hph) – hygromycin B antibiotic resistance gene from Escherichia coli 			
Genetic modification method	Agrobacterium-mediated transformation			
Number of events	Up to six events			
Principal purpose	To evaluate the increased metabolisable energy content trait under field conditions			
Previous releases	There have been no previous releases of these GMOs in Australia.			
	The GMOs have been evaluated in the field in the United States.			
Proposed limits				
Proposed use of GM	Animal feeding trials may be conducted with GM perennial ryegrass silage.			
plants	No use as human food or commercial animal feed is proposed for the GM perennial ryegrass.			

¹ Original title: Limited and controlled release of *Lolium perenne* L genetically modified for increased metabolizable energy content

Proposed location/s	Up to 7 trial sites per year to be selected from 119 possible local government areas in New South Wales, Victoria, Western Australia, and Queensland
Proposed release size	Up to 2.5 ha per year with a maximum of 12.5 ha over the period of release
Proposed period of release	From April 2023 to December 2028

Risk assessment

The risk assessment concludes that risks to the health and safety of people and animals are negligible to low and the risks to the environment from the proposed release are negligible. Specific risk treatment measures are included in the licence to manage these low 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 risks are considered.

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

The principal reason for the conclusion of a substantive risk to the health and safety of people and animals is that the introduced cysteine oleosin is derived from a known allergenic oleosin in sesame. Risk characterisation was performed to further consider the likelihood and consequences of potential harm related to allergenicity to humans and animals. In the specific context of this proposed limited and controlled release, the risk was characterised as negligible to low. The principal reasons for this characterisation are that the proposed limits and controls will minimise exposure of people and animals to the GMOs and that the number of people with an allergy to sesame oleosin is expected to be relatively low. As the risk to people is greater than negligible, specific risk treatment was considered as part of the risk management.

The remaining risk scenarios were found to pose negligible risks. The principal reasons for the conclusion of negligible risks are that the proposed limits and controls will effectively minimise dispersal and persistence of the GMOs, and there is no evidence to suggest the introduced genetic modifications would lead to toxicity to people or animals, or increase pest fitness.

Risk management

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

As the level of risk to the health and safety of people is considered to be low in regard to allergenicity, a licence condition is proposed to prevent people with a known sesame allergy from working with the GMOs in situations where they may be exposed to the introduced cysteine oleosin. In addition, since this is a limited and controlled release, the draft licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food or commercial animal feed, to minimise dispersal of the GMOs or GM pollen from the trial sites, to transport the GMOs in accordance with the Regulator's guidelines, to destroy GMOs at the end of the trial and to conduct post-harvest monitoring at the trial sites to ensure the GMOs are destroyed.

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Abbreviations

the Act	Gene Technology Act 2000
APVMA	Australian Pesticides and Veterinary Medicines Authority
САВ	Chlorophyll a/b-binding protein
DAFF	Department of Agriculture, Fisheries and Forestry
DGAT1	Diacylglycerol o-transferase 1
DIR	Dealings involving Intentional Release
dw	Dry weight
ELISA	Enzyme-linked immunosorbent assay
FSANZ	Food Standards Australia New Zealand
GM(0)	Genetically modified (organism)
ha	Hectare(s)
HGT	Horizontal gene transfer
hph/HPH	Hygromycin B phosphotransferase
ISAAA	International Service for the Acquisition of Agri-Biotech Application
km	Kilometre(s)
LGA	Local government area
m	Metres(s)
mm	Millimetre(s)
NOS	Nopaline synthase
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PPE	Personal protective equipment
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
RBSC3A	Ribulose bisphosphate carboxylase small subunit, chloroplastic 3
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
SA	South Australia
TAG	Triacylglycerol
TGA	Therapeutic Goods Administration
UK	United Kingdom
US(A)	United States (of America)
USDA	United States Department of Agriculture
WA	Western Australia
WHO	World Health Organization
WHO/IUIS	World Health Organization and International Union of Immunological Societies

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) <u>website</u>.

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

6. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

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

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

7. 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 it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

Section 2 The proposed dealings

8. Grasslanz Technology Australia Pty Limited (Grasslanz) proposes to release up to six GM perennial ryegrass events² into the environment under limited and controlled conditions. The GM plants have been genetically modified for increased metabolisable energy content.³

9. The purpose of the release is to evaluate the increased metabolisable energy content trait under field conditions. The objectives of the field trial are collection of regulatory data, agronomic assessment of the GM perennial ryegrass, and seed production to advance breeding and to enable forage production for feeding trials with perennial ryegrass silage.⁴ At some field trial sites, the GM perennial ryegrass would be allowed to flower and set seed, while at other field trials sites the applicant proposes to prevent the GMOs from flowering.

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- growing the GMOs

² An **event** is when DNA is inserted into the plant genome as a result of a single transformation process. Each time the transformation process occurs it is a new event, even if the same plasmid is used. The DNA may be inserted at a different location in the plant genome in a new event.

³ **Metabolisable energy** is defined as the energy contained in feed, minus the energy lost in faeces, urine, and gaseous emissions (Waghorn, 2007).

⁴ **Silage** is animal feed made from pasture plants that have been harvested, wilted, then preserved by fermentation. Further information about silage practices can be found on the NSW Department of Primary Industries: <u>Silage and hay</u> webpage. The process of making silage is known as 'ensiling'.

- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

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

11. The GM perennial ryegrass may be planted on its own, or into mixed swards with non-GM perennial ryegrass and/or non-GM white clover. The GM perennial ryegrass would be compared to elite non-GM perennial ryegrass genotypes or the non-GM counterpart (null segregant).⁵

12. The primary perennial ryegrass transformation events would be crossed into a range of elite founder plants, all non-GM perennial ryegrasses.

13. GM plant material from the field trial would not be used in human food or commercial animal feed.

14. GM perennial ryegrass from the field trial may be used to produce baled silage for animal feeding trials. These trials would only occur if Grasslanz has the appropriate approvals for each study in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.

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

15. The field trial is proposed to take place between April 2023 and December 2028. In each year up to 2.5 ha may be planted, with a maximum of 12.5 ha planted over the duration of the trial. Most sites would have a single planting in a year during autumn, however, in areas that are suitable there may be both an autumn and spring planting.

16. Up to seven sites per year would be selected from 119 possible local government areas (LGAs) in New South Wales (NSW), Victoria (Vic), Western Australia (WA), and Queensland (Qld) (Table 1). The trial sites would be located on private land in rural areas. Details of site locations would be provided to the Regulator prior to each planting season.

NSW	Vic	WA	Qld	
Armidale Regional	Ballarat City	Albany	Gympie Regional	
Bathurst Regional	Bass Coast	Augusta-Margaret River	Ipswich City	
Bega Valley	Baw Baw	Bridgetown- Greenbushes	Lockyer Valley Regional	
Bellingen	Benalla Rural City	Busselton	Logan City	
Berrigan	Campaspe	Capel	Moreton Bay Regional	
Blayney	Cardinia	Carnarvon	Scenic Rim Regional	
Byron	Casey City	Busselton	Somerset Regional	
Cabonne	Colac Otway	Dardanup	South Burnett Regional	
Central Coast	Corangamite	Denmark	Southern Downs Regional	
Cessnock	East Gippsland	Harvey	Tablelands Regional	
Clarence Valley	French Island	Manjimup	Toowoomba Regional	
Coffs Harbour	Gannawarra	Murray		
Cootamundra-Gundagai Regional	Glenelg	Nannup		
Cowra	Golden Plains	Nedlands		

Table 1. Local government areas where proposed trial sites may be located

⁵ The **null segregant** is the progeny of the GM perennial ryegrass that has not inherited the introduced DNA.

NSW	Vic	WA	Qld
Dubbo Regional	Greater Shepparton City	Serpentine Jarrahdale	
Dungog	Hepburn	Subiaco	
Glen Innes Severn	Indigo	Swan	
Goulburn Walwaree	Latrobe City	Waroona	
Gwydir	Loddon	Wyndham-East Kimberley	
Hawkesbury	Macedon Ranges		
Hilltops	Mitchell		
Inverell	Moira		
Kempsey	Moorabool		
Kyogle	Mornington Peninsula		
Lake Macquarie City	Moyne		
Lismore	Pyrenees		
Lithgow City	South Gippsland		
Liverpool	Southern Grampians		
Maitland	Surf Coast		
Mid-Coast	Towong		
Mid-Western Regional	Wangaratta		
Muswellbrook	Warrnambool City		
Nambucca Valley	Wellington		
Narrabri	Wodonga City		
Oberon	Yarra Ranges		
Orange			
Port Macquarie-Hastings			
Port Stephens			
Queanbeyan-Palerang Regional			
Richmond Valley			
Shoalhaven			
Singleton			
Snowy Monaro Regional			
Snowy Valleys			
Tamworth Regional			
Tenterfield			
Tweed			
Upper Hunter			
Upper Lachlan			
Uralla			
Wagga Wagga			
Walcha			
Walgett			
Warrumbungle			
Wingecarribee			

Note: for length, 'City of', 'Council' and 'Shire'/'Shire of' have been omitted from the LGA names. French Island is an unincorporated territory with no local government.

17. Only trained and authorised staff would be permitted to deal with the GM plants.

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

18. 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 controls include:

- locating each proposed trial site at least 100 m away from the nearest natural waterway
- locating each proposed trial site away from stock camps
- selecting trial sites that do not have a history of being prone to flooding
- restricting gene flow from the GMOs using the controls shown in Figure 2
- treating any non-GM perennial ryegrass plants grown in planting areas like the GMOs
- covering the planting area in weed matting for planting areas where GMOs are allowed to flower and set seed
- surrounding the planting area and monitoring zone with a fence capable of excluding livestock and rabbits
- preventing the growth of sexually compatible species in the isolation zone
- setting an end date from the time of planting in the field (e.g., 10 months) to harvest or trial end for planting areas where GMOs are not allowed to flower
- hand harvesting small blocks to avoid large machinery where possible
- growing dense crops (e.g., cereals, canola, safflower) in the isolation zone to prevent the growth of weeds and provide a physical barrier to pollen flow
- where pollen control tents are used some GM plants may be grown in pots on plastic trays to minimise seed dispersal
- treating any soil or potting mixtures remaining in trays or pots to promote volunteers or destroy seeds prior to disposal
- rodent baiting
- cleaning equipment used in connection with the GMOs as soon as practicable and before use for any other purpose
- only authorised people would access the site
- physically checking personnel and clothing before leaving the trial site each time to ensure no unintentional movement of GM material
- transporting and storing GMOs in accordance with the current Regulator's <u>Guidelines for the</u> <u>Transport, Storage and Disposal of GMOs</u>
- post-harvest monitoring of the planting area at least once every 35 days for at least 12 months after harvest and until the site is free of volunteer perennial ryegrass plants for at least six consecutive months, with any volunteer plants destroyed prior to flowering
- after harvest, destroying GMOs not required for further experimentation or future planting
- post-harvest tilling and irrigation of planting areas, and any other areas where GMOs were dispersed to encourage seed germination.



Figure 2. Options proposed by applicant for restricting gene flow from the GM perennial ryegrass (not to scale)

19. The site layout options (Figure 2, above) proposed to be used at each site are dependent on the experiments (objectives) at that particular site. The objectives are:

- Figure 2 i) Objective 1: Regulatory data collection and agronomic assessment. Planting area up to 0.25 ha in size and the GMOs would not be allowed to flower;
- Figure 2 ii) Objective 2a: Production of seed to use in Objective 3. Planting area located in area free of perennial ryegrass and related species and where the GMOs would be allowed to flower and set seed;
- Figure 2 iii) Objective 2b: Breeding of GM perennial ryegrass events into elite ryegrass genotypes. GMOs in the planting area would be covered in pollen control tents prior to flowering;
- Figure 2 iv) Objective 3: Forage production to create silage for the animal feeding trials. Planting area ≥ 0.25 ha and the GMOs would not be allowed to flower.

20. The proposed pollen control tents (Figure 2iii) consist of non-woven spun-bound polyester of no more than 0.4 mm thickness and pore size of approximately 215 μm (Trammell et al., 2020).

21. GMOs would be prevented from flowering (Figure 2i and 2iv) by cutting to remove pre-pollen dehiscing inflorescences, physically removing whole plants, or destroying via herbicide application.

22. The proposed limits and controls are taken into account in the risk assessment (Chapter 2) and their suitability for containing the release is considered in the risk management plan (Chapter 3).

Section 3 The parent organism

23. The parent organism is *Lolium perenne* L., also known as perennial ryegrass. Perennial ryegrass is exotic to Australia, but is commonly used for pasture and turf in Australia, predominately in the temperate, southern regions (NSW, Vic, and Tasmania). As a pasture for grazing dairy cattle and sheep, it is generally used in combination with other pasture species such as Kentucky bluegrass (*Poa pratensis*), white clover (*Trifolium repens*), and fescues (*Festuca spp*.). For turf, it is often used in

combination with other grasses such as ryegrasses (*Lolium spp.*), fescues (*Festuca spp.*) and buffalo grasses (*Stenophrum spp.* and *Buchloe spp.*) (Lamp et al., 2001).

24. Perennial ryegrass is naturalised throughout the higher rainfall temperate areas of Australia, particularly Vic and NSW, and extending into southern Qld (Figure 3). There have been some limited occurrences in north-east Qld and the Northern Territory (<u>Atlas of Living Australia</u>, accessed 4 October 2022). Perennial ryegrass is considered to be a weed in many parts of Australia, primarily in agricultural areas (Groves et al., 2003).



Figure 3: Distribution of L. perenne L. in Australia

Source: Atlas of Living Australia (accessed 4 October 2022).

25. Detailed information about the parent organism is contained in the document *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum (Schreb.) Darbysh (tall fescue)* (OGTR, 2022), which was produced to inform the risk analysis process, available from the <u>OGTR website</u>. Baseline information from that document will be used and referred to throughout this RARMP.

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

26. The applicant proposes to grow up to six events of GM perennial ryegrass with increased metabolisable energy content.

4.1 The genetic modifications in the GMOs proposed for release

27. The GMOs would contain two co-expressed genes to increase lipid content in leaf tissue and an introduced selectable marker gene, introduced via a single binary vector.

28. The two lipid-related genes were sourced from garden nasturtium (*Tropaeolum majus*) and sesame (*Sesamum indicum*), see Table 2. The selectable marker gene was sourced from *Escherichia coli*. The introduced regulatory elements were sourced from rice (*Oryza sativa*), *Agrobacterium tumefaciens*, soybean (*Glycine max*), and cauliflower mosaic virus (CaMV).

Gene	Source	Protein produced; function	Promoter (source)	Terminator (source)
DGAT1	T. majus	DGAT1; TAG synthesis	RBSC3A green tissue specific promoter (<i>O. sativa</i>)	NOS poly(A) signal (Agrobacterium tumefaciens)
Cysteine oleosin	S. indicum	Cysteine oleosin; oil body structural protein	CAB green tissue specific promoter (<i>O. sativa</i>)	vspB (G. max)
hph	E. coli	HPH; hygromycin B resistance (selectable marker)	CaMV 35S constitutive promoter (Cauliflower mosaic virus)	CaMV poly(A) signal (Cauliflower mosaic virus)

Table 2. Introdu	ced genes in t	the GM peren	nial ryegrass
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CAB = chlorophyll a/b-binding protein; CaMV = cauliflower mosaic virus; DGAT1 = diacylglycerol o transferase 1; hph/HPH = hygromycin B phosphotransferase; NOS = nopaline synthase; RBSC3A = ribulose bisphosphate carboxylase small subunit, chloroplastic 3; TAG = triacylglycerol; vspB = vegetative storage protein-acid phosphatase B.

29. The purpose of the introduced genes is to increase the amount of lipids, including triacyclglycerols (TAGs), in the perennial ryegrass leaf tissue, in order to improve the metabolisable energy content of the pasture for livestock. Triacylglycerols (TAGs), also known as triglycerides, are major storage lipids that are present in eukaryotes, including plants, algae, mammals, and insects, as well as some prokaryotes (Turkish and Sturley, 2007; Maurya et al., 2018). In plants, TAGs predominately accumulate in the seeds, fruit, flower petals, and pollen (Cagliari et al., 2011). TAGs consist of a 3-carbon glycerol and three fatty acids (Olzmann and Carvalho, 2019).

30. Lipid droplets are storage organelles for neutral lipids and originate from the endoplasmic reticulum (Olzmann and Carvalho, 2019). The exterior of the droplet comprises a phospholipid monolayer embedded with proteins, surrounding a core of predominately TAGs and steryl esters (Tauchi-Sato et al., 2002; Olzmann and Carvalho, 2019). Oleosin is a distinctive structural protein in oil bodies, a traditional name for plant lipid droplets (Tzen J et al., 1993; Huang, 2018).

31. The *DGAT1* gene encodes production of the DGAT1 enzyme, which catalyses the final and only committed step in TAG synthesis using diacylglycerol and acyl CoA as substrates. The *DGAT1* gene in the GM perennial ryegrass is sourced from *T. majus* and has reduced phosphoregulation and increased TAG formation due to an introduced S197A mutation (Xu et al., 2008). The gene has been codon optimised for expression in rice.

32. The *cysteine oleosin* gene encodes the production of cysteine oleosin, which is an oil body structural protein that has been modified with 6 cysteine substitutions to enable crosslinking and thereby protect oils (such as TAGs) from degradation (Winichayakul et al., 2013). The *cysteine oleosin* gene in the GMOs has been sourced from *S. indicum* and has been codon optimised for expression in rice.

33. The GMOs also contain the *hygromycin phosphotransferase (hph)* gene from *E. coli* which is used as a selectable marker during transformation. This gene confers resistance to the antibiotic hygromycin B. More information on marker genes may be found in the document *Marker Genes in GM Plants* which is available from the <u>Resources</u> page on the OGTR website.

34. Short regulatory sequences that control gene expression have also been introduced into the GMOs (Table 2). The expression of the introduced lipid-related genes is controlled by green tissue-specific promoters, while the expression of the *hph* gene is driven by a constitutive promoter which is active in all plant tissues. Other short regulatory elements used include terminators.

35. The gene construct was introduced into the GMOs using *Agrobacterium*-mediated transformation. This method has been widely used in Australia and overseas for introducing genetic modifications into plants. More information can be found in the document *Methods of Plant Genetic Modification* which is available from the <u>Resources</u> page on the OGTR website.

4.2 Toxicity and allergenicity of the proteins associated with the introduced genes

36. None of the source organisms of the introduced genes are known to be toxic or pathogenic, however some are known to be allergenic.

37. The introduced *DGAT1* gene is derived from garden nasturtium. Nasturtium contains oil of mustard (isothiocyanate) which can cause contact dermatitis (Morisset et al., 2003; Perez-Crespo et al., 2009). DGAT1 from nasturtium is not known to be associated with the production of isothiocyanate or to be allergenic.

38. The introduced cysteine oleosin gene is derived from sesame. In Australia, it is estimated that 0.6% of 1-year-old and 0.4% of 4-year-old children are allergic to sesame (Peters et al., 2017). Sesame allergy usually presents in the first few years of life and persists into adulthood in the majority of cases (Cohen et al., 2007). Allergic symptoms to sesame can include hives, gastrointestinal upset, and difficulty breathing (Warren et al., 2019).

39. In a double-blind placebo controlled oral food challenge in people who were allergic to sesame, the lowest dose of sesame protein that elicited an allergic response was between 1 to 2.4 mg. A single sesame seeds weighs 3.2 mg and contains 0.544 mg of sesame protein, therefore the threshold dose to elicit a response in the most sensitive patient in that study would be consuming between 2 and 4.4 sesame seeds (Dano et al., 2015).

40. Sesame contains several major classes of allergens, including oleosins, and food labels in Australia are required to identify sesame as an ingredient (<u>FSANZ Allergen Labelling</u>, accessed 20 October 2022). The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee lists four foods as containing oleosin proteins known to cause allergic reactions after consumption of food; peanut, hazelnut, Tartarian buckwheat and sesame (<u>Allergen.org</u>, accessed 11 October 2022). For sesame, the oleosins Ses i 4 and Ses i 5 have been identified as allergenic (Leduc et al., 2006).

