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

May 2025

Risk Assessment and Risk Management Plan for

DIR 212

Limited and controlled release of canola genetically modified for increased photosynthesis and photorespiration

Applicant: The University of Adelaide

Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 212

Decision

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

The application

Applicant	The University of Adelaide
Project Title	Limited and controlled release of canola genetically modified for increased photosynthesis and photorespiration
Parent organism	Canola (*Brassica napus* L.)
Genetic modifications	
Introduced genes	Introduced genes conferring increased photosynthesis and photorespiration: • *GhPGLP1* gene from *Gossypium hirsutum* (cotton) • *AtPetC* gene from *Arabidopsis thaliana* • *AtPip1;3* gene from *A. thaliana* Introduced marker genes: • *hptII* gene from *Escherichia coli* for antibiotic resistance • *bar* gene from *Streptomyces hygroscopicus* for tolerance to the herbicide glufosinate
Genetic modification method	*Agrobacterium*-mediated transformation
Number of lines	Up to 15 lines
Principal purpose	To evaluate the performance of the GM canola under field conditions
Previous releases	There have been no previous releases of the GMOs
Proposed limits	
Proposed use of GM plants	No use in human food or animal feed proposed
Proposed location	The trial is proposed to take place at one site in South Australia (Light Regional Council)
Proposed release size	Up to 2 ha per year
Proposed period of release	From April 2025 to January 2030

Risk assessment

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

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM canola plants. Potential harms associated with these pathways included adverse health effects to people, toxicity to desirable animals, and environmental harms due to weediness.

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks. The principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as not using GM plant material in human food or animal feed, will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

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.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food and animal feed, to minimise dispersal of the GMOs or GM pollen from the trial site, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs at the end of the trial and to conduct post-harvest monitoring at the trial sites to ensure the GMOs are destroyed.

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Abbreviations

2PG	2-phosphoglycolate
AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bar	Bialaphos resistance
CaMV35S	Cauliflower mosaic virus 35S
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving intentional release
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare(s)
HPT	Hygromycin phosphotransferase
mas	Mannopine synthase
nos	Nopaline synthase
ocs	Octopine synthase
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin N-acetyltransferase
PGLP1	Phosphoglycolate phosphatase 1
Pip	Plasma membrane intrinsic protein
RARMP	Risk Assessment and Risk Management Plan
RbcS2B	Rubisco small subunit 2B
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator

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) website.

5. 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
Modified genes
Novel traits

Parent organism (comparator)
Origin and taxonomy
Cultivation and use
Biology

Proposed GMO dealings
Activities
Limits
Controls

Previous releases
Australian approvals
International approvals

Receiving environment
Environmental conditions: abiotic and biotic factors
Production practices
Related organisms
Similar genes and proteins

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

6. 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.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian

Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Two public submissions were received and their consideration is summarised in Appendix B.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. 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), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry (DAFF). These dealings may also be subject to 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.

9. To avoid duplication of regulatory oversight, risks that will be considered by other regulatory agencies would not be assessed by the Regulator.

Section 2 The proposed dealings

10. The University of Adelaide (the applicant) proposes to release up to 15 lines of canola genetically modified for increased photosynthesis and photorespiration.

11. The purpose of the trial is to evaluate the agronomic performance of the GM canola under Australian field conditions. The performance of the GM canola will be assessed in both a rain-fed and water limited environment. The proposed release would also be used to produce sufficient grain for further replicated trials. The GM canola would not be used for human food or animal feed.

12. The dealings involved in the proposed intentional release are to:

- conduct experiments with the GMOs
- breed the GMOs
- propagate the GMOs
- grow the GMOs
- transport the GMOs
- dispose of the GMOs

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

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

13. The release is proposed to take place at one site, a field trial facility at Rosedale in South Australia (SA), Light Regional Council. The release is proposed to take place between April 2025 and January 2030. To account for seasonal variation and to vary the stresses that plants are exposed to, the applicant proposes that more than one planting area could be established at the trial site however, the total planting area will be no more than 2 hectares (ha) per year.

14. Only trained and authorised staff would be permitted to deal with the GM canola.

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

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

- not locating the trial site in a flood prone area

- restricting gene flow from the GMOs by locating the site at least 400 m away from other canola crops by either:
    a. covering the GMOs with an insect proof tent prior to flowering and surrounding the planting area with a 10 m monitoring zone and a 390 m isolation zone (Figure 2a); or
    b. surrounding the planting area with a 15 m pollen trap of non-GM canola, a 35 m monitoring zone, and a 390 m isolation zone (Figure 2b).
- treating any non-GM canola plants grown in planting areas or pollen traps as if they are GMOs
- after harvest, destroying GMOs not required for further evaluation or future trials
- cleaning equipment used in connection with the GMOs as soon as practicable and before use for any other purpose
- transporting and storing GMOs in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- surrounding the site with livestock proof fencing
- post-harvest tilling of planting areas, pollen traps and other areas where GMOs were dispersed to encourage seed germination
- post-harvest monitoring of each trial site at least every 35 days for at least 24 months and until the site is free of volunteer canola plants for at least 12 months, with any volunteer plants destroyed prior to flowering.