41. The cysteine oleosin in the GM perennial ryegrass is derived from known sesame allergen Ses i 5. The amino acid sequences of the proteins encoded by the introduced genes were compared to sequences of known allergens using the <u>AllergenOnline</u> database, which contains data for over 2200 known allergens. In a full-length homology search of the introduced cysteine oleosin, 96% homology was found to Ses i 5 sesame oleosin (information supplied by applicant). Homology to known allergens was not found for any of the other proteins encoded by the introduced genes.

42. Diagnostic tools for sesame allergy include skin prick tests, measurement of serum concentrations of IgE antibodies specific to a food antigen, and oral food challenge. Double-blind, placebo-controlled food challenge is considered to be the gold standard for diagnosing sesame allergy (Adatia et al., 2017). Sesame oleosin is recognised by IgE antibodies in serum enzyme-linked immunosorbent assay (ELISA) tests, but there may be false negatives in a skin prick test (Leduc et al., 2006).

43. When sesame seeds and/or oil have been used in a skin- prick test rather than a traditional commercial sesame extract, a localised allergic reaction has been observed (Leduc et al., 2006; Alonzi et al., 2011). This has been attributed to the absence of lipophilic antigens from the commercial sesame extract, including fat soluble oleosins. In an immediate read "contact test" (where filter paper dipped in sesame oil was placed on the intact skin for 20 minutes) localised allergic reactions have also been observed in 3 patients with a known sesame allergy. This test was negative in healthy subjects and in the sesame allergic subjects when other tolerated oils were tested. The component of the sesame oil responsible for the allergic reaction was hypothesised to be oleosins as the patients had

IgE specific antibodies to these proteins (Alonzi et al., 2011). It should be noted that oleosins are approximately 15 kDa in size (Leduc et al., 2006), therefore are too large to pass through the skin barrier (Bos and Meinardi, 2000) and illicit an allergic contact dermatitis reaction. In two individual case reports, patch testing using sesame lignin extracts resulted in positive skin reactions (Hayakawa et al., 1987; Kubo et al., 1987). This could suggest an alternative cause of sesame allergic contact dermatitis rather than oleosins.

44. These positive skin prick and skin contact tests led to a localised allergic skin reaction (wheal) in patients but did not result in anaphylaxis or other systemic allergic reactions.

45. Blood tests and skin prick tests support the diagnosis of sesame allergy, however they should not be used in isolation to confirm a diagnosis as people may have a positive allergy test but not get a reaction from eating the food (Bernhisel-Broadbent and Sampson, 1989).

46. Cross reactivity, where an antibody raised to a particular antigen recognises more than one antigen, is a well-recognised phenomenon for homologous proteins in foods and aeroallergens (Cox et al., 2021). While it is generally accepted that more than 70% sequence identity is required for cross-reactivity, non-homologous proteins have also been demonstrated to have cross reactivity (reviewed in Bublin and Breiteneder (2020)). Foods that show possible cross-reactivity with sesame include kiwi fruit, peanut, poppy seed, rye grain, and tree nuts (Patel and Bahna, 2016). The serum of sesame allergic patients has shown potential *in vitro* cross reactivity between sesame oleosin and oleosins from walnut, hazelnut, and peanut, however confirmation of clinical cross-allergenicity by oral challenge was not performed (Ehlers et al., 2019).

47. There have been several case reports of allergy to inhaled sesame flour or crushed sesame seeds. In one case, an apparent allergy to sesame flour inhaled in a bakery was supported by the results of an inhalation challenge (Caimmi et al., 2011). Two other cases of inhalation allergy resulting from occupational exposure of sesame flour or seed dust have been reported, with symptoms including asthma, rhinitis, and shortness of breath (Keskinen et al., 1991; Alday et al., 1996). The component of the sesame flour or seed dust responsible for the reaction was not identified in any of these three cases.

48. Sesame oil is used as an ingredient in cosmetics and has been found to not be a skin irritant, teratogen, or carcinogen at levels used in cosmetic products (summarised in Johnson et al. (2011)). However, there have been individual case reports of skin reactions following exposure to sesame lignins (Hayakawa et al., 1987; Kubo et al., 1987), which may indicate an uncommon skin irritant effect of sesame lignins in some people.

49. 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). Estimates suggest that as many as 37% of individuals with any allergic disease are immunoreactive to perennial ryegrass pollen (Scala et al., 2010). Perennial ryegrass is considered the main contributor to grass pollen in the Australian cities of Canberra, Adelaide, Melbourne, and Perth (Davies et al., 2015).

50. 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 grass allergic 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).

51. The introduced perennial ryegrass regulatory sequences and lipid-related genes are not associated with the known allergenic determinants of ryegrass pollen.

52. The GM perennial ryegrass may contain the *hph* selectable marker gene which confers resistance to the antibiotic hygromycin B. Regulatory agencies in Australia and other countries have found no evidence that the HPH protein is toxic or allergenic (FSANZ, 2004; EFSA, 2009). Food derived from GM cotton and GM safflower with the *hph* gene has been approved for sale in Australia (FSANZ, 2004, 2018).

53. Dietary intake of the protein products of antibiotic selection genes could conceivably reduce the therapeutic efficacy of antibiotics taken orally (Nap et al., 1992), however HPH protein is rapidly inactivated in simulated mammalian gastric juice (Lu et al., 2007). In addition, no hygromycin B products are currently registered for veterinary use in Australia (<u>APVMA PubCRIS database</u>, accessed 19 September 2022). Hygromycin B is not used in humans.

4.3 Toxicity and allergenicity due to the increased metabolisable energy content trait

54. Perennial ryegrass has a mutualistic symbiotic relationship with the endophyte fungus *Neotyphodium lolii* (alternatively known as *Epichloë festucae* var. *lolii*), which deters insect attack (Hettiarachchige et al., 2015). Endophytes produce a range of alkaloid metabolites which vary among endophyte species and can have various detrimental effects on the health of grazing animals depending on the level of associated alkaloid toxicity (Schardl et al., 2004). A neurological condition known as perennial ryegrass staggers can affect livestock that ingest large quantities of perennial ryegrass infected with *N. lolii*, and is characterised by muscle tremors and loss of coordination (Imlach et al., 2008). Horses, deer, and sheep are more susceptible to the condition as they graze close to the crown of the plant where the fungus predominately resides. Cattle are less susceptible to ryegrass staggers as long as they are regularly moved to fresh pasture to avoid grazing to the base of the plant (Prestidge, 1993). No literature could be found to indicate that ryegrass toxicity occurs in non-livestock animals, including native mammals such as kangaroos. This is possibly related to the quantity of perennial ryegrass consumed, as wild animals have access to a mixture of vegetative species to consume.

55. Perennial ryegrass can be intentionally inoculated with beneficial strains of *N. lolii*, with cultivars available that are either infected with novel strains that do not produce the alkaloids detrimental to livestock health, or with no endophyte (Tian et al., 2013). The applicant has not specified which, if any, strains of *N. lolii* the GM perennial ryegrass will be inoculated with. Infection with endemic *N. lolii* could also occur in the field.

56. Nitrogen and carbohydrate levels of perennial ryegrass have been shown to affect *N. lolii* and alkaloid levels (Rasmussen et al., 2007). A higher carbohydrate perennial ryegrass cultivar and a lower carbohydrate cultivar were infected with different strains of N. *lolii* and grown under high and low nitrogen conditions. Both the endophyte and alkaloid concentrations were significantly lower in association with the higher carbohydrate cultivar and also with higher nitrogen conditions. Some GM perennial ryegrass lines have shown a decreased proportion of carbon from water soluble carbohydrates compared to lipids (Beechey-Gradwell et al., 2020; Cooney et al., 2021) (see Section 4.4 for further information) which may encourage endophyte growth compared to the wildtype, but this has not been confirmed. It is not known whether the increased metabolisable energy content trait in the GM perennial ryegrass events proposed for release in this application will affect the endophyte and alkaloid levels of the plants.

57. The purpose of the introduced genes for lipid metabolism or storage is to increase the amount of lipids, including TAGs, in the vegetative tissue. TAGs are already present in plants species, although accumulate more in the seeds, pollen, petals, and fruit compared to vegetative tissue (Cagliari et al., 2011). TAGs are major storage lipids in a range of insects such as mealworms, crickets, cockroaches, and earthworms (Tzompa-Sosa et al., 2014). TAGs are also major storage lipids in mammals. TAGs are used as important energy source during bird migration, for example the migratory black tailed godwits, use TAGs as a fuel source for their migratory movements (Araujo et al., 2019). TAGs also comprise approximately 95% of milk fat from dairy cows (Jensen, 2002). Supplemental fats may be fed to dairy cows as a high energy substitute for cereal grains (Palmquist and Jenkins, 2017), although more than 7 to 8% fat in the total diet can negatively affect the fat and/or protein concentration in the milk.

58. Previous studies of GM perennial ryegrass with the same introduced genes as proposed in this application has shown a maximum of 5.5% dry weight fatty acids compared to approximately 3 to 4% in the control (Beechey-Gradwell et al., 2020) and TAG levels of 2.5% dry weight (dw) in the GM

perennial ryegrass compared to 0.18% dw in the wildtype (Beechey-Gradwell et al., 2018), see Section 4.4 for further information. As the total increase in lipids, including TAGs, is expected to be small and TAGs are commonly present in the environment and not known to be toxic, the increased lipid content in the GM perennial ryegrass is not expected to lead to toxicity.

59. The increase in lipids, including TAGs, in the GM perennial ryegrass is not expected to lead to allergenicity. The increased metabolisable energy content trait increases levels of lipids through increasing activity of a metabolic pathway that is already present in perennial ryegrass. In addition, TAGs have not been associated with allergenicity.

4.4 Characterisation of the GMOs

60. GM perennial ryegrass plants with the metabolisable energy content genes (*DGAT1* and *cysteine oleosin*) have been evaluated in glasshouses, controlled temperature rooms, and growth chambers (Beechey-Gradwell et al., 2018; Beechey-Gradwell et al., 2020; Cooney et al., 2021; Beechey-Gradwell et al., 2022).

61. Leaf fatty acids, growth rate, biomass and gross energy of the GM perennial ryegrass were evaluated in controlled environments. In one growth chamber study, the GM perennial ryegrass contained 5% dry weight leaf fatty acids, compared to 3.25% in the control (Beechey-Gradwell et al., 2018). In another study, the GM perennial ryegrass grown in a glasshouse contained 23 to 100% more leaf fatty acids, which equates to 4.3% to 7.0% dw, compared to 3.5% in the wildtype control. The leaf TAGs were 2.5% dw compared to 0.18% in the wildtype control (Beechey-Gradwell et al., 2020). In a third study, GM perennial ryegrass cultivated in controlled temperature rooms had a 118 to 174% increase in leaf fatty acids compared to the respective non-transformed controls (4.8 to 5.5% leaf dw in the GM perennial ryegrass compared to 2.9 to 4% dw in the control), 6 to 10% faster whole-plant relative growth rates, and 6 to 7% higher gross energy compared to the non-transformed control plants. When grown in spaced pots indoors, GM perennial ryegrass seedlings exhibited 13% higher total plant dw than the controls. This increased biomass translated into 6 to 10% greater GM perennial ryegrass herbage production (yield) when grown in mini swards arranged in spaced rows indoors, but this trend did not occur when the GM perennial ryegrass was grown in dense swards indoors (Beechey-Gradwell et al., 2022).

62. As discussed in Section 4.1, DGAT1 catalyses the production of TAGs and oleosin is a key structural protein in oil bodies (which contain predominately TAGs), therefore the introduction of these genes to the GM perennial ryegrass is expected to increase the amount of TAGs in the vegetative tissue. The results of a glasshouse trial in which two GM genotypes were compared to the wildtype and vector control, indicate that the increase in total leaf fatty acids appears to be predominately related to an increase in TAGs (see supplementary information in Beechey-Gradwell et al. (2022)).

63. Further analysis of GM perennial ryegrass plants grown in growth chambers showed a shift in carbon storage in the vegetative tissue. In one GM perennial ryegrass line, the proportion of carbon from water soluble carbohydrates was approximately 65% compared to 90% in the wildtype control, and the proportion from fatty acids was approximately 35% compared to 10% in the wildtype control. The lower proportion of water-soluble carbohydrates was predominately related to lower levels of high molecular weight water soluble carbohydrates (Beechey-Gradwell et al., 2020). In a subsequent analysis of multiple GM perennial ryegrass lines, three of the lines had a 57 to 69% reduction in total leaf water soluble carbohydrates. Other lines showed no significant difference in the total water-soluble carbohydrates (Cooney et al., 2021).

64. Molecular characterisation of two of the events proposed for release in this application indicates the leaf fatty acid content is approximately 5% of the dry weight compared to approximately 3.75-4% in the non-transformed control (information provided by applicant).

65. Fresh and ensiled GM perennial ryegrass forage was also evaluated for *in vitro* gas production and its rumen fermentation profile. When GM perennial ryegrass, either fresh or ensiled, was

incubated *in vitro* with rumen fluid, there was a 10 to 15 % decrease in the proportion of methane gas in the total gas production, as well as a decrease in the actual concentration of methane, and greater percentage of unsaturated fatty acids compared to the wildtype controls (Winichayakul et al., 2020).

66. A field trial of GM perennial ryegrass with the increased metabolisable energy content trait was conducted in the US (Beechey-Gradwell et al., 2022). The GM perennial ryegrass swards contained 25 to 34% higher herbage fatty acid content (0.8 to 1% dry weight) at the end of the season compared to the control swards. Growth was similar between the GM perennial ryegrass and the control.

67. No secondary effects, other than the increased lipid content (indoor and field swards) and biomass (indoor mini swards only), have been observed for the GM perennial ryegrass plants grown in controlled environments or during the field trials in the US (information provided by applicant).

68. No adverse effects, including allergic contact dermatitis or other allergic events, have been observed so far in staff handling the GM perennial ryegrass plants in controlled environments or during the field trial in the US conducted in 2019 and 2020 (information provided by applicant).

69. The expression of the introduced genes for increased metabolisable energy content are controlled by a green tissue specific promoter and therefore expression is expected to be confined to the green tissue. However, the applicant has not yet tested other tissues (for example, pollen and seeds) for expression of the introduced genes and resulting proteins and lipids.

70. Following the initial introduction of the gene constructs into perennial ryegrass using *Agrobacterium*—mediated transformation, the events would be crossed into a range of elite non-GM perennial ryegrass founder plants. *Agrobacterium* is not normally transmitted from one generation to the next via seed, so is not expected to be present in the GMOs proposed for release.

Section 5 The receiving environment

71. 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 GMOs; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

72. Detailed information about the commercial cultivation and distribution of perennial ryegrass in Australia is presented in the document *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum (Schreb.) Darbysh (tall fescue)* (OGTR, 2022).

5.1 Relevant abiotic factors

73. There are 119 LGAs where proposed trial sites may be located. The majority of these LGAs are located in the cooler, temperate parts of southern Australia which have a suitable climate for the growth of perennial ryegrass and related species (see Section 5.4 for further information).

74. The applicant has proposed Kununurra (Western Australia) as a suitable site for the open block pollination experiments as it is free of perennial ryegrass and related species due to unfavourable climatic conditions. Table 3 shows the minimum temperature data for Kununurra. The minimum temperatures do not meet the perennial ryegrass vernalisation requirement of at least two weeks at less than 4°C (Cooper, 1957; Fejer, 1960; Cooper and Calder, 1963), with the lowest daily temperature observed being 6°C and the mean minimum temperature noticeably higher.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean min temp (°C)	25.1	24.7	24.2	21.8	18.6	15.7	14.7	15.5	19.8	23.4	25.1	25.5
Lowest daily temp (°C)	18.8	19.5	16.1	11.9	8.6	6.7	6.0	6.1	9.3	13.9	16.3	19.0

Source: Bureau of Meterology – <u>Climate Data Online</u>. Accessed 30 September 2022. Data from Kununurra Aero station from 1986 – 2022.

75. Perennial ryegrass has begun to show extensive herbicide resistances (Ghanizadeh et al., 2015; Heap, 2022), although no records have been listed for Australia (Heap, 2022). Glyphosate resistance in perennial ryegrass has been shown to be a heritable trait able to be transmitted to *L. perenne × L. multiflorum* hybrids (Yanniccari et al., 2015). However, an analysis of the glyphosate resistance trait demonstrated that it confers a burden on the organism (Yanniccari et al., 2017) which suggests that in the absence of herbicide selection the trait may be self-limiting. The GM perennial ryegrass does not contain a herbicide tolerant trait.

5.2 Relevant biotic factors

76. Common pests of perennial ryegrass in Australia include the black field cricket (*Teleogryllus commodus*), black headed pasture cockchafer (*Aphodius tasmaniae*), red headed pasture cockchafer (*Adoryphorus coulonii*), common army worm (*Mythimna convecta*), common cutworm (*Agrostis infusa*), pasture tunnel moth (*Philobota productella*), red legged earth mite (*Halotydeus destructor*), lucerne flea (*Sminthurus viridis*) and cereal rust mite (*Abacarus hystrix*) (Cunningham et al., 1994; Anon., 2020).

77. The major fungal diseases of perennial ryegrass in Australia are crown rust (*Puccinia coronata*) and stem rust (*Puccinia graminus*), which can reduce dry matter and seed yield significantly. Other fungal pathogens include blind seed disease (*Gloeotinia dictyoides*) which can significantly reduce seed quality and yield (Cunningham et al., 1994).

5.3 Relevant agricultural practices

78. Standard cultivation practices for perennial ryegrass in Australia are discussed in the document *The Biology of Lolium multiflorum Lam. (Italian ryegrass), Lolium perenne L. (perennial ryegrass) and Lolium arundinaceum (Schreb.) Darbysh (tall fescue)* (OGTR, 2022).

79. The applicant has proposed that for some sites the GMOs would be prevented from flowering by cutting pre-pollen dehiscing inflorescences, physically removing whole plants, or destroying via herbicide application.

80. On sites where the GMOs are allowed to flower and set seed, the applicant has proposed that the planting area would be located on a site free of perennial ryegrass and related species, or the GMOs would be covered by a pollen control tent prior to flowering. Weed matting would be used across the planting area. Pots and trays may be used within the pollen control tents.

81. Small areas would be hand planted and larger areas would be planted with commercial equipment. Harvesting may occur by hand for smaller plots or with commercial equipment. Harvested forage would be wrapped in plastic to form a silage bale on-site and bales labelled and stored in an approved facility until required for feeding trials. Herbicides, pesticides, and irrigation may be used to manage the health of the GM plants.

5.4 Presence of related plants in the receiving environment

82. As discussed in Section 4, perennial ryegrass (*Lolium perenne* L.) is widely cultivated in Australia for grazing and as turf. It is also considered a weed throughout many parts of Australia

(Groves et al., 2003; Randall, 2017). As noted in Section 5.3, the trial sites would be selected from a range of locations across Vic, NSW, Qld and WA. Perennial ryegrass can hybridise with other grass species present in the majority areas of Australia proposed for release (Figure 4), 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*). Some of these hybrids are sterile including *L. perenne* × *L. loliaceum* and *L. perenne* × *L. remotum* (OGTR, 2022). However, *L. perenne* hybridises readily with *L. multiflorum* and *L. rigidum*, to produce vigorous, fertile progeny (Wipff, 2002).



Figure 4. (A) Location of proposed trial sites compared to the distribution (B) *Festuca* L. spp. and (C) *Lolium* L spp.in Australia

Proposed LGAs for trial sites mapped on <u>MapCustomizer</u> (accessed on 5 October 2022). Distribution of *Festuca* L spp. and *Lolium* L spp. from the <u>Atlas of Living Australia</u> (accessed on 5 October 2022).

83. Of particular note, annual ryegrass (*L. rigidum* Gaud.), with which *L. perenne* can hybridise readily to produce fertile offspring, is a serious and costly weed of cropping systems in southern Australia, where it is widespread throughout the temperate areas (GRDC, 2019).