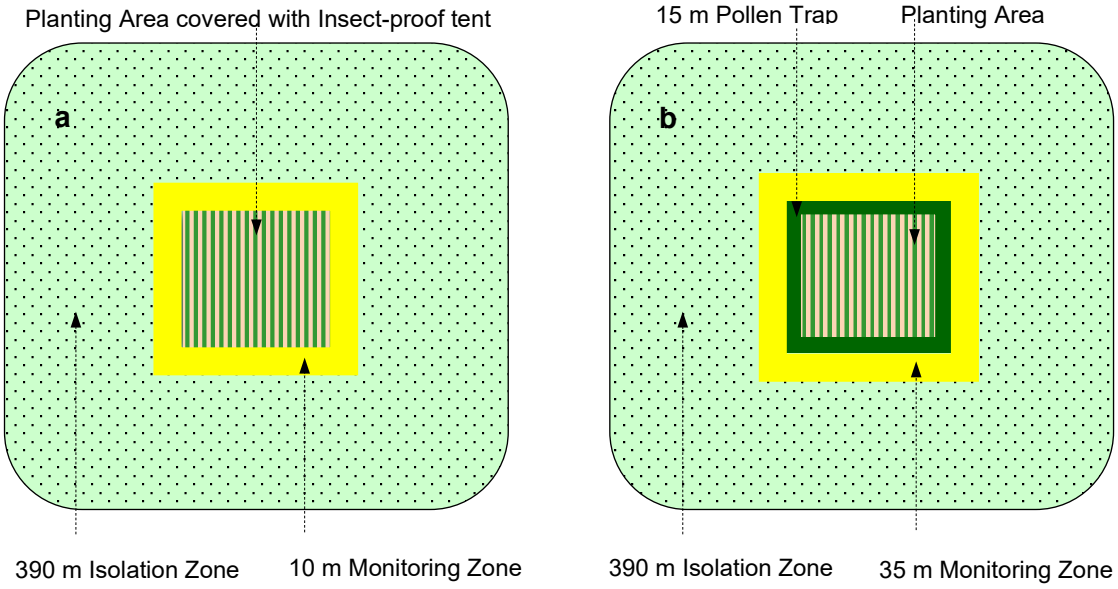

Figure 2. Applicant's proposed options for restricting gene flow from the GM canola (not to scale)

Site layout (a) with Insect-proof tent, and (b) with Pollen Trap.

16. The proposed limits and controls are taken into account in the risk assessment (Chapter 2) and their suitability for containing the release will be evaluated in the risk management plan (Chapter 3).

Section 3 The parent organism

17. The parent organism is *Brassica napus* L., which is commonly known as canola, rapeseed or oilseed rape. *B. napus* is exotic to Australia.

18. Canola is the third-most widely grown crop in Australia. It is grown mainly in Western Australia (WA), New South Wales (NSW), Victoria (Vic) and SA (ABARES, 2024). Canola oil is used as food and the canola meal remaining after oil extraction is used as animal feed.

19. *B. napus* is naturalised in Australia. In areas where it is grown, it can be an agricultural weed in subsequent crops. There are isolated reports of *B. napus* as an environmental weed in WA and Vic (Randall, 2017). However, the most recent Western Australian state government environmental weed risk assessment gives *B. napus* a weed risk rating of negligible to low (Moore and Nazeri, 2022), and the most recent Victorian state government environmental weed list gives *B. napus* a risk ranking score of zero and classified as 'lower risk' (White et al., 2022).

20. Detailed information about the parent organism is contained in the document *The Biology of* Brassica napus *L. (canola)* and Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2024a), which was produced to inform the risk analysis process and is available from the Resources page on the OGTR website. Baseline information from this document will be used and referred to throughout the RARMP.

21. While non-GM canola is not generally regarded as allergenic or toxic to humans or animals, it does produce some toxins and anti-nutritional factors such as erucic acid and glucosinolates, and some cases of canola food, pollen and dust allergies have also been reported (OGTR, 2024a).

22. The specific parental canola variety from which the GMOs are derived is called 'Oscar'.

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

23. The applicant proposes to release 15 lines of canola genetically modified for increased photosynthesis and photorespiration. The applicant expects these modifications to increase the yield of the GM canola in the field.