84. The applicant has proposed Kununurra (Western Australia) as the site for the open pollination experiments due to the absence of perennial ryegrass and related species. Neither *Festuca* L. *spp*. (fescue species) or *Lolium* L. *spp*. (ryegrass species) have been observed in Kununurra based on records from the <u>Atlas of Living Australia database</u> (see Figure 5, below). This lack of occurrence is consistent with the vernalisation requirements for fescues and ryegrasses (OGTR, 2022) and the climate of Kununurra (see Section 5.1 above).



Figure 5. Location of Kununurra (WA) compared to the distribution of (A) *Festuca* L. and (B) *Lolium* L in Australia

Source: Atlas of Living Australia. Red star identifies the location of Kununurra. Image supplied by the applicant.

5.5 Presence of similar genes and their products in the environment

85. The introduced genes (Table 2) were originally isolated from naturally occurring organisms most of which are widespread and prevalent in the environment. Therefore, humans and animals have been exposed to these genes and their encoded proteins either through consumption of the parent organisms or through other exposures in the environment.

86. The *DGAT1* gene is derived from nasturtium, a common ornamental plant that can be used as a food, naturopathic medicine, and source of bioactive compounds (Niizu and Rodriguez-Amaya, 2005; Albrecht et al., 2007).

87. The *cysteine oleosin* gene encodes a modified oil body structural protein (oleosin) that is derived from sesame. Sesame seeds and sesame oil are common food ingredients. Approximately 13,000 tonnes of whole sesame seeds and sesame products (for example, tahini) are imported each year (Reynolds and Robinson, 2021). Limited amounts of sesame are grown in Australia, with only 525 ha of sesame grown in Australia in 2020 (Rahman et al., 2020).

88. The GM perennial ryegrass plants may also contain the *hph* selectable marker gene derived from *E. coli*, a common bacterium that is widespread in human and animal digestive systems and/or in the environment. More information on marker genes is available in the document Marker Genes in GM Plants, available from the <u>OGTR website</u>.

89. The regulatory sequences are derived from either crop plants (rice and soybean) or common plant pathogens (CaMV and *A. tumefaciens*). Humans and animals have been exposed to these plants and plant pathogens for centuries and all these source organisms are present in Australia.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

6.1.1 Approvals by the Regulator

90. The GM perennial ryegrass in this application has not previously been approved for release in Australia.

91. The Regulator has previously issued two licences for field trials of other GM perennial ryegrasses in Australia (DIR 082/2007 and DIR 160). The GMOs authorised under those licences contained genes for modified fructan or lignin content (DIR 082/2007), or altered fructan metabolism (DIR 160).

92. There have been no approvals for commercial release of GM perennial ryegrass in Australia.

6.1.2 Approvals by other government agencies

93. There have been no approvals by any other Australian government agencies to date. The Department of Agriculture, Fisheries and Forestry (DAFF) Biosecurity regulates importation into Australia of all animal, plant, and biological products that may post a quarantine pest and/or disease risk. It is the applicant's responsibility to seek approval of a relevant permit before importing the GM seed or tissue culture material into Australia.

6.2 International approvals

94. GM perennial ryegrass with the increased metabolisable energy content trait, including some of the events proposed for release in this application, was approved for field evaluation in the United States (US) in 2019 and 2020. Results of the US field trial have been published (Beechey-Gradwell et al., 2022) and are briefly described in Section 4.4, above.

95. There have been no approvals internationally for the commercial release of the GM perennial ryegrass in this application or any other GM perennial ryegrass (European Union <u>GM register</u>;

International Service for the Acquisition of Agri-Biotech Application (ISAAA) <u>GM Approval database;</u> <u>Biosafety Clearing House</u>; all accessed 29 August 2022).

Chapter 2 Risk assessment

Section 1 Introduction

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



Figure 6. The risk assessment process

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

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

lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 6), that is, the risk is considered to be no greater than negligible.

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

Section 2 Risk identification

100. Postulated risk scenarios are comprised of three components (Figure 7):

- I. the source of potential harm (risk source)
- II. a plausible causal linkage to potential harm (causal pathway)



III. potential harm to people or the environment.

Figure 7. Risk scenario

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

plausible causal linkage

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

2.1 Risk source

102. 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.

103. As discussed in Chapter 1, the GM perennial ryegrass has been modified by the introduction of DGAT1 derived from garden nasturtium and cysteine oleosin from sesame, intended to increase the metabolisable energy content of the pasture grass. These introduced genes are considered further as potential sources of risk.

104. The GM perennial ryegrass also contains the *hph* gene which confers resistance to antibiotic hygromycin B and was used as a selectable marker gene. This gene and its product have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document Marker genes in GM plants available from the <u>OGTR website</u>. 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.

105. The introduced genes are controlled by introduced regulatory sequences. These were derived from *O. sativa* (rice), *A tumefaciens*, *G. max* (soybean), and Cauliflower mosaic virus. Regulatory sequences are naturally present in all plants, and the introduced sequences are expected to operate in similar ways to endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

106. The genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. 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

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

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

108. The potential for 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 (OGTR, 2011). 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.

109. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs. 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

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

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems

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

111. 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

112. Five risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 4 and examined in detail in Sections 2.4.1 - 2.4.5 and Section 3.

113. In the context of the activities proposed by the applicant and considering both the short and long term, only Risk Scenario 2 gave rise to a substantive risk.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
1	GM perennial ryegrass	Cultivation of GM perennial ryegrass at trial sites Exposure of people and other desirable organisms to the products of the introduced genes	Increased toxicity to people or other desirable organisms	No	 GM perennial ryegrass would not be used for human food or commercial animal feed The other proposed limits and controls would further restrict exposure of people and other desirable organisms to the GM perennial ryegrass The introduced genes are under the transcriptional control of a green tissue
					 specific promoter and are not expected to be expressed in the pollen or seeds of the GM plants The products of the genes
					 are not expected to be toxic There were no adverse health effects on people handling the GM plants in the controlled environment trials (non-flowering and flowering) or non-flowering overseas field trials

Table 4. Summary of risk scenarios from the proposed dealings with GM perennial ryegrass

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
2	GM perennial ryegrass	Cultivation of GM perennial ryegrass at trial sites Exposure of people and other desirable organisms to the products of the introduced genes	Increased allergenicity to people or other desirable organisms	Yes	 The cysteine oleosin in the GM perennial ryegrass is derived from sesame oleosin, a known dietary allergen in humans It has not been reported that sesame oleosin is an allergen in animals, however this is an area of uncertainty See Section 3 for risk characterisation
3	GM perennial ryegrass	Cultivation of GM perennial ryegrass at trial sites Consumption by pest organisms of the GM perennial ryegrass enriched in lipids, including TAGs Increased fitness of pests	Reduced establishment of desirable vegetation OR Reduced biodiversity	No	 The proposed limits and controls would restrict exposure of pest organisms to the GM perennial ryegrass Lipids, including TAGs, are common in the environment, from sources containing much higher concentrations
4	GM perennial ryegrass	Cultivation of GM perennial ryegrass at trial sites Dispersal of GM seeds or vegetative propagules outside trial limits Establishment of populations of volunteer GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for people or other desirable organisms OR reduced establishment or yield of desirable plants OR increased fitness of pests	No	 The controls of the field trial would minimise dispersal or persistence of GM seeds and vegetative propagules, and subsequent exposure to people and other organisms

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
5	GM perennial ryegrass	Cultivation of GM perennial ryegrass at trial sites Pollen from GM plants dispersed outside the trial sites Pollen flow to non- GM perennial ryegrass or related species outside the trial site Production of hybrid seed with GM traits	Increased toxicity or allergenicity for people or other desirable organisms OR reduced establishment or yield of desirable plants OR increased fitness of pests	Νο	The controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites

2.4.1 Risk Scenario 1

Risk source	GM perennial ryegrass	
Causal pathway	 ↓ Cultivation of GM perennial ryegrass at trial sites ↓ Exposure of people and other desirable organisms to the products of the introduced genes 	
Potential harm	Increased toxicity to people or other desirable organisms	

Risk source

114. The source of potential harm for this postulated risk scenario is the GM perennial ryegrass.

Causal pathway

115. The GM perennial ryegrass would be grown at the trial sites. As the introduced genes for increased metabolisable energy content are controlled by green tissue specific promoters, the encoded proteins are expected to be produced only in the vegetative material. The vegetative material is expected to contain the expressed proteins, DGAT1 and cysteine oleosin, as well as higher concentrations of lipids, including TAGs (see Chapter 1, Section 4.4 for further information).

116. Perennial ryegrass is not used as food and therefore the GM perennial ryegrass would not be used for human food. The trial is to be conducted at sites on private properties in rural areas and only authorised and trained trial staff would be permitted to deal with the GM plants. Therefore, there is little potential for the public to be exposed to GM vegetative material grown at the trial sites.

117. Trial staff would handle the GM vegetative material and could be exposed to the introduced proteins by dermal contact and/or inhalation of released components such as oil bodies when the vegetative material is cut, for example, during mowing or hand harvesting. Trial staff could also accidentally ingest small pieces of cut GM vegetative material that becomes airborne during mowing.

118. The GM perennial ryegrass would not be used for commercial animal feed, and livestock would not be permitted to graze the trial sites, including through use of livestock-proof fences. The applicant proposes to conduct animal feeding trials with silage produced from the GM forage. The experimental animals would ingest plant material containing the introduced proteins and the increased TAGs. These feeding trials would be subject to approval by an Animal Ethics Committee operating under the Australian Code for the Care and Use of Animals for Scientific Purposes.

119. Desirable organisms, such as native mammals, birds, and insects could enter the trial sites and consume GM vegetative material. Soil organisms, such as earthworms, might come into contact with decomposing GM vegetative material. The limited size and duration of the field trial would minimise the number of desirable organisms exposed to GM vegetative material grown at the trial sites.

120. The GM perennial ryegrass would be allowed to flower and set seed at some of the trial sites. As perennial ryegrass is wind pollinated, people working on the trial site or in the vicinity of the trial site could inhale airborne pollen during flowering of the GM perennial ryegrass. At some sites the applicant has proposed to cover the GMOs with pollen control tents before flowering, however there are some uncertainties in the efficacy of the pollen control tents in preventing the flow of pollen (discussed in Chapter 3 Section 3.1.1). Trial staff at the sites where the GMOs are allowed to flower would also be exposed to the seeds during harvesting. As the introduced metabolisable energy content genes are under the control of a green tissue specific promoter, expression of the proteins encoded by these genes is not expected to occur in the pollen and seeds of the GM perennial ryegrass. However, the applicant has not tested the pollen or seeds for expression of these genes or proteins, or the levels of TAGs.

121. The applicant has proposed that, where appropriate, personnel would wear protective clothing, eye wear, and dust masks to minimise exposure.

122. A mechanism whereby the oleosin and/or increased TAGs could occur in the pollen or seeds is through nutrient transport. In *Arabidopsis thaliana*, oleosin has been detected in the phloem, which plays an important role in nutrient transport (Guelette et al., 2012). However, no literature was found to indicate that in sesame plants oleosin-coated oil bodies are known to travel from the vegetative tissue to the pollen and seeds.

123. Therefore, it is uncertain whether people or other desirable organisms could be exposed to the products of the introduced metabolisable energy content genes in pollen or seeds.

Potential harm

124. As discussed in Chapter 1, Section 4.2, none of the introduced proteins are expected to be toxic to people or animals.

125. As discussed in Chapter 1, Section 4.3, low levels of TAGs are already present in non-GM ryegrass and the increased levels of TAGs in the vegetative tissue of the GM ryegrass are not expected to be toxic to desirable terrestrial animals, including livestock and beneficial insects, as TAGs are major storage lipids in a variety of organisms. TAGs are readily available in the environment from other sources which may contain much higher levels than those expected in the GMOs.

126. The GM vegetative tissue is not intended for human consumption and the other proposed limits and controls would further restrict exposure of people to the GM perennial ryegrass (see Chapter 3 for further discussion of the proposed limits and controls). There were no adverse health effects on people handling the GM plants in the controlled environment trials (non-flowering and flowering) or previous non-flowering field trials conducted overseas (information supplied by applicant).

127. As discussed in Chapter 1, Section 4.3, changes in the metabolic profile of perennial ryegrass, specifically increased nitrogen and carbohydrates, have been shown to decrease endophyte and alkaloid levels (Rasmussen et al., 2007). As some of the GM perennial ryegrass lines have shown a decreased proportion of carbon from water soluble carbohydrates compared to lipids (Beechey-Gradwell et al., 2020; Cooney et al., 2021), there is a possibility that some of the events proposed for release could result in decreased levels of carbohydrates. This could lead to increased endophyte and alkaloid levels and increased potential of toxicity to livestock consuming the GM perennial ryegrass. As discussed in Chapter 1

Section 4.3, no scientific literature was identified to indicate that ryegrass alkaloid toxicity occurs in other animals, including native mammals. Livestock would not be allowed to access the trial site. The applicant proposes to conduct feeding trials with silage produced from the perennial ryegrass forage, which must be conducted under animal ethics guidelines as noted in paragraph 118. The trials would provide important information on any potential alkaloid toxicity. The increased metabolisable energy content may instead have no effect or a negative effect on endophyte and alkaloid levels. This remains an area of uncertainty.

Conclusion

128. Risk Scenario 1 is not identified as a substantive risk because the GM perennial ryegrass would not be used for human food or commercial animal feed, the other proposed limits and controls would further restrict exposure of people and other desirable organisms to the GM perennial ryegrass, the products of the genes are not expected to be toxic, and no adverse health effects were observed for people handling the GM plants in the controlled environment trials or field trials. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

129. Risk Scenario 2 considers the potential harm of increased allergenicity to people or other desirable organisms from the introduced genes. As Risk Scenario 2 is considered to be a substantive risk due to the potential allergenicity of the introduced cysteine oleosin, a risk characterisation was conducted as detailed in Section 3.

2.4.3 Risk Scenario 3

Risk source	GM perennial ryegrass			
Causal pathway	Cultivation of GM perennial ryegrass at trial sites			
	Consumption by pest organisms of the GM perennial ryegrass enriched in lipids, including TAGs			
	↓ Increased fitness of pests			
Potential harm	Reduced establishment of desirable vegetation OR			
	Reduced biodiversity			

Risk source

130. The source of potential harm for this postulated risk scenario is the GM perennial ryegrass.

Causal pathway

131. The GM perennial ryegrass would be grown at the trial sites. Pest animals such as rabbits, rats, mice, birds, or insects may access the trial sites and consume the GM perennial ryegrass.

132. As outlined in Risk Scenario 1, the introduced genes for increased metabolisable energy content are controlled by green tissue specific promoters. Expression of the introduced genes for increased metabolisable energy content is not expected to occur in the pollen and seeds, however this remains to be confirmed by the applicant. As a result of the genetic modification, the vegetative material is expected to have higher levels of fatty acids and TAGs (Chapter 1, Section 4.4). Consumption of GM perennial ryegrass with increased lipid content could provide additional energy to the pest organisms and potentially increase their fitness. There is no literature to indicate that consumption of the proteins encoded by the genes for increased metabolisable energy content could increase pest fitness and these will not be considered further in this risk scenario.

133. The limits and controls of the trial, including the limited duration and size of the trial and pest management practices like rodent baiting and fencing, would minimise exposure of pest species to the

GM perennial ryegrass. At sites where pollen control tents are used, these would further limit pest access to the GMOs.

Potential harm

134. If consumption of the GM perennial ryegrass with increased levels of TAGs resulted in increased fitness of pest animals, this could lead to a greater negative impact of these animals on native or other desirable vegetation, or increased competitiveness with desirable animals.

135. Lipids play an important role in enabling insects to meet energy demands for reproduction (Ziegler, 1997), during a deep resting stage known as diapause (Lehmann et al., 2020), and for prolonged flight (Haunerland, 1997). If pest insects feeding on the GM perennial ryegrass enriched in TAGs have increased fitness, this may result in a competitive advantage over desirable insects, or increased damage to crops or other desirable vegetation including native species.

136. However, as TAGs are major storage lipids in plants and animals, pest species normally have access to TAGs from a wide range of commonly available food sources.

137. In studies of GM perennial ryegrass expressing the introduced increased metabolisable energy content genes, the leaf material had TAG content of approximately 2.5% dw and total fatty acids of 6.7% dw. There are much richer sources of TAGs in the environment, including pollen, fruit, flower petals, and seeds (Cagliari et al., 2011). For example, sunflower and safflower seed lipids consist of up to 97% TAGs at the later stages of seed development (Banas et al., 2013). As such it is unlikely that consumption of the GM ryegrass would provide significant levels of additional energy to the pest organisms and potentially increase their fitness.

Conclusion

138. Risk scenario 3 is not identified as a substantive risk because TAGs are normally available to pest species, the final concentration of TAGs in the GM perennial ryegrass is relatively small, and exposure of pest animals and insects to the GM perennial ryegrass is limited. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	GM perennial ryegrass		
Causal pathway	Cultivation of GM perennial ryegrass at trial sites		
	Dispersal of GM seeds or vegetative propagules outside trial limits OR		
	Persistence of GM perennial ryegrass at trial sites		
	Establishment of populations of volunteer GM plants expressing the introduced genes in the environment		
Potential harm	Increased toxicity or allergenicity for people or other desirable organisms		
	OR Reduced establishment or yield of desirable plants		
	OR Increased fitness of pests		

Risk source

139. The source of potential harm for this postulated risk scenario is the GM perennial ryegrass.

Causal pathway

140. The GM perennial ryegrass would be grown at the trial sites and produce vegetative propagules. At some sites the GM perennial ryegrass would also be allowed to flower and set seed. GM seeds or

GM vegetative material could potentially be dispersed outside the trial site by wind or water, by human activity or by animal activity. Non-GM perennial ryegrass is well-adapted to the southern parts of Australia where it has become naturalised and widespread (see Chapter 1, Section 3). Therefore, it is plausible that if GM perennial ryegrass seeds or propagules spread outside the trial limits, that volunteer populations could establish.

141. Most perennial ryegrass seeds fall adjacent to the parent plant (DiTomaso and Healy, 2007). Shattering seed heads may aid in short distance dispersal, although the number of seeds lost to shattering depends on the genotype and abiotic conditions (Elgersma et al., 1988; Fu et al., 2019).

142. Livestock could enter a site where the GMOs are allowed to flower and access the GM perennial ryegrass seeds. Grass seeds, including perennial ryegrass, are capable of germination after passing through the digestive systems of grazing animals. In two cattle feeding trials, perennial ryegrass seeds were recovered in faeces 12-24 hours after feeding and seedlings started to emerge after 1 week (Yamada and Kawaguchi, 1972; Yamada et al., 1972). In goats, 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 hours post-ingestion. Perennial ryegrass seeds have also been shown to be transported on the wool of grazing sheep, remaining in the wool for 1-2 months, but the viability of the seeds was not determined. (Fischer et al., 1996). However, as noted in Risk Scenario 1, livestock would not be permitted to graze the GM ryegrass growing in the field trial, including through use of livestock-proof fences. In addition, the feeding trial proposed as part of this application is for silage made from forage cut pre-flowering and therefore should not contain seeds.

143. Terrestrial pests could consume and/or remove the seeds from the site. A United Kingdom (UK) study in grassland showed removal of perennial ryegrass seeds by small rodents, with a 20-40% probability of at least one seed being removed and 80-95% of seeds being removed once the seeds had been encountered (Hulme, 1994). Removal was similar regardless of whether the seeds were on the surface or buried 1 cm under the soil. The applicant has proposed measures to reduce rodents at the site such as the use of rodenticides. There was no significant predation of the seeds by molluscs or arthropods (Hulme, 1994).