4.1 The genetic modifications in the GMOs proposed for release

24. The GMOs will contain one of three different genes of interest for altered photosynthesis and photorespiration (Table 1). Up to 5 lines will be produced for each of these introduced genes. Gene pyramiding may be carried out by crossing plants containing combinations of the 3 genes of interest if individual transgenes are successful in producing improvements in yield.

Table 1. Introduced genes of interest and selectable markers

Gene	Source	Encoded protein	Intended function
GhPGLP1	*Gossypium hirsutum* (cotton)	Phosphoglycolate phosphatase 1 (PGLP1)	Enhanced photorespiration
AtPetC	*Arabidopsis thaliana*	Rieske FeS	Improved electron transport capacity in photosynthesis
AtPip1;3	*Arabidopsis thaliana*	Plasma membrane intrinsic protein 1;3 (Pip1;3)	Improved photosynthesis by improved CO_2 transport
hptII	*Escherichia coli*	Hygromycin phosphotransferase (HPT)	Selectable marker (antibiotic resistance)
bar	*Streptomyces hygroscopicus*	Phosphinothricin N-acetyltransferase (PAT)	Selectable marker (herbicide tolerance)

4.1.1 GhPGLP1

25. In organisms that undergo oxygenic photosynthesis, photorespiration is an important metabolic pathway for detoxifying metabolic intermediates and recycling carbon. The oxygenase activity of Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) results in the production of the metabolite 2-phosphoglycolate (2PG), a toxic intermediate that can inhibit carbon metabolism. As part of the first step in the photorespiratory C2 cycle in the chloroplast, the enzyme phosphoglycolate phosphatase 1 (PGLP1) dephosphorylates 2PG to glycolate. Glycolate is then exported to the peroxisome to participate in further metabolic reactions, eventually resulting in the Calvin cycle intermediate 3-phosphoglycerate. Photorespiration also influences other metabolic pathways, including sulphur and nitrogen metabolism (reviewed in Aroca et al., 2023).

26. The GMOs contain the *PGLP1* gene from *Gossypium hirsutum* (cotton; *GhPGLP*). In a laboratory study of *Arabidopsis thaliana* plants genetically modified to over-express the native *Arabidopsis* PGLP1 protein, the plants overexpressing PGLP1 showed slightly increased growth and biomass compared to the control in standard growth environment conditions (Flugel et al., 2017). In a different laboratory trial using GM *A. thaliana*, plants that overexpressed native PGLP1 had improved heat-stress tolerance compared to the wild-type control (Fu et al., 2024).

4.1.2 AtPetC

27. Located in the thylakoid membrane of plant chloroplasts, the photosynthetic electron transport chain comprises a series of 4 protein complexes where electron transfer sequentially occurs, resulting in the production of ATP and NADPH. These molecules are then used to build carbohydrates. One of the protein complexes in the electron transport chain is cytochrome b_6f, which consists of 8 subunits. The Rieske FeS protein is a key polypeptide of the cytochrome b_6f complex and is encoded by the *Photosynthetic electron transfer C* (*PetC)* gene (Ermakova et al., 2019).

28. The GMOs contain the *PetC* gene from *A. thaliana* (*AtPetC*). In *A. thaliana,* overexpression of the Rieske FeS protein from *Nicotiana tabacum* (tobacco) has been shown to enhance photosynthesis, biomass and seed yield in a laboratory setting (Simkin et al., 2017). Improvements in photosynthetic capabilities were also seen in a model of overexpression of the Rieske FeS protein from *Brachypodium* sp. in the C4 plants *Setaria viridis* (green foxtail) grown in the laboratory (Ermakova et al., 2019) and improvements in photosynthesis, biomass, and grain for *Sorghum bicolor* (sorghum) grown in the glasshouse (Ermakova et al., 2023).

4.1.3 AtPip1;3

29. Aquaporins are a major family of membrane channel proteins that are found in almost all species (reviewed in Kruse et al., 2006). Most aquaporins have a primary function of water transport, although permeability to other substrates has been demonstrated, including gases and nutrients (reviewed in Groszmann et al., 2017). Plasma membrane intrinsic proteins (Pips) are a subfamily of aquaporins found in plants, including canola (Sonah et al., 2017) and *A. thaliana* (Quigley et al., 2002), and are known to play an important role in plant responses to abiotic stresses, including drought and salt tolerance (Cao et al., 2020; Li et al., 2015; Tang et al., 2021). Based on sequence similarity, Pips are categorised into subgroups Pip1 and Pip2, with Pip2 proteins being considered to be the more efficient water channels (reviewed in Kapilan et al., 2018).