144. Birds can spread seeds through endozoochory (ingestion and transport in the gut) or through exozoochory (adhesion to the exterior of the animal). In the UK, geese and buntings have been shown to graze on the seeds of Lolium spp. (Patton and Frame, 1981; Buckingham et al., 2011). In a small, contained feeding study of bird species that have been identified as horticultural pests in Australia, 0.03% of perennial ryegrass seeds were excreted by corellas, 0% by galahs, and up to 0.4% by house sparrows (Woodgate et al., 2011). However, as only small amounts of perennial ryegrass seeds were consumed by the studied bird species, the germination potential of the excreted seeds could not be determined due to insufficient numbers of seeds excreted. No specific scientific literature was identified on the spread of seeds by exozoochory, however perennial ryegrass lacks structures to enhance dispersal by this method (for example, hooks). While no specific measures to exclude birds have been proposed, at the site where the pollen control tents are proposed to be used, the tents would limit access of birds to the GM seeds. At the Kununurra site where the GM perennial ryegrass will be allowed to set seed outside pollen control tents, some seed could be dispersed by birds. However, this site was specifically selected due to the lack of perennial ryegrass and related species in the area and because the environmental conditions are unsuitable for perennial ryegrass germination and persistence. As such, it is highly unlikely that if any of the GM ryegrass seed was dispersed by birds that it would result in a GM perennial ryegrass population establishing and persisting in the Kununurra area.

145. Earthworms can move seeds, and seeds can remain viable after passing through the earthworm gut. In a petri dish study, earthworm species *Lumbricus terrestris* consumed only 3% of perennial ryegrass seeds offered (McRill and Sagar, 1973). Of the perennial ryegrass seed consumed, two thirds were recovered in the worm casts with the remaining third presumably digested. This compares with 60% consumption of *Poa annua* (Annual bluegrass) seeds and 50% consumption of perennial grass *Agrostis tenuis* (now *Agrostis capillaris*) seeds. Viability of the perennial ryegrass seeds after passing through the earthworm gut was not studied. In a more recent study (Eisenhauer et al., 2010), earthworms consumed

approximately 50% of perennial ryegrass seeds offered and 31% of seeds were digested. This compares to 90% of *Poa trivialis* (rough bluegrass) and 65% of legume *Medicago varia* seeds being consumed. When a mixture of seeds, radicles (embryonic roots) and cotyledons (seed leaves) were offered, the earthworms showed a preference for consuming the radicles (50% consumed) over the seeds (30% consumed) or cotyledons (8% consumed). Taken together, these studies indicate that earthworms may consume perennial ryegrass seeds in their natural environment, however they are unlikely to be a preferred food source. If earthworms were to consume the GM perennial ryegrass seeds, the seeds would likely only be moved a small distance and there are conditions in the draft licence to monitor for volunteers post-harvest. In addition, the applicant proposes to cover the planting areas with weed matting for sites where the GMOs are allowed to flower and set seed, which would reduce dispersal of seeds on the soil surface where they could be accessed by soil organisms.

146. While perennial ryegrass seeds lack structures to enhance their dispersal by water, GM seeds on the soil surface could be transported by water during heavy runoff or 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. In a study of seed dispersal in irrigation water in Chile, viable seeds from *Lolium* spp. were recovered from the irrigation water (Tosso et al., 1986). The applicant has proposed to locate the sites 100 m from waterways and in areas that do not have a history of flooding.

147. Human activity is also a likely source of seed dispersal. Grass seeds can be dispersed on cars (Hodkinson and Thompson, 1997). It is possible that the GM perennial ryegrass seeds could be dispersed via equipment used on the trial site, such as harvesters, or that seeds could be spilled during transport. While perennial ryegrass seeds lack structures such hooks to assist dispersal through sticking to clothing, it is possible than GM seeds could be spread on clothes or footwear. The applicant has proposed that clothing and equipment be cleaned to reduce the spread of GM material.

148. 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. Perennial ryegrass seeds have been reported to have high germination rates at a broad range of temperatures (Lodge, 2004). Vegetative propagules may also remain at the site after harvest and could grow into volunteer GM perennial ryegrass plants. The applicant has proposed post-harvest monitoring to facilitate identification and destruction of volunteers at the site. The applicant has also proposed hand harvesting of small blocks and inclusion of weed matting on flowering sites to reduce loss of seed and development of a seedbank. The applicant has proposed that post-harvest tillage and watering would promote the germination of volunteers. These measures are expected to minimise persistence of GM plants or seeds at the trial site.

149. At some sites the GMOs would be prevented from flowering and setting seed by cutting off prepollen dehiscing inflorescences, physically removing whole plants, or destroying via herbicide application, so it would be highly unlikely that there would be GM seeds at these sites.

150. While perennial ryegrass is a bunchgrass where the primary form of vegetative propagation is through the production of upright tillers, it is also able to form clones with adventitious roots from stolons or tillers (OGTR, 2022). Livestock trampling has been shown to play a role in perennial ryegrass sward renewal through burial of the tillers and induction of aboveground stolons in a localised manner (Matthew et al., 1989). It is also theoretically possible that animals, most likely livestock, could transport small pieces of perennial ryegrass stems with adventitious roots away from the trial site, however no literature is available on the likelihood of vegetative dispersal occurring in this manner under field conditions. The applicant has proposed that livestock would not be permitted to access the site.

151. Vegetative propagules could also be spread by people or equipment. The applicant has proposed clothing and equipment be cleaned to reduce the spread of GM material.

Potential harm

152. As discussed in Risk Scenario 1, it is not expected that the GM perennial ryegrass would have increased toxicity for people or other desirable organisms.

153. As discussed in Risk Scenario 2 and further characterised in Section 3, there is a negligible to low risk of allergenicity to people and a negligible risk of allergenicity to animals from the introduced cysteine oleosin in the GM perennial ryegrass.

154. GM ryegrass spread either through dispersal of seed or viable vegetative material could establish and persist outside the field trial sites. As the introduced genes are associated with energy metabolism, it is theoretically possible that the traits could increase the competitiveness of the GM perennial ryegrass and provide a competitive advantage over agricultural crops, pastures, or native vegetation. As a result, populations of volunteer GM perennial ryegrass could reduce establishment or yield of desirable plants.

155. While increased biomass was observed when the GM perennial ryegrass was grown in mini swards in controlled conditions, this was not observed when the GM perennial ryegrass was grown in dense swards in controlled conditions or during the field trials in the US (Chapter 1, Section 4.4). Other than the increased lipids, including TAGs, and increased biomass only in mini swards grown under controlled conditions, no other phenotypes have been observed, including any changes to the growth rate or time to flowering. In addition, modifications of lipid metabolism can be associated with plant growth penalties (Vanhercke et al., 2017). Therefore, it is unlikely that the introduced genes will increase the competitiveness of the GM perennial ryegrass with desirable plants, however this is an area of uncertainty.

156. GM volunteers could also reduce the yield of commercial perennial ryegrass by providing a reservoir for pests or pathogens, such as the important fungal rust diseases (see Chapter 1, Section 5.2). No changes have been reported in the susceptibility of the GM perennial ryegrass to pests or pathogens. The applicant has stated that no secondary effects, other than the increased lipid content (indoor and field swards) and biomass (indoor mini swards only), have been observed for the GM perennial ryegrass plants grown in controlled environments or during the field trials in the US. Perennial ryegrass is widespread in the environment currently, and as discussed, the introduced genes are not expected to increase the competitiveness of the GM ryegrass compared to non-GM ryegrass, 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.

157. It is expected that the GM perennial ryegrass would be susceptible to standard weed management practices. As none of the introduced genes encode for herbicide resistance, it is not expected that there will be any change to herbicide susceptibility for the GM ryegrass as a result of the modification.

158. As discussed in Risk Scenario 3, pest consumption of the GM perennial ryegrass is not expected to increase the fitness of pest species.

Conclusion

159. Risk scenario 4 is not identified as a substantive risk because the proposed controls would minimise dispersal and persistence of GM seed or vegetative parts. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.
2.4.5 Risk Scenario 5

Risk source	GM perennial ryegrass				
Causal pathway	Cultivation of GM perennial ryegrass at trial sites				
	Pollen from GM plants dispersed outside the trial sites				
	Pollen flow to non-GM perennial ryegrass or related species outside the trial site				
	Production of hybrid seed with GM traits				
Potential harm	Increased toxicity or allergenicity for people or other desirable organisms				
	OR				
	Reduced establishment or yield of desirable plants				
Increased fitness of pests					

Risk source

160. The source of potential harm for this postulated risk scenario is the GM perennial ryegrass.

Causal pathway

161. The GM perennial ryegrass would be grown at the trial sites. At some sites the GM perennial ryegrass would also be allowed to flower and set seed. Pollen from the GM plants could be dispersed from the trial sites by wind and could fertilise sexually compatible plants. Hybrid seeds containing the introduced genes could be harvested by farmers and planted as pasture or could grow as volunteers.

162. Perennial ryegrass is self-incompatible, highly outcrossing, wind pollinated, and produces large volumes of pollen.

163. The GM perennial ryegrass could outcross with nearby naturalised or cultivated perennial ryegrass, if there is synchronicity of flowering. As discussed in Chapter 1, Section 5.4, perennial ryegrass can hybridise with some other grasses that are expected to be found at the majority of the proposed trial sites. While some hybrids crosses are known to be sterile, perennial ryegrass hybridises readily with Italian ryegrass and annual ryegrass to produce vigorous, fertile progeny (Wipff, 2002).

164. If pollen from GM ryegrass fertilised sexually compatible plants growing as cultivated pasture, as volunteers or as weeds, the hybrid GM seeds could grow as volunteers. However, the introduced genes are not expected to alter the potential for dispersal of GM perennial ryegrass pollen or the likelihood of hybridisation with other non-GM ryegrass or sexually compatible species.

165. Populations of hybrid GM volunteers could be consumed by other desirable organisms or could reduce the establishment or yield of desirable plants.

166. The applicant has proposed control measures to minimise pollen flow from GM plants growing on the trial sites to sexually compatible plants outside the trial sites (Chapter 1, Section 2.2). At some sites the GMOs would be prevented from flowering, and on sites where the GMOs are allowed to flower, either the planting area would be located in an area free of perennial ryegrass or related species, or the GMOs would be covered with pollen control tents prior to flowering. In addition, any GM volunteers growing on the trial sites after harvest would be destroyed prior to flowering. However, the available literature discussing the use of the proposed fabric for the pollen control tents provides little information about the flow of pollen, so this is an area of uncertainty. Further discussion of the efficacy of the pollen control tents is provided in Chapter 3.

Potential harm

167. As discussed in Risk Scenario 1, it is not expected that the GM perennial ryegrass would have increased toxicity for people or other desirable organisms.

168. As discussed in Risk Scenario 2 and further characterised in Section 3, there is a negligible to low risk of allergenicity to people and a negligible risk of allergenicity to animals from the introduced cysteine oleosin in the GM perennial ryegrass.

169. As discussed in Risk Scenario 3 consumption of the GM perennial ryegrass enriched in TAGs by pest animals is not expected to increase the fitness of pest species. Similarly, consumption of hybrids with other perennial ryegrass or related species with the GM traits would not be expected to increase the fitness of pest species.

170. As discussed in Risk Scenario 4, the GM perennial ryegrass is not expected to be more competitive outside cultivation compared to non-GM perennial ryegrass, although this is an area of uncertainty. The GM perennial ryegrass is also expected to be able to be controlled by standard weed management practices. Similarly, an increase in competitiveness of any GM hybrids is not expected.

Conclusion

171. Risk scenario 5 is not identified as a substantive risk because pollen dispersal and hybridisation are not expected to be altered in the GM perennial ryegrass compared to non-GM perennial ryegrass, and the proposed controls would minimise dispersal of GM pollen. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Risk characterisation

172. Five risk scenarios were postulated and evaluated, as summarised in Table 4. The second risk scenario was identified as posing a substantive risk which warrants further assessment. This section provides more detail on the characterisation of this risk.

Risk source	GM perennial ryegrass		
Causal pathway	 Cultivation of GM perennial ryegrass at trial sites Exposure of people and other desirable organisms to the products of the introduced genes 		
Potential harm	Increased allergenicity to people or other desirable organisms		

3.1 Risk Scenario 2

Risk source

173. The source of potential harm for this postulated risk scenario is the GM perennial ryegrass.

174. The risk of harm to people and animals as a result of increased allergenicity related to expression of the sesame-derived cysteine oleosin in the GM perennial ryegrass was considered to be a substantive risk. Therefore, a risk characterisation was performed for this risk. Risk characterisation involves a likelihood assessment, a consequence assessment, a risk estimate, and a decision on whether risk treatment is required. See the Risk Analysis Framework for further information about the OGTR's approach to conducting risk analysis.

175. As discussed in Chapter 1, Section 4.2 and Section 4.3, none of the other proteins encoded by the introduced genes or the increased fatty acids and TAGs are expected to be allergenic, and therefore will not be considered further in this risk characterisation as the risk is considered to be negligible.

3.2 Likelihood assessment

176. A likelihood assessment determines the chance that harm may occur, ranging from highly unlikely to highly likely. The likelihood assessment is presented separately in the following sections for people (Section 3.2.1) and animals (Section 3.2.2).

3.2.1 Causal pathway and likelihood of allergenicity to people

177. The GM perennial ryegrass contains a modified cysteine oleosin which is derived from the known human sesame allergen Ses i 5 oleosin (Chapter 1, Section 4.2). It is possible that people who are allergic to sesame may have an allergic reaction if exposed to the GM plant material containing the cysteine oleosin. GM plant material includes vegetative material, pollen, and seeds. The routes of exposure could occur via the skin, mouth, or nose. Each of these pathways is discussed below (Tables 5, 6 and 7).

178. Given that the modified cysteine oleosin is a structural protein in oil bodies, there might also be the potential for the modified cysteine oleosin to end up in a human food product (such as meat, milk and eggs) if a food production animal consumes the GM perennial ryegrass. There is no literature or data to support this pathway and therefore, this is an area of uncertainty. However, sesame products are fed to livestock including lambs (Bonos et al., 2017; Obeidat et al., 2019), goats (Obeidat and Gharaybeh, 2011), and cows (Shirzadegan and Jafari, 2014), and no literature could be found to indicate that sesame oleosin in the consumed sesame feed was transferred into milk or meat products from these animals. Soy and peanut proteins, well recognised sources of allergenicity, have not been detected in chicken meat or eggs after the chickens were feed a diet including soybean meal or high-oleic acid peanuts (Toomer et al., 2020). As material from the GM perennial ryegrass will only be fed to a limited number of animals in controlled animal feeding trials and these animals will not enter the human food supply, exposure to the modified cysteine oleosin via this pathway will not be considered further in this assessment.

Pathway	Likelihood	
A person is handling or in the vicinity of the GM perennial ryegrass	Only authorised people are allowed to work with the GM perennial ryegrass, which means that a limited number of people would be on the trial sites or handling GM material.	
Small pieces of the GM material are generated	Small pieces of the GM vegetative material could be generated during machine harvesting or cutting of the GM material for silage. Such events will occur on a limited number of occasions.	
	Sampling/processing of the GM material will occur, such as tissue collection, and crushing and analysis of seeds. The amounts are expected to be small.	
	Some of the trials will be allowed to flower and therefore people could be exposed to the pollen.	
GM plant material enters	The GM perennial ryegrass is not to be used as human food.	
the person's mouth	The applicant has stated that where appropriate, personnel working with the GMOs would wear protective clothing, eye wear, and dust masks to minimise exposure. Even without any PPE, the chance of material entering a person's mouth is highly unlikely.	
	People not dealing with GMOs but in the vicinity of the trial would be highly unlikely to be exposed to GM vegetative or seed material, or the GM pollen (as the flowering trials will either be tented or in an isolated area (Kununurra)), and have it enter their mouths.	
Amount of cysteine oleosin is sufficient to trigger an immune	Expression of the modified cysteine oleosin is driven by a green tissue specific promoter. Therefore, it is not expected that the pollen or seeds will contain the protein (although this is an area of uncertainty).	
reaction •	While no quantitative information is available on the amount of cysteine oleosin in the GM perennial ryegrass, it is expected that the GM vegetative material would not contain a high proportion of oleosin compared to sesame seeds or oil.	

Table 5. Likelihood assessment for an allergic reaction occurring in people following ingestion of
GM plant material containing the cysteine oleosin

	And given the indigestibility of grass, very little of the cysteine oleosin would be expected to be released from the plant tissue.
The person is allergic to sesame, specifically Ses i 5	It is estimated that less than 0.4% of Australian adults are allergic to sesame (Peters et al., 2017). The proportion of these people who are specifically allergic to Ses i 5 is likely to be even lower. However, potential cross reactivity between sesame oleosin and nut oleosins may slightly increase the proportion of reactions to Ses i 5, although the evidence is limited (Ehlers et al., 2019).
	Therefore, considering both specific allergy to Ses i 5 and the potential for cross- reactivity, it is highly unlikely that the person exposed would have an allergic reaction to Ses i 5.
	Overall likelihood: Highly unlikely

Table 6. Likelihood assessment for an allergic reaction occurring in people following inhalation ofGM plant material containing the cysteine oleosin

Pathway	Likelihood		
A person is handling or in the vicinity of the GM	Only authorised people are allowed to work with the GM perennial ryegrass, which means that a limited number of people would be on the trial sites.		
perennial ryegrass	There are only a limited number of trial sites and these are likely to be in areas with very few bystanders.		
GM material is airborne	Pollen is the most likely material to be airborne and inhaled as part of the trial.		
and inhaled by people	The amount of pollen will be minimal as only one site in Kununurra, WA, will be allowed to flower in the open and, at the other sites where flowering is allowed, pollen movement will be restricted by tents.		
	The applicant has stated that where appropriate, personnel working with the GMOs would wear protective clothing, eye wear, and dust masks to minimise exposure.		
	The amount of pollen in the air will reduce with increasing distance from the sites where the GM plants are allowed, and therefore exposure to bystanders outside of the trials is highly unlikely.		
Amount of cysteine oleosin is sufficient to trigger an immune reaction	There have been limited individual case reports of inhalation allergies to sesame flour or crushed sesame seeds (Keskinen et al., 1991; Alday et al., 1996; Caimmi et al., 2011), however the component of the flour or crushed seeds responsible for the reactions was not confirmed.		
¥	Expression of the modified cysteine oleosin is driven by a green tissue specific promoter, and therefore it is not expected that the pollen will contain the protein (although this is an area of uncertainty).		
The person is allergic to sesame, specifically Ses i 5	It is estimated that less than 0.4% of Australian adults are allergic to sesame (Peters et al., 2017). The proportion of these people who are specifically allergic to Ses i 5 is likely to be even lower. However, potential cross reactivity between sesame oleosin and nut oleosins may slightly increase the proportion of reactions to Ses i 5, although the evidence is limited (Ehlers et al., 2019).		
	Therefore, considering both specific allergy to Ses i 5 and the potential for cross reactivity, it is highly unlikely that the person exposed would have an allergic reaction to Ses i 5.		
	Overall likelihood: Highly unlikely		

Table 7. Likelihood assessment for an allergic reaction occurring in people following skin exposure to GM plant material containing the cysteine oleosin

Pathway	Likelihood			
The GM vegetative material is cut	The GM perennial ryegrass would be cut during mowing, harvesting, or sampling of plant tissues.			
A person handles the cut GM vegetative material ↓	Only authorised people allowed to access the site and deal with the GMOs, which means that a limited number of people would be handling the GM vegetative material.			
A sufficient quantity of cysteine oleosin crosses	The applicant has stated that where appropriate, personnel would wear protective clothing, eye wear, and dust masks to prevent exposure.			
the skin barrier to trigger an immune reaction ŧ	However, it is not expected that the cut material would contain high levels of oleosin compared to sesame oil and unlikely that the cut material would be in contact with the bare skin for a significant amount of time whether people were wearing PPE or not.			
	Allergic reactions have been reported in several patients when sesame oil is placed on intact skin (Alonzi et al., 2011), however the component of the oil responsible for the reactions was not confirmed. Two case reports indicate that sesame lignins may play an important role in sesame-induced contact dermatitis (Hayakawa et al., 1987; Kubo et al., 1987).			
	In order to cause an immune reaction following skin contact the cysteine oleosin protein must penetrate the skin. The molecular weight of a compound needs to be less than 500 daltons in size in order to penetrate intact skin (Bos and Meinardi, 2000). Sesame oleosin Ses i 5 is 15 kDa (Leduc et al., 2006), and therefore it is too large to penetrate the skin.			
The person is allergic to sesame, specifically Ses i 5	It is estimated that less than 0.4% of the Australian population is allergic to sesame (Peters et al., 2017). The proportion of these people who are specifically allergic to Ses i 5 is likely to be even lower. However, potential cross reactivity between sesame oleosin and nut oleosins may slightly increase the proportion of reactions to Ses i 5, although the evidence is limited (Ehlers et al., 2019).			
	Therefore, considering both specific allergy to Ses i 5 and the potential for cross reactivity, it is highly unlikely that the person exposed would be specifically allergic to Ses i 5.			
	Overall likelihood: Highly unlikely			

179. Overall, the likelihood assessment found that an allergic reaction occurring in people from exposure to GM plant material containing the cysteine oleosin is **highly unlikely**. This conclusion is influenced strongly by the low percentage of people that are sesame allergic (and likelihood of an even lower proportion of people allergic to Ses i 5) and the limited number of people that would be accessing the trial site and being exposed to the GMO.

3.2.2 Causal pathway and likelihood of allergenicity to animals

180. As sesame oleosin is a known allergen in humans, it is possible that animals may also be/become allergic to the cysteine oleosin in the GM perennial ryegrass.

181. The limits and controls of the trials would restrict the access of some wild animals to the GM perennial ryegrass.

182. As livestock graze predominately on grass species and can consume large quantities of grasses, they are considered to be of particular interest regarding potential allergenicity. Livestock sensitivity to food-derived allergens has been previously demonstrated, for example, some pigs may develop a transient hypersensitivity to soy (Li et al., 1990). Sesame products are fed to livestock including lambs (Bonos et al., 2017; Obeidat et al., 2019), goats (Obeidat and Gharaybeh, 2011), and cows (Shirzadegan and Jafari, 2014). No literature could be found to indicate that sesame oleosin is a known allergen in livestock. This is an area of uncertainty.

183. Livestock would not be allowed to access the trial site, however animal feeding trials will be conducted with livestock being fed silage created from the GM perennial ryegrass. The feeding trials would only occur subject to approval from an Animal Ethics Committee operating under the Australian Code for the Care and Use of Animals for Scientific Purposes. A standard condition of the draft licence is that any unintended effects of the dealings authorised by the licence need to be reported to the Regulator as soon as possible. This would include any adverse effects noted in the animal feeding trials.

184. As the limits and controls of the trial would restrict access of animals to the GM perennial ryegrass and sesame oleosin has not been reported to be allergenic in animals, the likelihood assessment of an allergic reaction occurring in animals from exposure to GM plant material containing the cysteine oleosin is considered to be **highly unlikely**.

3.3 Consequence assessment

185. A consequence assessment determines the degree of seriousness of harm to people or the environment, ranging from marginal to major. The consequence assessment relates to the degree of harm if a human or animal who is allergic to sesame oleosin Ses i 5 had an allergic reaction following exposure to GM plant material containing the cysteine oleosin. Consequence assessment is presented separately in the following sections for people (Section 3.3.1) and animals (Section 3.3.2).

3.3.1 Consequence to people

186. In the case of consumption, a range of allergic symptoms could occur. The most common symptoms reported in allergic individuals as a result of sesame consumption are skin reactions such as hives (approximately 70%), with other reported symptoms (< 20%) including trouble breathing, chest tightening, nasal congestion, belly pain, cramps, nausea, fainting or dizziness, and chest pain (Warren et al., 2019). An allergic reaction is expected to occur rapidly, over the space of minutes to hours. Treatment ranges from antihistamines, epinephrine, glucocorticoids, and inhaled beta-agonists. Anaphylaxis is a systemic, rapid onset hypersensitivity reaction (Cardona et al., 2020). While anaphylaxis is a serious condition that may require hospitalisation, fatal and near-fatal events are rare (Umasunthar et al., 2013; Turner et al., 2017). The standard of care for anaphylaxis is an intramuscular injection of adrenaline (epinephrine) to reduce the risk of death (Prince et al., 2018).

187. Considering the reported range of severity of outcomes from dietary exposure of allergic individuals to sesame oleosin, including the consequences of anaphylactic reactions requiring hospitalisation, the consequence assessment for potential allergenicity to people from the introduced cysteine oleosin is considered to be **intermediate** (serious illness/injuries usually requiring hospitalisation; treatment is usually available; prevention may be available).

188. In the case of inhalation of GM plant material, an individual sensitive to the cysteine oleosin might be expected to develop hay fever symptoms or could potentially develop more serious asthmatic symptoms (Schäppi et al., 1999). Treatments, such as antihistamines, are available and therefore the consequence is considered **minor**. It should be noted that, as discussed in Chapter 1, Section 4.2, non-GM perennial ryegrass pollen is allergenic, therefore allergy symptoms from the standard perennial ryegrass pollen antigens and any potential symptoms from the cysteine oleosin may be indistinguishable.

189. In the case of skin exposure, the allergic individual might be expected to develop a localised wheal where the contact occurred, similar to findings in skin prick and contact tests (Leduc et al., 2006; Alonzi et al., 2011). The consequence of this is **minor** (minor illness/injury requiring medical treatment) and could be readily treated.

3.3.2 Consequence to animals

190. As discussed in Section 3.2.2, there is no literature to indicate that animals are allergic to sesame oleosin, even in situations where sesame meal is used as feed. Considering other food allergens, it has been shown that some pigs may develop a transient hypersensitivity to soy and displayed depressed weight gain from three to four weeks of age (Li et al., 1990).

191. As no allergies to sesame oleosin have been reported in animals, the consequence of a hypothetical allergic reaction is uncertain. If the reaction was to be a similar hypersensitivity reaction to soy, the symptoms would be very mild. Therefore, the consequence is expected to be **marginal** (ailment not requiring medical treatment), and the symptoms would likely cease upon removal of the source of the hypersensitivity.

3.4 Risk estimate

192. The risk estimate is based on a combination of the likelihood and consequence assessments, using the Risk Estimate Matrix (see Chapter 2, Section 1), as described in the Regulator's Risk Analysis Framework (OGTR, 2013).

193. The potential consequence to people of an allergic reaction following exposure to GM plant material containing the cysteine oleosin is considered to be **minor** to **intermediate**, with a probability of **highly unlikely**. The overall risk is therefore considered to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation) to **low** (risk is of minimal concern but may invoke actions for mitigation beyond standard practices).

194. The potential consequence to animals of an allergic reaction following exposure to GM plant material containing the cysteine oleosin is considered to be **marginal**, with a probability of **highly unlikely**. The overall risk is therefore considered to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation).

Risk scenario that may give risk to allergenicity	Consequence Likelihood assessment		Risk estimate	Does risk require treatment?
Expression of the introduced sesame- derived cysteine oleosin leading to increased allergenicity in people	 Minor to Intermediate An allergic reaction following inhalation or skin exposure would be expected to be localised and minor Ingestion could lead to an anaphylactic reaction which may require hospitalisation 	 Highly unlikely The GMOs are not to be used for human food A limited number of people would be accessing the trial site Exposure amounts of cysteine oleosin are expected to be very low There is a low frequency of people that are sesame allergic (and presumably even lower to Ses i 5), although there may be some cross-reactivity with nut oleosins 	Negligible to Low	Yes
Expression of the introduced sesame- derived cysteine oleosin leading to increased allergenicity in animals	 Marginal No reports have been found of animals being allergic to sesame oleosin If a reaction were to occur it would be expected to be very mild 	 Highly unlikely The limits and controls of the trial would restrict the access of animals to the trial site The animal feeding trials would only be conducted subject to approval by an Animal Ethics Committee No reports have been found of animals being allergic to sesame oleosin 	Negligible	Νο

Table 8: Summary of risk characterisation for Risk scenario 2

Section 4 Uncertainty

195. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's <u>Risk Analysis Framework</u> document.

196. 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.

197. 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.

- 198. For DIR 194, uncertainty is noted particularly in relation to:
 - potential expression of the introduced increased metabolisable energy content genes and proteins, and increased lipid levels in the pollen and seeds
 - the quantitative amount of cysteine oleosin in the GM perennial ryegrass and the impact this may have on allergenicity to people
 - potential allergenicity to animals
 - effects of the increased metabolisable energy content trait on the levels of endophytic fungus and alkaloids
 - whether the increased biomass seen in the controlled environment mini swards, but not in controlled environment dense swards or the field trial to date, may be observed in this trial and whether this indicates a competitive advantage
 - efficacy of the pollen control tents to control the flow of pollen.

199. 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.

200. Chapter 3 Section 4 discusses information that may be required for future releases.

Section 5 Risk evaluation

201. 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.

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

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

203. Five risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment.

204. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process

205. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, four of the risk scenarios were not identified as substantive risks. The principal reasons for these conclusions are summarised in Table 4 and include:

- the GM plants would not be used as human food or commercial animal feed
- limits on the size and duration of the proposed release
- controls proposed by the applicant to restrict the spread and persistence of the GM perennial ryegrass plants and their genetic material (see Chapter 3 for discussion of their suitability)
- the products of the introduced genes are not expected to be toxic
- GM perennial ryegrass volunteers are expected to be controlled by standard weed management measures.

206. Risk Scenario 2 was identified as a substantive risk due to the potential for allergenicity to people from the introduced cysteine oleosin. Therefore, further assessment of the risk of allergenicity to people was required. The likelihood and consequences of the substantive risk was characterised (Chapter 2,

Section 3), and the level of risk estimated using the Risk Estimate Matrix, as described in the Regulator's Risk Analysis Framework 2013 (OGTR, 2013). Following risk characterisation, the risk of allergenicity to people following exposure to GM plant material containing cysteine oleosin was considered to be negligible to low, and the risk of allergenicity to animals was considered to be negligible. The Risk Analysis Framework defines low risks as risks of minimal concern, but which may invoke actions for mitigation beyond standard practices. Therefore, measures to mitigate the identified risk are considered in Chapter 3.

207. Determination of whether a risk is considered to be significant, and therefore whether a longer consultation period is required for the consultation RARMP, are made on a case-by-case basis. As the risk of allergenicity to people is considered to be negligible to low, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.⁶

⁶ As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

Chapter 3 Risk management plan

Section 1 Background

208. 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.

209. 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.

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

211. 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

212. The risk assessment of Risk Scenario 2 in Chapter 2 concluded that there is a negligible to low risk to people from the proposed field trial of GM perennial ryegrass. This risk stems from the introduction of the cysteine oleosin gene to the GM perennial ryegrass, which is derived from a known dietary sesame allergen.

213. Two conditions have been included in the draft licence to manage the risk of harm to staff working with the GMOs as a result of possible allergenicity. The first is that the licence holder must make reasonable inquiries to establish that the person does not have a known sesame allergy. The second is that the licence holder must not knowingly permit a person with a sesame allergy to conduct any dealing which may expose the person to small pieces of green vegetative plant material from the GM perennial ryegrass, as the vegetative material is the tissue type most likely to have oleosin and the small pieces could inadvertently be ingested. "Known sesame allergy" has not been further defined, for example physician diagnosed. It is estimated that more than 50% of people with a convincing sesame allergy have not been diagnosed by a physician (Warren et al., 2019), and therefore have self-identified their allergy based on symptoms post exposure. Given the widespread occurrence of sesame in food products, it is expected that people with serious sesame allergy (for example, leading to anaphylaxis) would be aware that they are allergic, even if they have cross-reactivity with other food allergens, and as such could be excluded from the trial site. In combination with the small scale, short duration, and proposed controls for this release, this risk treatment measure is considered sufficient to manage the risk of allergenicity to sensitive workers.

214. The risk assessment of the remaining four risk scenarios listed in Chapter 2 concluded that there are negligible risks posed by these scenarios. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed control measures (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both

the short and the long-term risks. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks.

215. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

216. 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 proposed 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 draft licence.

3.1 Draft licence conditions to limit and control the release

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

3.1.1 Considerations of the limits proposed by Grasslanz

218. The application proposes that the trial would take place at up to seven sites per year. Sites would be chosen from the 119 LGAs listed in Chapter 1 Section 2.1, across NSW, Vic, Qld and WA. The duration of the trial would be from April 2023 and December 2028. In each year, up to 2.5 ha may be planted, with a maximum of 12.5 ha over the duration of the field trial. The small size and short duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk Scenarios 1 and 2), as well as pest species (Risk Scenario 3).

219. At most sites, a single planting at a site would be made in each year, and the perennial ryegrass would be harvested as an annual crop. However, the applicant has also indicated that at some sites where conditions are suitable, two plantings – autumn and spring – may be made at the same site. The applicant has indicated that more than one planting area may be established at a site, each with a distinct monitoring and isolation zone. The applicant has also indicated that one site would be managed as a "breeding nursery" for crossing of the GMOs into elite lines, with the same planting area used over multiple years.

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

220. The applicant proposes that GM plant material would not be used as human food or commercial animal feed. The applicant proposes to use GM plant material in animal feeding trials. The draft licence requires that GM plant material must not be used as food for humans or feed for animals, except for use in specified animal feeding trials. Animal feeding trials must be approved by an Animal Ethics Committee operating under the Australian Code for the Care and Use of Animals for Scientific Purposes. These conditions would restrict the exposure of people and other desirable animals to the GMOs (Risk Scenarios 1 and 2).

221. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard conditions have been included in the draft licence that require that only authorised people are permitted to undertake any activity authorised by the licence and that all people dealing with the GMOs must be trained and informed of the relevant licence conditions. These measures are considered appropriate to limit the potential exposure of people to the GMOs (Risk Scenarios 1 and 2). Where used, the pollen control tents would also limit the exposure of people to the GMOs (Risk Scenarios 1 and 2) and pest access to the GMOs (Risk Scenario 3).

222. Risk Scenario 2 was found to be a negligible to low risk due to the potential for allergenicity to people from the introduced cysteine oleosin. As discussed in Section 2 of this chapter, a specific condition has been proposed to prevent people with a known sesame allergy from being exposed to the GM plant material containing cysteine oleosin.

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

223. The potential for outcrossing of perennial ryegrass has been discussed in Chapter 1 and in Risk Scenario 5. As noted there, perennial ryegrass is wind pollinated, highly outcrossing, and can hybridise with a number of sexually compatible species which are commonly found in the majority of the areas proposed for release.

224. The applicant has proposed a number of containment measures for the GM perennial ryegrass with four possible site layout options. The following site areas have been defined by the applicant:

- Planting Area—an area of land where the GMOs and non-GM perennial ryegrass or non-GM white clover may be planted and grown pursuant to a licence.
- Monitoring Zone—an area of land extending 10 m or 40 m outwards from the Planting Area, depending on the planting option used.
- Isolation Zone–an area of land extending outwards by 100 m in all directions from the outer edge of the monitoring zone. The isolation zone is proposed to be maintained in a manner to prevent flowering of grasses (e.g., mown, grazed or treated with selective grass herbicides) while the GM ryegrass is flowering. To prevent the growth of weeds and provide a physical barrier to pollen flow, the applicant proposed that a dense crop could be planted in the isolation zone (e.g., cereals, canola, safflower).

225. The applicant has noted that the GMOs may be grown in blocks or rows with spaces in between (information supplied by the applicant). In the draft licence, the definition of the planting area includes all land within the inner edge of the monitoring zone, where the GMOs and non-GM perennial ryegrass and white clover may be intentionally planted and grown.

226. The applicant has proposed to grow dense crops in the isolation zone that are not sexually compatible with perennial ryegrass. Dense crops may act as a physical barrier to limit pollen dispersal and the dense planting could suppress the growth of weeds, including pasture grasses. However, if dense crops were being grown in the isolation zone, it may hinder identification of flowering perennial ryegrass and related species and therefore use of a selective grass herbicide may be necessary to destroy perennial ryegrass and related species before flowering. The applicant will need to consider this in their management approach for the isolation zone. The draft licence includes a condition requiring that the isolation zone must be managed in a manner that enables prevention of flowering of any perennial ryegrass and related species from 14 days prior to the expected flowering of the GMOs until the planting area has been cleaned.

227. In order to maintain the isolation zone in a manner that prevents flowering of perennial ryegrass and related species, the applicant has proposed that mowing may be one option of achieving this. It should be noted that previous experience of the OGTR indicates that repeated mowing may encourage tillering and flowering at a shorter height, which may create a challenge for monitoring. The applicant will need to consider this in their management approach for the isolation zone.

228. In the licence application, four site layout options (shown below in Figure 8) were proposed by the applicant and labelled Objective 1, Objective 2a, Objective 2b, and Objective 3 based on the different objectives of the trial:

- Figure 8 i) Objective 1: Planting Area up to 0.25 ha in size and GMOs are not allowed to flower;
- Figure 8 ii) Objective 2a: Planting Area located in area free of perennial ryegrass and related species and where GMOs are allowed to flower;

- Figure 8 iii) Objective 2b: Planting Area where GMOs are covered in Pollen Control Tents prior to flowering;
- Figure 8 iv) Objective 3: Planting Area \geq 0.25 ha and GMOs are not allowed to flower.



Figure 8. Options proposed by applicant for restricting gene flow from the GM perennial ryegrass (not to scale)

229. The applicant has proposed Objectives 1 and 3 as separate site layout options (Figure 8i and Figure 8iv), however these are considered to be a single (non-flowering) option and will be referred to as Option A in the remainder of this RARMP. Objective 2a (Figure 8ii) will henceforth be referred to as Option B, and Objective 2b (Figure 8iii) as Option C.

230. The perennial ryegrass inflorescence is a distinctive spike up to 20 cm in length (Lamp et al., 2001). The Kangaroo Valley cultivar of perennial ryegrass takes approximately 132 days from seedling emergence to spike emergence, then a further 16 days for anthesis (flowering) to occur when planted in NSW (Shah et al., 1990). In controlled environment studies, the GM perennial ryegrass did not display differences in time to flowering compared to the non-GM counterparts (information supplied by applicant). However, given the vernalisation requirement of perennial ryegrass and the range of locations proposed for the trial (and as a consequence, the varying climatic conditions under which the trials may be planted), time from spike emergence to flowering may vary across trial sites.

231. In trials with perennial ryegrass, the amount of pollen deposition declined with increasing distance (measured up to 80 m), although there was considerable variation in dispersal of pollen from early to late anthesis and detection of dispersed pollen varied when traps of various orientations (forward, backward and upward) are used (Giddings et al., 1997a, b). Further trials with perennial ryegrass showed that the amount of pollen deposited does not always decrease smoothly with increasing distance from the source. It is suggested that in some conditions pollen clouds are taken high up into the atmosphere, move with weather and are deposited in times of calm weather, so it is therefore conceivable that pollen could move significant distances from the source. Both wind speed and turbulence are expected to be important factors (among others) in this process (Giddings et al., 1997b).

232. Studies on pollen flow from fields of Italian ryegrass to adjacent fields of perennial ryegrass in the United States have shown that little outcrossing occurred beyond 6 m from the field border in perennial ryegrass (Copeland and Hardin, 1970). A later study in Australia (Cunliffe et al., 2004) showed that gene flow in perennial ryegrass had a leptokurtic distribution with high gene flow close to the source which declines to a horizontal asymptote at 36 m. Beyond this, levels decreased from < 5% at 36 m to < 2% at 144 m (the maximum distance studied), depending on wind direction. In this study, the recipient plants were isolated from any sexually compatible pollen that could compete with the pollen from the source plants. These results are supported by a more recent study of perennial ryegrass in Argentina (Yanniccari et al., 2018), which examined pollen-mediated gene flow from glyphosate resistant plants to receptor plants that were glyphosate susceptible. Effective gene flow was detected in receptor plants that were less than 35 m from the source plants, while no gene flow was detected in receptor plants that were greater than 35 m from the source plants. Pollen-mediated gene flow between Italian ryegrass (source plants) and perennial ryegrass (receptor plants) was shown to decrease exponentially with increased distance from the pollen source at distances of up to 32 m in field studies in Ireland. This study also found hybridisation at stochastic low levels at distances between 64 m and 192 m, the maximum distance studied (Mullins et al., 2009).

233. In the USA, the isolation standard required by the United States Department of Agriculture (USDA) for foundation seed of cross-pollinated grasses is 274 m (2016), and the allowed level of seed contamination of other species is 0.1% (Montana Seed Growers Association, 2008). In South Australia, the isolation distance for basic seed is 200 m from other grasses if the area is less than 2 ha, or 100 m if greater than 2 ha, and the basic seed must be 99% pure at minimum (Seed Services Australia, 2020).

234. Considering currently available literature, it appears that most gene flow would be likely to occur within 36 m of the edge of the planting area, although there is a small chance that it could occur at greater distances (for example, < 2% at 144 m in Cunliffe et al. (2004)). Given the large amount of non-GM perennial ryegrass/other sexually compatible species pollen available in the surrounding areas compared to GM perennial ryegrass pollen from the trial site, this may influence the potential for gene flow from the GM perennial ryegrass to non-GM perennial ryegrass or related species. The total distances from the outer edge of the planting area to the outer edge of the isolation zone are considered under each site design option, below.

235. The potential for gene flow has also been considered in determining appropriate inspection frequencies for the monitoring and isolation zone for perennial ryegrass and related species. The applicant has proposed that the monitoring zone for all planting layouts would be inspected every 14 days (commencing 14 days before expected flowering of the GMOs and continuing until the GMOs are harvested) for perennial ryegrass or related species. If detected, these would be destroyed or prevented from flowering. The isolation zone for all planting setups is proposed to be inspected every 35 days (commencing from planting of the GMOs and continuing until 60 days after the GMOs are harvested or destroyed) for intentionally planted perennial ryegrass or related species. If perennial ryegrass or related species were found, these would be destroyed before flowering or prevented from flowering, or the GMOs would be destroyed.

236. Inspection of the monitoring and isolation zones is considered important for identifying plants with which the GM perennial ryegrass could outcross. The GM perennial ryegrass could outcross with any perennial ryegrass plants, whether these plants were intentionally planted or not. Therefore, to limit pollen flow, conditions requiring inspection for any perennial ryegrass plants or related species in both the monitoring zone and isolation zone, and the destruction or prevention of flowering of these plants, are included in the draft licence (Risk Scenarios 4 and 5).

237. Due to the potential for short distance vegetative reproduction and seed spread (see Section 3.1.4 for further information), inspection of the monitoring zone every 14 days is considered to be reasonable. Given there is approximately 150 days from perennial ryegrass seedling emergence to flowering (Shah et al., 1990), inspection of the isolation zone every 35 days, starting 14 days before the GMOs are expected to flower, to detect any perennial ryegrass and related species and prevent them from flowering, is considered appropriate. However, as the licence condition requires that any

perennial ryegrass or related species detected in the isolation zone must be prevented from flowering, if management practices in the isolation zone result in shorter flowering periods, inspections would need to be conducted more frequently to ensure compliance with the condition.

Option A – GMOs prevented from flowering

238. Under Option A, the applicant has proposed that the planting area would be surrounded by a 10 m or 40 m monitoring zone, with the different sizes of monitoring zone based on the size of the planting area. Discussion of the size of the monitoring zones is in Section 3.1.4, below. They also propose that the monitoring zone would be surrounded by an isolation zone, extending to a distance of 100 m from the outer edge of the monitoring zone. The GMOs would be prevented from flowering.

239. The applicant has proposed that the GMOs would be prevented from flowering by cutting to remove pre-pollen dehiscing inflorescences, physically removing whole plants, or destroying plants via herbicide application. The planting area is proposed to be inspected every 14 days (commencing 14 days before expected flowering of the GMOs and continuing until all the GMOs have been harvested) for flowering GM perennial ryegrass or related species. If detected, these would be destroyed or prevented from flowering.

240. Given the widespread nature of perennial ryegrass and related species and the likelihood that these species may be present at many of the trial sites, preventing the GM perennial ryegrass from flowering is a critical step in minimising pollen flow. The growth and reproductive stages of perennial ryegrass are well characterised (Moore et al., 1991; Lamp et al., 2001), and the applicant has advised that inspectors would be trained to be able to identify early signs of ryegrass flowering. However, considering it takes approximately 16 days from spike emergence to flowering (Shah et al., 1990) and that the varying climatic conditions at the sites may influence time to flowering, inspections of the planting area every 14 days (starting 14 days before expected flowering) as proposed by the applicant may not be sufficient to ensure that the GM perennial ryegrass would not progress to flowering without being detected. Therefore, an inspection frequency of the planting area every 7 days, starting 14 days before expected flowering area every 7 days, starting 14 days before expected flowering area every 7 days, starting 14 days before expected appropriate for this option.

241. The applicant has proposed different sizes of monitoring zone based on the size of the planting area; 10 m < 0.25 ha or 40 m for \ge 0.25 ha, with a 100 m isolation zone, to give 110 m or 140 m total distance from the outer edge of the planting area to the outer edge of the isolation zone. The GMOs will be prevented from flowering and setting seed at these sites. Therefore, it is not considered necessary to have the combined distance of the monitoring and isolation zone differ based on the size of the planting area. The draft licence also specifies that the GMOs would be inspected to detect potential flowering in the monitoring zone every 14 days. In the unlikely circumstance that one or a small number of GMOs progress to flowering undetected, low levels of gene flow have been shown to occur at 144 m (Cunliffe et al., 2004). Therefore, the total distances from the outer edge of the planting area to the outer edge of the isolation zone proposed by the applicant are not considered to be sufficient and 150 m total is considered more appropriate to manage pollen flow at sites with design Option A. The size of the monitoring zone is discussed in Section 3.1.4, below.

Option B – open block flowering in area free of perennial ryegrass and related species

242. Under Option B, the applicant proposed that the planting area would be located in an area free of perennial ryegrass and related species. The planting area would be surrounded by a 40 m monitoring zone. The monitoring zone would be surrounded by an isolation zone, extending to a distance of 100 m from the outer edge of the monitoring zone, to give a total distance of 140 m from the edge of the planting area to the edge of the isolation zone (the size of the monitoring zone is in Section 3.1.4, below). The GMOs would be allowed to flower and set seed.

243. The applicant has proposed that a research station in Kununurra (Western Australia) is a suitable site for this option. As discussed in Sections 5.1 and 5.4, the minimum temperatures of Kununurra do not meet the perennial ryegrass vernalisation requirement, and perennial ryegrass and related species have not been reported in the area. The applicant has proposed to simulate the

required vernalisation period by using cool rooms at the site. As the climatic conditions of the site play a critical role in the pollen flow control for Option B, the draft licence states that this option must be conducted at Kununurra.

As the site would be located at Kununurra, it is unlikely that naturalised perennial ryegrass or related species will be present at the site due to the unsuitable climate. In the unlikely circumstance that perennial ryegrass or related species are present on site, low levels of gene flow have been shown to occur at 144 m (Cunliffe et al., 2004). Therefore, a total distance of 140 m from the outer edge of the planting area to the outer edge of the isolation zone is not considered to be quite sufficient. Instead, 150 m is considered to be more appropriate. The size of the monitoring zone is discussed in Section 3.1.4, below.

Option C – GMOs covered by pollen control tents prior to flowering

245. Under Option C, the applicant has proposed that the GMOs would be allowed to flower and set seed and would be covered in pollen control tents during flowering. In addition, the planting area would be surrounded by a 10 m monitoring zone. The monitoring zone would be surrounded by an isolation zone, extending to a distance of 100 m from the outer edge of the monitoring zone to give a total distance of 110 m from the edge of the planting area to the edge of the isolation zone.

246. The pollen control tents proposed for use are comprised of a non-woven spun-bound polyester of no more than 0.4 mm thickness with a pore size of approximately 215 μ m (Trammell et al., 2020). The fabric would be attached to a frame made of PVC piping and secured to the ground by wire pegs and weighted bags would be placed on each side of the bottom pipe of the frame. Additional dirt can be placed around all sides of the pollen control tent. If needed, soaker hoses will be supplied to each tent for supplementary irrigation. At the end of the trials, the pollen control tent fabric would be washed using a bleach solution to clean and remove any contaminants before re-use. Duct tape would be used on any fabric seams of the pollen control tents to make minor repairs.

247. The fabric of the proposed pollen control tents has been described as having a greater pore size than some other pollen proofing fabric in order to increase air permeability and reduce heat stress, but a complex fibre structure to optimise pollen proofing (Townson et al., 2020). Perennial ryegrass pollen is 23 to $60 \,\mu\text{m}$ in size (Jansen and Den Nijs, 1993) which is smaller than the pore size of the fabric the tent (215 μ m). However, the fibre section is complex (Townson et al., 2020; Trammell et al., 2020), see Figure 9 below, and the spun bound layers of the material would be expected to disrupt the flow of pollen through the fabric.



Figure 9. Scanning electronic microscope image showing arrangement of fibres of nonwoven spunbond fabric layer (Townson et al., 2020)

248. Trammell et al. (2020) assessed the potential for pollen entry by bagging 20 reproductive tall fescue panicles in an existing tall fescue population in the field with the proposed material. Tall fescue is a wind pollinated plant and the pollen is approximately 30 μm in size. No seeds were subsequently

detected in any of the bags. This experiment did not consider pollen flow out of the pollen control bags and details of pollen flow into or out of tents of the same fabric were not reliably reported. Another study from the same group used mini tents of the proposed material and assessed seed formation in single cytoplasmic male sterile sugar beet plants within the tents. Sugar beet is wind pollinated and the pollen is approximately 20 to 25 μ m in size. The experiment was conducted in the field with donor pollen from flowering sugar beets in adjacent polly tunnels placed up-wind of the tents. The conclusion of that study that the tents completely prevented pollen flow is not considered to be reliable as a small number of viable seeds were collected from the tented plants (Townson et al., 2020).

249. While the proposed pollen control tents appear likely to reduce the flow of pollen due to the complex fabric structure, from the limited information available there is no definitive evidence that the pollen control tents will fully control the flow of pollen. In the event that the pollen control tents fail to control the GM pollen, the potential for gene flow has been considered in conjunction with the proposed monitoring and isolation zones. Given the widespread distribution of perennial ryegrass and related species at many of the proposed sites, the uncertainty regarding the ability of the pollen control tents to completely prevent pollen flow, and the potential for small amounts of outcrossing to occur at 144 m (Cunliffe et al., 2004), the total distance of 110 m proposed by the applicant is not considered to be sufficient. As the pollen control tents are expected to reduce and disrupt the flow of the pollen, it is considered unlikely that pollen clouds could form and travel great distances. Therefore, a distance of 150 m from the outer edge of the planting area to the outer edge of the isolation zone is considered appropriate for this site setup.

250. As the material of the tents forms an important part of the risk context, the draft licence includes a condition that the tents must be constructed with the material proposed by the applicant, or another material approved in writing by the Regulator.

251. As discussed in Option A (above), different conditions at the trial sites may influence the time to flowering. As covering the GMOs with a pollen control tent prior to flowering is a critical part of the pollen flow control for Option C and considering that the planting area for this site setup may be located in an area with perennial ryegrass or related species, the draft licence includes a condition that the GMOs must be covered with pollen control tents at least 14 days before the GMOs are expected to commence flowering and until all GMOs have finished flowering.

252. While the pollen control tents are in use, the applicant has proposed to inspect them every 3 days to identify any damage that may render them unable to control pollen and to perform repairs or replacements, as applicable. Given the pollen control tents play a critical role in controlling pollen flow for this site option and that the planting area for this site setup may be located in an area with perennial ryegrass or related species, inspections of the tents every 3 days is considered appropriate. It has also been specified in the draft licence that the tents should be inspected after any extreme weather event to check and repair any damage to the tents. As discussed in paragraph 246, the applicant proposed to secure the tents to the ground by wire pegs, weighted bags would be placed on each side of the bottom pipe of the frame, and additional dirt can be placed around all sides of the tent. These measures would help secure the tents from being moved in high wind conditions.

3.1.4 Consideration of proposed controls regarding dispersal of the GMOs

253. The applicant proposes to treat any non-GM perennial ryegrass and white clover plants grown in planting areas like the GMOs. These non-GM plants may be fertilised by (in the case of non-GM perennial ryegrass) or mingled with the GM ryegrass plants and it is therefore necessary to handle the non-GM plants in the same way as the GMOs to manage the dispersal or persistence of GM seed.

254. The applicant has proposed that all equipment, tools, shoes and other clothing would be inspected for GM seeds or vegetative material and cleaned before using it for any other purpose. Such measures are considered appropriate to ensure seed or viable vegetative material is not unintentionally dispersed by people or equipment (Risk Scenario 4). The draft licence contains a

condition that requires any equipment used in connection with the GMOs to be cleaned as soon as practicable after use and before use for any other purpose. Requirements for cleaning of equipment associated with transport and storage of the GMOs would need to be conducted according to the requirements set out in the Regulators <u>Guidelines for the Transport</u>, <u>Storage and Disposal of GMOs</u>.

255. The applicant has proposed to make round baled silage on site, then transport the bales to a storage facility for the animal feeding trials. The silage is not expected to contain reproductive GM material as the forage will be harvested from sites where the GMOs are prevented from flowering and the higher vegetative material will be cut rather than the base of the tillers. To maintain the context of how the applicant proposes to produce the silage, the draft licence includes a condition that silage must be produced and baled on site. As the silage is not expected to contain reproductive material, dispersal of the GMOs is not expected to occur in the animal feeding trials.

256. The applicant has proposed that the planting area and monitoring zone be surrounding with a fence capable of excluding livestock. As outlined in Risk Scenario 4, livestock have been shown to be capable of spreading perennial ryegrass seeds some distance, and so particular consideration is given for sites where the GMOs are allowed to flower and set seed. It is also possible that livestock may spread vegetative propagules, a consideration for all sites. Due to the clear and likely mechanism of dispersal, the draft licence includes a condition that the planting area must be surrounded by a fence capable of excluding livestock.

257. The applicant has proposed that the fence would be inspected for damage at least every 14 days. However, inspections at least every 35 days and after any extreme weather event are considered to be sufficient to identify any damage to the fence.

258. The applicant has also proposed to locate the planting area away from stock camps. This has not been specifically conditioned in the draft licence as the planting area is required to be surrounded by a livestock-proof fence.

259. GM perennial ryegrass seeds could be dispersed short distances from the trial sites during sowing or harvest activities, by seed shattering, by seed-hoarding behaviours of animals such as rodents, or by strong winds or runoff after heavy rain.

260. Perennial ryegrass has shattering seed heads (Elgersma et al., 1988; Fu et al., 2019), which may aid in dispersal over short distances. The applicant has proposed that at sites where the GMOs are allowed the flower and set seed, the planting area would be covered in weed matting. The weed matting would assist in reducing the number of seeds lost to the soil through shattering. The proposed pollen control tent material has a pore size of 215 μ m. 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). Where there are small blocks of GMOs, the GM perennial ryegrass seed would be hand harvested to minimise the loss of seed.

261. GM perennial ryegrass seeds could be dispersed short distances from the trial sites by small animals, including through seed-hoarding behaviours of rodents. The applicant has proposed rodent baiting to reduce the number of rodents at the site. The draft licence includes a condition requiring measures to be implemented to control rodents within each planting area while GMOs are being grown and until the planting area is cleaned. This could include rodent baiting, but also gives flexibility as to the most effective rodent control option for each site.

262. The applicant has also proposed to surround the planting area and monitoring zone with a fence capable of excluding rabbits. Rabbits are not prone to seed hoarding behaviours in the same manner as rats and mice, and are more likely to feed on vegetative material from the GM perennial ryegrass. Therefore, rabbits are not considered to be likely method of dispersal of seeds and a condition requiring a rabbit-proof fence has not been included in the draft licence.

263. The applicant proposes to locate trial sites at least 100 m away from waterways. The draft licence requires that planting areas must be at least 100 m from waterways and must not be located in

flood-prone areas, and that any extreme weather events must be reported to the Regulator. These measures would minimise dispersal of GM seeds by flooding (Risk Scenario 4).

264. Irrigation is another potential mechanism of dispersal, as perennial ryegrass seeds have been found in irrigation water. Irrigation methods such as flood irrigation may wash seeds off the planting area, particularly for sites where the GMOs are allowed to flower and set seed. The proposed weed matting for these trial sites could also enhance run-off during irrigation. The draft licence includes a condition that any area outside the planting area and monitoring zone where the GMOs have dispersed must be cleaned as soon as practicable and is subject to post cleaning monitoring until the site is signed off.

265. As described in Section 3.1.3, the planting areas would be surrounded by monitoring zones that are inspected while the GMOs are growing, so any volunteers (or related species) detected during this period would be detected and destroyed. The applicant also proposes to inspect the monitoring zones after harvest to destroy any volunteers growing from dispersed GM seeds. The proposed size of the monitoring zones is 10 m or 40 m, depending on the measures used to control pollen flow (the appropriateness of size of the monitoring zones is discussed in the individual option sections, below).

266. The draft licence includes additional conditions to manage short-distance dispersal of GM seeds. These include requiring the trial site to be cleaned within 14 days after harvest by a method that removes GM seeds from the soil surface, and requiring post-harvest inspections of any area used to clean equipment or any other area where GMOs are known to have dispersed. This combination of controls would minimise short-distance dispersal of GM seeds leading to establishment of volunteer populations outside the trial sites (Risk Scenario 4).

267. Standard conditions have been included in the draft licence that require that only authorised people are permitted to undertake any activity authorised by the licence and that all people dealing with the GMOs must be trained and informed of the relevant licence conditions. These measures would limit the opportunity for seed and vegetative material spread outside the trial area (Risk Scenario 4).

Option A - GMOs prevented from flowering

268. At sites with design Option A, the GMOs would be prevented from flowering. The applicant has proposed different sizes of monitoring zones (10 m or 40 m) depending on the size of the planting area. As the GMOs would be prevented from flowering and setting seed, no risk-based rationale is present for having these varying monitoring zone sizes. Therefore, regardless of the size of the planting area, a 10 m monitoring zone is considered appropriate for sites where the GMOs are prevented from flowering and the only likely means of spread would be through vegetative reproduction.

Option B - open block flowering in area free of perennial ryegrass or related species

269. Option B site design involves a large open block where the GMOs are allowed to flower and set seed. The applicant has proposed this site would have a monitoring zone of 40 m. As Option B allows flowering and seed production, there is a potential for seed dispersal by equipment, people, and potential run-off from the weed matting. The draft licence includes conditions to manage potential dispersal through cleaning of equipment, and to clean and monitor the planting area, monitoring zone, and other areas where GMOs have been dispersed. Vegetative dispersal is expected to only occur over short distance. Therefore, a monitoring zone of 10 m, rather than the 40 m proposed by the applicant, is considered to be sufficient.

Option C - GMOs covered by pollen control tents prior to flowering

270. Sites with design Option C are proposed to be managed as breeding nurseries over several years, where the GMOs are covered with pollen control tents prior to flowering. The sites are proposed to be harvested and replanted each year. The applicant has proposed these sites would have a monitoring zone of 10 m. At sites with Option C layout, the GMOs would be allowed to flower and

set seed, however as outlined in Option B above, there are conditions in the draft licence to minimise the dispersal and persistence of GM seeds. The pollen control tents would also likely limit the dispersal of seeds. Vegetative dispersal is expected to only occur over short distance. Therefore, the proposed monitoring zone of 10 m is considered to be appropriate.

3.1.5 Consideration of proposed controls regarding persistence of the GMOs

271. 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 (e.g., by herbicide application) within 14 days following the completion of a harvest. This is considered appropriate to minimise persistence of the GMOs.

272. The applicant has 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.

273. Perennial ryegrass forms a transient type I seed bank; a transient type I seed bank enables a species to take advantage of seasonal gaps in vegetation cover (Thompson and Grime, 1979). This is due to their large seed, lack of pronounced dormancy mechanisms, and ability to germinate in a range of temperatures or in light and dark (Thompson and Grime, 1979). 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).

274. A field experiment in NSW indicated that 14 months after seed production the seed bank contained 14% of total perennial ryegrass seeds released, and after 26 months no seed bank remained (Lodge, 2004). 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. Therefore, the seeds present in the seed bank after 14 months may have been second generation seed and/or seeds incapable of germination. Perennial ryegrass seed has been seen to persist in the soil for less than 5 years (Thompson et al., 1993) and seeds were not found six years after burial in a seed bank study in the UK (Akinola et al., 1998). Another UK study found that in a permanent pasture perennial ryegrass comprised 7.5% of the vegetative cover and 0.6% of the transient seedbank during April prior to ryegrass seed set, but when seed set was prevented over the summer, no perennial ryegrass was found in the seed bank by October (Williams, 1984). These findings indicate that the vegetative material plays a greater role in perennial ryegrass persistence in a sward compared to the seeds.

275. The applicant has proposed measures to reduce the amount of GM seed that would come into contact with the soil and potentially form a seedbank. At some sites, the applicant has proposed to prevent the GMOs from flowering (Option A), therefore the GMOs are not expected to successfully flower and produce seeds. The applicant has proposed to cover the planting area of sites where GMOs are allowed to flower (Options B and C) with weed matting to reduce the number of seeds that would come into contact with the soil.

276. The use of weed matting has not been included as a condition in the draft licence. It may be challenging to use at larger sites and there are some uncertainties about the impact it may have on dispersal and persistence of the GMOs. Although weed matting may reduce the number of seeds in the soil and resulting volunteers it is still possible that some seeds will reach the soil through any holes cut for the GMOs, joins, or through any damage to the matting, and therefore measures will still be required to manage even a small seed bank. The proposed 12-month post-harvesting monitoring period in the draft licence will manage any seed bank that forms, regardless of the seed bank size. Weed matting may increase the likelihood of seeds being spread through runoff of rain or irrigation water and removal of weed matting may inadvertently spread seeds. The draft licence includes conditions to manage potential dispersal of seeds outside the planting area or monitoring zone. Following use in the planting area, the weed matting would be considered a piece of equipment used

in connection with the GMOs and must be cleaned as soon as practicable and before use for any other purpose. If GM material were to be dispersed outside the monitoring zone during removal of the weed matting the area would have to be cleaned and monitored, as conditioned in the draft licence.

277. At sites where the GMOs are covered in pollen control tents prior to flowering, the applicant has stated that they may grow the GMOs in pots on trays to reduce the dispersal of GM seeds. The applicant has proposed that any soil/potting mixtures remaining in the pots or trays after use for the GMOs would be treated prior to disposal. This may, for example, include promoting the germination of volunteers in pots, composting or autoclaving of media or any other methods agreed to by the Regulator. Pots and trays would be considered equipment used in connection with the GMOs and must be cleaned as soon as practicable and before use for any other purpose.

278. Post-harvest, the applicant has proposed to till the soil to the depth of the original planting (to a depth of no more than 5 cm) and irrigate to promote the germination of volunteers. Burial depth may also influence the persistence of viable seeds in the soil seed bank. A recent study of the small-plot field studies of the burial of perennial ryegrass seeds in temperate conditions in Denmark found reduced seed survival when seeds were left at the soil surface compared to deep burial (Jensen, 2010). This study simulated soil tillage practices and found that leaving seeds at the soil surface or buried up to 5cm depth had a survival rate of less than 1% after 12 months. Conversely, seeds buried at a depth of 25 cm, immediately or shortly after seed-shedding, were found to have a higher survival rate after 12 months (Jensen 2010). Germination of perennial ryegrass seed in glasshouse experiments found that 90% of seed emerged at 1 cm seed burial depth, while no seed emerged at the soil surface or at depths of 6 - 7 cm (Javaid et al. 2022).

279. Given the fast germination rate of perennial ryegrass seeds and the transient nature of the seedbank, the proposed monitoring period of 12 months after harvest with at least six months volunteer free would minimise the likelihood of persistence of GM perennial ryegrass at the trial site and has been proposed in the licence.

280. The applicant has proposed that GM vegetative material may be destroyed through burial at locations notified and approved by the Regulator, to a depth of at least 1 m. This is considered to be sufficient to prevent persistence of seeds and vegetative propagules.

281. At sites where the GMOs are prevented from flowering, the applicant has proposed to harvest or destroy all GMOs 10 months after planting. The justification is that this would further restrict the potential for seeds to be created and released into the site seed bank. This requirement is not considered to be necessary as there is a condition in the draft licence for this site setup that the GMOs must be prevented from flowering which would limit the potential production of seed. In addition, the draft licence includes conditions that the site must be cleaned after harvesting and that the site must be monitored for volunteers post-cleaning, which would manage persistence of the GMOs.

282. The applicant has proposed the following methods of destruction of the GMOs:

- destructive analysis (e.g., plant material would be harvested for analysis)
- herbicide application
- root cutting and shredding/mulching
- hand weeding/uprooting
- burning/incineration
- light tillage to a depth of no more than 5 cm
- autoclaving or
- burial of seed or other plant material to a depth of at least 1 m.

283. Of these proposed methods of destruction, herbicide application, hand weeding, burning/incineration, autoclaving, and burial of GM material are considered to be appropriate methods of destruction and have been included in the draft licence, noting that in some circumstances

a combination of methods might be required to completely destroy the GMOs. Destructive analysis is also considered to be an acceptable method of destruction if the process of analysing the reproductive material causes the material to become non-viable.

284. Considering the potential for vegetative dispersal, root cutting and shredding/mulching, and light tillage are not considered to be effective methods of destruction and have not been included in the draft licence.

285. The combination of control measures described in this section would minimise the persistence of GM seeds and plants leading to establishment of GM volunteer populations in the environment (Risk Scenario 4).

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

286. A number of licence conditions are proposed to limit and control the release, based on the above considerations. These include requirements to:

- limit the duration of the release to the period from April 2023 and December 2028
- limit the size of the release to a maximum of seven sites per year, with up to 2.5 ha each year and up to 12.5 ha over the duration of the release
- limit the location of the release to nominated local government areas in New South Wales, Victoria, Queensland and Western Australia
- not allow GM plant material to be used in human food or animal feed, except for specified animal feeding trials
- control pollen flow from the trial sites using a monitoring zone of 10 m and an isolation zone of 140 m, and one of the following options
 - Option A: GMOs prevented from flowering.
 - Option B: Planting area located in Kununurra (Western Australia) and GMOs allowed to flower and set seed.
 - Option C: GMOs covered with pollen control tents prior to flowering and setting seed.
- surround sites with a fence suitable to exclude livestock
- take measures to control rodents within the planting area
- treat any non-GM perennial ryegrass and white clover grown in planting areas like the GMOs
- harvest the GM perennial ryegrass separately from other crops
- clean the areas after use including the planting area and any area in which seed has been dispersed
- clean equipment used with the GMOs before use for any other purpose
- transport and store the GMOs in accordance with the Regulator's guidelines
- locate planting areas at least 100 m from any natural waterways
- destroy all GMOs not required for further evaluation or future trials
- till and irrigate the planting area to promote the germination of any perennial ryegrass volunteers
- monitor for at least 12 months after harvest and destroy any perennial ryegrass plants that may grow and until no GM volunteers have been detected for a continuous 6-month period prior to the end of monitoring
- monitor any site used to bury GM material for at least 12 months to detect any disturbance or volunteers
- destroy all GMOs not required for further analysis or future trials
- transport and store the GMOs in accordance with the Regulator's guidelines.

3.2 Other risk management considerations

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

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

3.2.1 Applicant suitability

288. 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.

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

290. 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

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

292. Before planting the GMOs, Grasslanz would also be required to provide the Regulator with a method to reliably and uniquely detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

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

3.2.4 Reporting requirements

294. The draft 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.

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

- details of site management choice to manage pollen flow
- expected and actual dates of planting
- details of areas planted to the GMOs
- expected dates of flowering
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

296. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

297. 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.

298. 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

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

- additional molecular and biochemical characterisation of the GM perennial ryegrass events, particularly with respect to expression of the introduced genes, proteins, and lipid levels in the pollen and seeds
- additional phenotypic characterisation of the GM perennial ryegrass events, particularly with respect to any changes in biomass and the effect of the increased metabolisable energy content on the endophyte fungus and alkaloid concentrations
- allergenicity information for people with respect to the introduced cysteine oleosin
- results from the animal feeding trials, particularly with respect to the potential for increased toxicity from the endophyte alkaloids and allergenicity from the cysteine oleosin
- information on the efficacy of the pollen control tents to control pollen flow of perennial ryegrass.

Section 5 Conclusions of the consultation RARMP

300. The risk assessment concludes that the proposed limited and controlled release of GM perennial ryegrass poses negligible to low risk to the health and safety of people and animals, and negligible risk to the environment.

301. The risk management plan concludes that the identified negligible to low risks can be managed to protect the health and safety of people and the environment. Conditions are proposed to limit the release to the size, location and duration of the trial, 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. Specific risk treatment measures are proposed in the licence to manage the potential for allergenicity to people working with the GMOs.

Chapter 4 Proposed licence conditions

Section 1 Interpretations and definitions

- 1. In this licence:
 - (a) unless defined otherwise, words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;
 - (b) words denoting a gender include any other gender;
 - (c) words in the singular include the plural and words in the plural include the singular;
 - (d) words denoting persons include a partnership and a body whether corporate or otherwise;
 - (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
 - (f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;
 - (g) specific conditions prevail over general conditions to the extent of any inconsistency.
- 2. In this licence:

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

'Clean' means, as the case requires:

- (a) in relation to Equipment or a facility, remove and/or Destroy the GMOs; or
- (b) in relation to an area of land specified in this licence as requiring Cleaning:
 - i. Destroy Perennial Ryegrass plants, if present, to the reasonable satisfaction of the Regulator, and
 - ii. remove Perennial Ryegrass seeds from the soil surface to the reasonable satisfaction of the Regulator.

Note: The intent of removing seeds from the soil surface is to minimise seed dispersal. One method of removing seeds from the soil surface is Tillage, which moves seeds to under the soil. Tillage must be in accordance with condition 53.

'Common Conditions' means Conditions 1 to 26 and 42 to 63.

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

- (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event; and
- (b) recover and/or Destroy the GMOs to the reasonable satisfaction of the Regulator; and
- (c) inspect for and Destroy any Volunteers that may exist as a result of the event to the reasonable satisfaction of the Regulator.

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

- (a) uprooting;
- (b) treatment with herbicide;
- (c) burning/incineration;
- (d) autoclaving;
- (e) crushing or grinding of seed;
- (f) burial, but only in accordance with condition 54;
- (g) destructive analysis;
- (h) a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, treatment with herbicide would not successfully kill GM seeds.

'Equipment' includes, but is not limited to, seeders, harvesters, Pollen Control tents, transport equipment (e.g. bags, containers, trucks), weed matting, clothing, footwear and tools.

'Extreme Weather' includes, but is not limited to, fires, flooding, cyclones or torrential rain, that could disperse GMOs or affect the licence holder's ability to comply with licence conditions.

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

'GM' means genetically modified.

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

'Isolation Zone' means an area of land extending outwards from the outer edge of the Monitoring Zone, as shown in Figures 1-3.

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

'Monitoring Zone' means an area of land extending outwards from the outer edge of a Planting Area, as shown in Figures 1-3.

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

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

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

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

'Planting Area' means all land, within the inner edge of the Monitoring Zone, where the GMOs and non-GM Perennial Ryegrass and White Clover may be intentionally planted and grown pursuant to this licence, as shown in Figures 1-3.

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

'Pollen Control' means designed to control the movement of pollen from the GMOs to other Related Species.

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

'Regulator' means the Gene Technology Regulator.

'Related Species' means plants of the species *Lolium perenne* L (perennial ryegrass), *L. multiflorum* Lam. (Italian ryegrass), *L. rigidum* Gaud. (annual ryegrass), *L. loliaceum* (rigid ryegrass), *L. remotum* (hardy ryegrass), *Festuca pratensis* (meadow fescue), *F. rubra* L. (red fescue) and *F. arundinaceum* (tall fescue), but does not include plants intentionally grown in the Planting Area in accordance with licence conditions.

'Sesame' means plants of the species Sesamum indicum.

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

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

Note: Tillage must be in accordance with condition 53.

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

White Clover means plants of the species Trifolium repens L.

Section 2 General conditions and obligations

- 3. This licence does not authorise dealings with the GMOs that are otherwise prohibited as a result of the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.
- 4. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMOs are authorised during any period of suspension.

Note: Although this licence has no expiry date, the period when GMOs may be grown is restricted in accordance with Condition 18.

- 5. The licence holder is Grasslanz Technology Australia Pty Limited.
- 6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by this licence.
- 7. The GMOs with which dealings are authorised by this licence are those listed at Attachment A.
- 8. The dealings authorised by the licence are to:
 - (a) conduct experiments with the GMOs;
 - (b) breed the GMOs;
 - (c) propagate the GMOs;
 - (d) grow the GMOs;
 - (e) import the GMOs;

- (f) transport the GMOs;
- (g) dispose of the GMOs;

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

9. This licence does not apply to dealings with the GMOs conducted as a Notifiable Low Risk Dealing (NLRD) or pursuant to another authorisation under the Act.

Note: Dealings conducted as an NLRD must be assessed by an Institutional Biosafety Committee (IBC) before commencement and must comply with the requirements of the Regulations.

General obligations of the licence holder

- 10. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.
- 11. The licence holder must be able to access and control all Planting Areas, Monitoring Zones, Isolation Zones and approved facilities to the extent necessary to comply with this licence.

Note: Arrangements to access and control these areas must be notified to the Regulator as part of each planting notification (Condition 62(a)).

- 12. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:
 - (a) the particular condition, including any variations of it;
 - (b) the cancellation or suspension of the licence;
 - (c) the surrender of the licence.
- 13. The licence holder must not permit a person covered by this licence to conduct any dealing with the GMOs unless:
 - (a) the person has been informed of any applicable licence conditions, including any variation of them; and
 - (b) the licence holder has obtained from the person a signed and dated statement that the person:
 - i. has been informed by the licence holder of the licence conditions including any variation of them; and
 - ii. has understood and agreed to be bound by the licence conditions, or variation.
- 14. The licence holder must inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator.

General obligations of persons covered by the licence

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

Note: Under the Act, the definition of premises includes a building, area of land or vehicle.

Section 3 Limits and control measures

3.1 Limits on the release

The following licence conditions impose limits on where and when the GMOs may be grown.

- 16. The only plants that may be intentionally grown at a Planting Area are:
 - (a) the GMOs covered by this licence; and
 - (b) non-GM Perennial Ryegrass and non-GM White Clover; and
 - (c) plants approved in writing by the Regulator.
- 17. Non-GM Perennial Ryegrass and non-GM White Clover plants grown in a Planting Area must be handled as if they were the GMOs.
- 18. Planting and growing of the GMOs may only occur within the following limits:

Area and duration

Period	Maximum number of Planting Areas per year	Maximum combined size of Planting Areas per year	Maximum combined size of Planting Areas for the trial
April 2023 to December 2028	7	2.5 ha	12.5 ha

Local Government Areas in which Planting Areas may be located

New South Wales Victoria		Western Australia	Queensland
Armidale Regional	Ballarat City	Albany	Gympie Regional
Bathurst Regional	Bass Coast	Augusta-Margaret River	Ipswich City
Pogo Vallov	Pow Pow	Bridgetown-	Lockyer Valley
bega valley		Greenbushes	Regional
Bellingen	Benalla Rural City	Busselton	Logan City
Berrigan	Campaspe	Capel	Moreton Bay Regional
Blayney	Cardinia	Carnarvon	Scenic Rim Regional
Byron	Casey City	Busselton	Somerset Regional
Cabonne	Colac Otway	Dardanup	South Burnett Regional
Central Coast	Corangamite	Denmark	Southern Downs Regional
Cessnock	East Gippsland	Harvey	Tablelands Regional
Clarence Valley	French Island	Manjimup	Toowoomba Regional
Coffs Harbour	Gannawarra	Murray	
Cootamundra-Gundagai Regional	Glenelg	Nannup	
Cowra	Golden Plains	Nedlands	
Dubbo Regional	Greater Shepparton City	Serpentine Jarrahdale	
Dungog	Hepburn	Subiaco	
Glen Innes Severn	Indigo	Swan	
Goulburn Walwaree	Latrobe City	Waroona	
Gwydir	Loddon	Wyndham-East Kimberley	
Hawkesbury	Macedon Ranges		
Hilltops	Mitchell		
Inverell	Moira		
Kempsey	Moorabool		
Kyogle	Mornington Peninsula		

Lake Macquarie City	Moyne
Lismore	Pyrenees
Lithgow City	South Gippsland
Liverpool	Southern Grampians
Maitland	Surf Coast
Mid-Coast	Towong
Mid-Western Regional	Wangaratta
Muswellbrook	Warrnambool City
Nambucca Valley	Wellington
Narrabri	Wodonga City
Oberon	Yarra Ranges
Orange	
Port Macquarie-Hastings	
Port Stephens	
Queanbeyan-Palerang	
Regional	
Richmond Valley	
Shoalhaven	
Singleton	
Snowy Monaro Regional	
Snowy Valleys	
Tamworth Regional	
Tenterfield	
Tweed	
Upper Hunter	
Upper Lachlan	
Uralla	
Wagga Wagga	
Walcha	
Walgett	
Warrumbungle	
Wingecarribee	

Note: for length, 'City of', 'Council' and 'Shire'/'Shire of' have been omitted from the LGA names. French Island is an unincorporated territory with no local government.

3.2 Control measures

The following licence conditions restrict the spread or persistence of the GMOs and their genetic material in the environment.

Conditions to restrict exposure to the GMOs

- 19. Subject to condition 20, Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in its use as food for humans or feed for animals.
- 20. Silage produced from the GMOs may be fed to livestock for experimental purposes, subject to those experiments being approved by an Animal Ethics Committee operating under the Australian Code for the Care and Use of Animals for Scientific Purposes.
- 21. Each Planting Area must be inside a fence that is capable of excluding livestock while GMOs are being grown and until the Planting Area is Cleaned.

22. While a fence is required under the preceding condition, the fence must be inspected for damage at least once every 35 days and after any Extreme Weather event, and if damage is found, must be repaired as soon as practicable.

Note: Details of any inspection activity must be recorded in a Logbook (condition 63) and reported to the Regulator (condition 62).

23. The licence holder must, before permitting a person covered by this licence to conduct any dealing with the GM Perennial Ryegrass, make reasonable inquiries that the person does not have a known allergy to Sesame.

Note: The licence holder must document compliance with this condition, as per Condition 13.

- 24. The licence holder must not knowingly permit a person covered by this licence to conduct any dealing which may expose the person to small pieces of green vegetative Plant Material from the GM Perennial Ryegrass if that person has an allergy to Sesame.
- 25. GMOs must be planted using Option A or Option B or Option C site layout (as shown in Figures 1, 2, and 3, respectively).

Note: Prior to planting the GMOs, the licence holder must notify the Regulator which option is proposed to be used for each Planting Area (Condition 62).

- 26. The licence holder must comply with all Common Conditions.
 - (a) Sites planted under Option A must also comply with conditions 27 to 31;
 - (b) Sites planted under Option B must also comply with conditions 32 to 36;
 - (c) Sites planted under Option C must also comply with conditions 37 to 41.

Note: conditions relevant for each option are detailed separately in the following sections. The sections are colour coded for clarity. Orange for Option A; Purple for Option B; Yellow for Option C.

Option A: GMOs prevented from flowering



Figure 1. Diagram (not to scale) showing the relationship between the Planting Area, Monitoring Zone and Isolation Zone for **Option A** site layout. GMOs planted under this option must not be allowed to flower or set seed.

Conditions to restrict pollen flow for Option A

- 27. The GMOs must be prevented from Flowering.
- 28. A Planting Area must be surrounded by a Monitoring Zone of at least 10 metres (as shown in Figure 1). All land within the inner edge of the Monitoring Zone is included in the Planting Area and must be managed as such whether planted or not.
- 29. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and Destruction of Volunteers and Related Species while the GMOs are growing in the Planting Area and until the Planting Area is Cleaned.

Note: Condition 63 requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

- 30. The Monitoring Zone must be surrounded by an Isolation Zone of at least 140 metres (as shown in Figure 1) that is managed in a manner that enables compliance with Condition 31.
- 31. While the GMOs are growing in a Planting Area, the Planting Area, Monitoring Zone and Isolation Zone must be inspected by people trained to recognise Perennial Ryegrass and Related Species, and actions must be taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs have been harvested or Destroyed	At least once every 7 days	Perennial Ryegrass & Related Species	Destroy before Flowering or prevent from Flowering
Monitoring Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs have been harvested or Destroyed	At least once every 14 days	Perennial Ryegrass & Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs
Isolation Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs have been harvested or Destroyed	At least once every 35 days	Perennial Ryegrass & Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs

*Condition 62(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.

Note: Details of any inspection activity must be recorded in a Logbook (Condition 63) and reported to the Regulator (Condition 62).



Option B: Open flowering in area free of perennial ryegrass or related species

Figure 2. Diagram (not to scale) showing the relationship between the Planting Area, Monitoring Zone and Isolation Zone for **Option B** site layout. The Planting Area must be located in Kununurra (Western Australia). GMOs planted under this option will be allowed to flower and set seed.

Conditions to restrict pollen flow for Option B

- 32. The Planting Area must only be located at a single site in Kununurra in the Local Government Area Shire of Wyndham-East Kimberley in Western Australia.
- 33. A Planting Area must be surrounded by a Monitoring Zone of at least 10 metres (as shown in Figure 2). All land within the inner edge of the Monitoring Zone is included in the Planting Area and must be managed as such whether planted or not.
- 34. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and Destruction of Volunteers and Related Species while the GMOs are growing in the Planting Area and until the Planting Area is Cleaned.

Note: Condition 63 requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

35. The Monitoring Zone must be surrounded by an Isolation Zone of at least 140 metres (as shown in Figure 2) that is managed in a manner that enables compliance with Condition 36.

36. While the GMOs are growing in a Planting Area, the Planting Area, Monitoring Zone and Isolation Zone must be inspected by people trained to recognise Perennial Ryegrass and Related Species, and actions must be taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Related Species	Destroy before Flowering or prevent from Flowering
Monitoring Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Perennial Ryegrass and Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs
Isolation Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 35 days	Perennial Ryegrass and Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs

*Condition 62(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.

Note: Details of any inspection activity must be recorded in a Logbook (Condition 63) and reported to the Regulator (Condition 62).

Option C: GMOs covered by Pollen Control tents prior to flowering



Figure 3. Diagram (not to scale) showing the relationship between Planting Area (with tents), Monitoring Zone and Isolation Zone for **Option C** site layout. GMOs planted under this option must be covered with Pollen Control tents prior to flowering.
Conditions to restrict pollen flow for Option C

- 37. All GMOs in a Planting Area must be covered with Pollen Control tents comprised of a material of non-woven spun-bound polyester of no more than 0.4 mm thickness with a pore size of approximately 215 μ m, or another material approved in writing by the Regulator, from at least 14 days prior to the expected commencement of Flowering of any GMOs and until all GMOs have completed Flowering.
- 38. A Planting Area must be surrounded by a Monitoring Zone of at least 10 metres (as shown in Figure 3). All land within the inner edge of the Monitoring Zone is included in the Planting Area and must be managed as such whether planted or not.
- 39. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and Destruction of Volunteers and Related Species while the GMOs are growing in the Planting Area and until the Planting Area is Cleaned.

Note: Condition 63 requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

- 40. The Monitoring Zone must be surrounded by an Isolation Zone of at least 140 metres (as shown in Figure 3) that is managed in a manner that enables compliance with Condition 40.
- 41. While the GMOs are growing in a Planting Area, the Planting Area, Monitoring Zone and Isolation Zone must be inspected by people trained to recognise Perennial Ryegrass and Related Species, and actions must be taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Related Species	Destroy before Flowering or prevent from Flowering
Monitoring Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Perennial Ryegrass and Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs
Isolation Zone	From 14 days prior to the expected commencement of Flowering of any GMOs* until all GMOs in the Planting Area have finished Flowering	At least once every 35 days	Perennial Ryegrass and Related Species	Destroy before Flowering or prevent from Flowering or Destroy GMOs
Pollen Control tents	While tents are in place	At least once every 3 days and after any Extreme Weather event	Damage that may affect the tent's ability to control pollen flow	Repair any damage or replace if repair not possible

*Condition 62(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.

Note: Details of any inspection activity must be recorded in a Logbook (Condition 63) and reported to the Regulator (Condition 62).

Conditions to restrict dispersal

- 42. Equipment used in connection with the GMOs must be Cleaned as soon as practicable after use with the GMOs and before use for any other purpose.
- 43. Planting Areas must be at least 100 metres away from any permanent natural watercourses or man-made drainage features that flow into natural watercourses.

Note: This includes irrigation channels or storm water drains that flow into a natural watercourse.

- 44. Planting Areas must not be located in flood prone areas.
- 45. Measures must be implemented to control rodents within each Planting Area while GMOs are being grown and until the Planting Area is Cleaned.

Note: Measures for rodent control may include, but are not limited to, traps and/or poison baits within and/or surrounding the Planting Area.

Conditions relating to harvesting

- 46. If all GMOs in a Planting Area have been Destroyed, then for the purposes of this licence:
 - (a) the GMOs are taken to have been harvested; and
 - (b) the Planting Area is taken to have been Cleaned.

Note: Cleaning activities must be reported to the Regulator (Condition 62). Areas of land that have been Cleaned are subject to inspections (Condition 51).

- 47. The GMOs must be harvested, threshed, or ensiled separately from any other crop.
- 48. Silage from harvested GM forage must be created and baled in the Planting Area, Monitoring Zone, or Isolation Zone.
- 49. Harvested GM Plant Material not required for experimentation or future planting must be Destroyed as soon as practicable.

Conditions to restrict persistence of GMOs on trial sites

50. Areas of land used in connection with the GMOs must be Cleaned as follows:

Areas of land to be Cleaned	When
Planting Area and Monitoring Zone	Within 14 days after harvest of the GMOs
Any area, outside a Planting Area or Monitoring Zone, used to Clean any Equipment used in connection with the GMOs e.g. machinery, weed matting, or pots	As soon as practicable
Any area, outside a Planting Area or Monitoring Zone, where the GMOs have dispersed, e.g. during planting, growing, harvest, ensiling or Destruction	As soon as practicable

Note: Cleaning activities must be reported to the Regulator (Condition 62). Areas of land that have been Cleaned are subject to inspections (Condition 51).

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

Area	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area, Monitoring Zone and other areas of land that were Cleaned in accordance with Condition 50	 From the day of Cleaning, until: i. the area is planted as a new Planting Area in accordance with condition 16; or ii. the Regulator has issued a Sign off for the area. 	At least once every 35 days	Volunteers	Destroy before Flowering

Note: Details of any inspection activity must be recorded in a Logbook (Condition 63) and reported to the Regulator (Condition 62).

- 52. While post-Cleaning inspection requirements apply to an area:
 - (a) the area must be maintained in a manner appropriate to allow identification of Volunteers; and
 - (b) no plants may intentionally be grown in the area unless:
 - i. the area is planted as a new Planting Area in accordance with condition 16; or
 - ii. the plants are agreed to in writing by the Regulator; and
 - (c) the area must not be used for grazing livestock; and
 - (d) the Area must receive at least two Tillage and watering events as described in **Attachment B**, where Tillage and watering both occur within a period of 14 days; and
 - (e) the final required Tillage and watering event must occur within the 6 months prior to submission of the Sign off application.

Tillage

53. Any Tillage of the Planting Area must be to a depth no greater than 5 centimetres.

Destruction by burial

54. If Destruction of GMOs occurs by burial:

- (a) the GMOs must be buried in a pit and covered by a layer of soil at least one metre in depth, the top of which is no higher than the surrounding soil surface; and
- (b) Plant Material must be wet when buried to encourage decomposition; and
- (c) the licence holder must take measures to ensure that the burial site is not disturbed for a period of at least 12 months from the date of burial.

Note: If GMOs are dispersed on the soil surface during the process of burial, the burial site becomes an area of land that requires Cleaning under Condition 50 and is subject to post-Cleaning requirements.

Note: The date and location of burial, and measures used to ensure that the burial site is not disturbed, must be reported to the Regulator (Condition 62(f)).

Processing or experimentation with the GMOs

- 55. Treatment, threshing or processing of GM seed or experimentation or analysis with the GMOs may only be undertaken within:
 - (a) a Planting Area before Cleaning; or
 - (b) a Monitoring Zone before Cleaning; or
 - (c) a facility approved in writing by the Regulator.

Note: This condition does not apply to dealings conducted as an NLRD (see Condition 9).

56. Within a facility approved in writing by the Regulator in accordance with Condition 55, any area that is used for treatment, threshing, processing, experimentation, or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

Transport or storage of the GMOs

- 57. Transport or storage of the GMOs must:
 - (a) only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation under the Act, or to the extent necessary to enable export of the GMOs; and
 - (b) be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal* of *GMOs* for PC2 GM plants as current at the time of transportation or storage; and
 - (c) comply with all other conditions of this licence.

Note: Activities with the GMOs within a Planting Area prior to Cleaning are not regarded as transport or storage.

Note: Condition 13 requires signed statements for persons transporting the GMOs.

Note: This condition does not apply to dealings conducted as an NLRD (see Condition 9).

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

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 59).

Contingency plan

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

Section 4 Sign off

- 60. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and other areas requiring Cleaning if:
 - (a) post-Cleaning inspection activities have been conducted for at least 12 months on the area; and
 - (b) conditions have been conducive for germination and detection of Volunteers; and
 - (c) no Volunteers have been detected in the area during the 6 months prior to the Sign off request; or
 - (d) molecular analysis of Volunteers demonstrates that no GMOs were detected in the area during the 6 months prior to the Sign off request.

Note: An area requires two Tillage and watering events prior to a Sign off application (Condition 52).

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

Section 5 Reporting and documentation

The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR.

61. General notifications must be sent to the Regulator as follows:

Note: please send all correspondence related to the licence to OGTR.M&C@health.gov.au.

Notice	Content of notice	Timeframe
(a) Changes to contact details	Changes to any of the contact details of the project supervisor that were notified in the licence application or subsequently	As soon as practicable
(b) Ongoing suitability to hold a licence	 i. any relevant conviction of the licence holder; or ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and 	As soon as practicable after any of these events occur
	 iv. any information related to the licence holder's ongoing suitability to hold a licence, that is requested by the Regulator 	Within the timeframe stipulated by the Regulator

(c) People covered by the licence	 i. names of all organisations and persons, or functions or positions of the persons, who will be covered by the licence, with a description of their responsibilities; and <i>Note: Examples of functions or positions are</i> <i>'project supervisor', 'site manager', 'farm</i> <i>labourer' etc.</i> ii. detail of how the persons covered by the licence will be informed of licence conditions 	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
(d) Testing methodology	A written methodology to reliably detect the genetic modifications described in this licence. The detection method must be capable of identifying the GM Perennial Ryegrass events planted under this licence.	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
(e) Contingency plan	A Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
(f) Training records	Copies of the signed and dated statements referred to in condition 13 if requested by the Regulator	Within the timeframe stipulated by the Regulator
(g) Additional information required by the Act	 i. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or ii. any contraventions of the licence by a person covered by the licence; or iii. any unintended effects of the dealings authorised by the licence Note: The Act requires, for the purposes of the condition 61.g, that: the licence holder will be taken to have become aware of additional information of a kind mentioned in Condition 61.g if he or she was reckless as to whether such information existed; and the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in Condition 61.g, if he or she was reckless as to whether such information existed; and 	Without delay after becoming aware of any new information <i>Note: An example of</i> <i>notification without delay is</i> <i>contact made within a day of</i> <i>a contravention of the licence</i> <i>via the OGTR free call phone</i> <i>number 1800 181 030, which</i> <i>provides emergency numbers</i> <i>for incidents that occur out of</i> <i>business hours. Notification</i> <i>without delay will allow the</i> <i>OGTR to conduct a risk</i> <i>assessment on the incident</i> <i>and attend the location, if</i> <i>required</i>
	Note: Contraventions of the licence may occur through the action or inaction of a person.	
(h) Further details regarding additional information	Any further details requested by the Regulator in relation to information provided under condition 61.g	Within the timeframe stipulated by the Regulator

62. Notifications relating to each trial site must be sent to the Regulator as follows:

Note: please send all correspondence related to the lice	cence to <u>OGTR.M&C@health.gov.au</u> .
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Notice	Content of notice	Timeframe
(a) Intention to plant	 Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the trial site (e.g. Google Maps) and any other descriptions 	At least 7 days prior to each planting (to be updated as soon as practicable if the notified details change)
	 Which planting option is being used at each trial site. 	
	 Detail of how the licence holder will access and control the Planting Area and the associated Monitoring Zone and Isolation Zone, in accordance with condition 11 	
	Note: this should include a description of any contracts, agreements, or other enforceable arrangements.	
	iv. Date on which the GMOs will be planted	
	v. Period when the GMOs are expected to Flower	
	vi. Period when harvesting is expected to commence	
	 vii. How all areas requiring post-Cleaning inspections are intended to be used until Sign off, including proposed post-harvest crops (if any) 	
	viii. Details of how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers	
	ix. History of how the trial site has been used for the previous two years	
(b) Planting	i. Actual date(s) of planting the GMOs	Within 7 days of any
	 Any changes to the details provided under part (a) of this condition 	planting
(c) Extreme Weather	Any Extreme Weather event that is expected to affect or has already affected an area where the GMOs are or may be present.	As soon as practicable
	Note: The Contingency Plan must be implemented if the GMOs are detected outside areas requiring Cleaning (Condition 59).	
(d) Harvest	Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting of the GMOs and within 7 days of completion of the final harvest of the GMOs
(e) Cleaning	 Date(s) on which required Cleaning was performed on any areas of land Method(s) of Cleaning 	Within 7 days of completion of Cleaning
(f) Destruction by burial	Date of burial, location of burial including GPS co-ordinates, and details of measures used to ensure that the burial site will not be disturbed for the period required by Condition 54	Within 7 days of burial of any GMOs

(g) Inspection activities	Information recorded in a Logbook as per the inspection requirements:	Within 35 days of inspection
	i. Option A: Conditions 22, 31, 51 and 63;	
	ii. Option B: Conditions 22, 36, 51 and 63	
	iii. Option C: Conditions 22, 41, 51 and 63.	

Note: Additional records must be provided to the Regulator, if requested, in accordance with condition 58.

- 63. Details of any inspection activity must be recorded in a Logbook and must include:
 - (a) date of the inspections; and
 - (b) name of the person(s) conducting the inspections; and
 - (c) details of the experience, training or qualification that enables the person(s) to recognise Perennial Ryegrass and Related Species, if not already recorded in the Logbook; and
 - (d) details of areas inspected including current land use (including any post-harvest crops) and recent management practices applied; and

Note: management practices include Tillage events, spraying or maintenance measures used to facilitate inspections.

- (e) details of the developmental stage of the GMOs while they are being grown; and
- (f) details of any post-Cleaning rainfall events including measurements at or near the area, or any irrigation events; and
- (g) details of any Volunteers and/or Related Species observed during inspections or during land-management activities, including number, developmental stage and approximate position of the Volunteers and/or Related Species within each area inspected[†]; and
- (h) date(s) and method(s) of Destruction of or preventing Flowering of any Volunteers and/or Related Species, including destruction of Volunteers and/or Related Species during landmanagement activities; and
- (i) details of any damage and any repairs to the fence surrounding the Planting Area, while the fence is required; and
- (j) details of any damage and any repairs to the Pollen Control tents, while Pollen Control tents are required; and
- (k) details of rodent control methods used and any evidence of rodent activity, while rodent control methods are required.

[†] Examples of acceptable ways to record the positional information for Volunteers and/or Related Species in the Logbook include:

- descriptive text

- marking on a diagram
- indicating grid references on a corresponding map/sketch.

Note: Details of inspection activities must be provided to the Regulator (Condition 62). The Regulator has developed a standardised proforma for recording inspection activities. This can be made available on request.

ATTACHMENT A

DIR No:	194	
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Full Title:Limited and controlled release of perennial ryegrass genetically modified for
increased metabolisable energy content

Organisation Details

Postal address:	Grasslanz Technology Australia Pty Limited
	PO Box 2064, Hilton Plaza
	Adelaide, SA 5033

GMO Description

GMOs covered by this licence

Perennial ryegrass plants genetically modified by introduction of only the genes and genetic elements listed below.

Parent Organism		
Common Name:	Perennial ryegrass	
Scientific Name:	Lolium perenne L	
Modified traits		
Category:	Composition – animal nutrition	
	Selectable marker – antibiotic resistance	
Description:	The perennial ryegrass has been genetically modified with two co-expressed genes to increase the fatty acid content in green tissue. The GMOs may also contain a selectable marker gene that confers antibiotic resistance. The introduced genes are listed in Table 1 and the associated regulatory sequences are listed in Table 2.	

Purpose of the dealings with the GMO

The purpose of the release is to evaluate the increased metabolisable energy content trait under field conditions. The objectives of the field trial are collection of regulatory data, agronomic assessment of the GM perennial ryegrass, and seed production to advance breeding and to enable forage production for feeding trials with ryegrass silage. The GM perennial ryegrass is not permitted to be used for human food or animal feed except in the proposed animal feeding trials.

Gene name	Source organism	Description
Diacylglycerol o transferase 1 (DGAT1)	<i>Tropaeolum majus</i> (garden nasturtium)	Enzyme that catalyses the final step in triacylglycerol synthesis
Cysteine oleosin	Sesamum indicum (sesame)	Modified oil structural body protein
Hygromycin B phosphotransferase (hph)	Escherichia coli	Hygromycin B antibiotic resistance (selectable marker)

Table 1. Introduc	ed genes in the GM	perennial ryegrass
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Table 2. Introduced regulatory	v sequences in th	he GM perennial	ryegrass

Element function	Genetic element	Source organism
Green tissue specific promoter	<i>Ribulose bisphosphate carboxylase small subunit, chloroplastic 3 (RBCS3A)</i> promoter	<i>Oryza sativa</i> (rice)
Green tissue specific promoter	Chlorophyll a/b-binding protein (CAB) promoter	<i>Oryza sativa</i> (rice)
Constitutive promoter	<i>Cauliflower mosaic virus (CaMV</i>) 35S promoter	Cauliflower mosaic virus
Terminator	Nopaline synthase (NOS) poly(A) signal	Agrobacterium tumefaciens
Terminator	<i>Vegetative storage protein-acid phosphatase B (vspB)</i> terminator	<i>Glycine max</i> (soybean)
Terminator	CaMV poly(A) signal	Cauliflower mosaic virus

ATTACHMENT B

A watering event is irrigation or natural rainfall that provides sufficient soil moisture to promote germination of Perennial Ryegrass seeds on a trial site.

Examples of acceptable watering events are:

- At least 26 millimetres of rainfall over one day; or
- At least 28 millimetres of rainfall over two days; or
- At least 30 millimetres of rainfall over three days; or
- At least 32 millimetres of rainfall over four days; or
- Irrigation that provides equivalent levels of soil moisture to one of the examples of rainfall above.

Rainfall measurements must be taken on the site or within 3 km of the site. An irrigation or natural rainfall that matches one of the examples listed above, and occurs during the time period specified for a watering event in Condition 52 of the licence, is considered a valid watering event. The licence holder should keep records of the date/s and amount of water applied during the watering event, and provide this information when requesting Sign off of the relevant site.

If an irrigation or natural rainfall does not match one of the examples listed above, the licence holder may submit a request to the Regulator for it to be considered a watering event. The request should provide:

- evidence of amount of water applied, such as rainfall measurements on the site or within 3 km of the site, and
- evidence that resultant soil moisture is suitable for germination, such as photos of germinating plants on the site.

It is recommended that any requests that an irrigation or natural rainfall be considered a watering event be submitted at the time of the event, to minimise potential delays to Sign off of the site.

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