30. The GMOs contain the *Pip1;3* gene from *A. thaliana* (*AtPip1;3*). The applicant states that *AtPip1;3* comes from a group of Pips reported to have activity as a CO_2 porin and hypothesises that insertion of *AtPip1;3* in the GMOs may improve photosynthesis by improving CO_2 transport. As CO_2 availability impacts photosynthesis, improving CO_2 flux within plant leaves has been a research target to improve crop yields (reviewed in Lundgren and Fleming, 2020). No literature could be found specifically examining the role of AtPip1;3 in CO_2 transport, however the related aquaporin Pip1;2 from *A. thaliana* has been shown to play an important role in CO_2 transport (Heckwolf et al., 2011; Uehlein et al., 2012). AtPip1;3 has been demonstrated to transport a number of non-CO_2 substrates. In a high-throughput screening assay in yeast, AtPip1;3 was shown to have strong permeability to hydrogen peroxide and moderate permeability to water

(Groszmann et al 2023). CO_2 transport was not examined. Although from a different species, overexpression of tobacco Pip1;3 in canola grown in the laboratory resulted in increased root oxygen concentrations and improved the tolerance of the plants to waterlogging compared with the wild-type controls, suggesting a role in O_2 transport (Liu et al., 2024).

31. Aquaporins are known to play an important role in pollen hydration, the second step in pollination following pollen recognition in the stigma. *A. thaliana* mutants with complete suppression of *Pip1;2* and/or *small basic intrinsic protein 1;1* (*Sip1;1*), which are normally relatively highly expressed in the stigmatic papillae cells, showed normal pollen viability and morphology, but decreased pollen hydration, germination and seed number compared to the wild-type (Windari et al., 2021). Reciprocal crossing between wild type and mutant lines demonstrated that decreased pollen hydration occurred only if the female side in the cross was a *Sip1;1* and/or *Pip1;2* mutant, which suggests that Sip1;1 and Pip1;2 play important roles in water supply from the papillae cells to the pollen grain, rather than water uptake in the pollen. Overexpression of the 2 aquaporins was not assessed. No literature could be found specifically examining the role of AtPip1;3 in pollen characteristics, including pollen hydration and pollen viability.

4.1.4 Selectable markers

32. The GMOs may contain up to 2 selectable markers that are used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes (Table 1). The *hygromycin phosphotransferase* (*hptII*) gene is derived from *Escherichia coli*. It encodes the hygromycin phosphotransferase (HPT) protein, which confers resistance to the antibiotic hygromycin B. The *bialaphos resistance* (*bar*) gene is derived from *Streptomyces hygroscopicus*. It encodes the phosphinothricin N-acetyltransferase (PAT) enzyme, which confers tolerance to the herbicide glufosinate. The applicant does not intend to apply glufosinate to the GM canola in the field.

33. The GMOs may also contain a small fragment of the *lacZ* gene (156 bp out of the 3072 bp full length gene) from *E. coli*. The *lacZ* gene encodes the enzyme β-galactosidase and can be used as a visual marker to confirm successful transformation of plasmids in bacteria. The fragment that the GMOs may contain is out of frame. Therefore, the *lacZ* gene is not expected to be functional in the GM canola and will not be considered further.

4.1.5 Regulatory sequences and tag

34. Short regulatory sequences that control expression of the genes are also present in the GM canola lines (Table 2). The expression of the *GhPGLP1*, *AtPip1;3*, and *hptII* genes are driven by a constitutive promoter *Cauliflower mosaic virus 35S* (*CaMV35S*), which is active in all plant tissues. The expression of the *AtPetC* gene is driven by the *Rubisco small subunit 2B* (*RbcS2B*) promoter, which is active in green tissues. Constitutive promoters *nopaline synthase* (*nos*) or *mannopine synthase* (*mas*) will be used to drive expression of the *bar* herbicide tolerance gene. Other short regulatory elements used include termination sequences.

35. A N-terminal Myc epitope tag will also be fused to the 3 genes of interest to identify the introduced proteins. The Myc tag is a 10 amino acid sequence derived from the human transcription factor c-Myc. The Myc tag is a common tag used to detect expression of recombinant proteins. Due to their small size, peptide tags, including the Myc-tag, generally do not disturb protein function (Thermo Fisher Scientific Inc.). The Myc tag fused to a carrier protein was shown to be able to induce immune responses in animals when emulsified in a suitable adjuvant (Chiarella et al., 2010). A search of the scientific literature found no reports of adverse immunogenic reactions to Myc tags fused to proteins.

Table 2. Introduced regulatory sequences and epitope tag

Element function	Genetic element	Source
Constitutive promoter	*CaMV35S*	*Cauliflower mosaic virus*
	nos	*Agrobacterium tumefaciens*
	mas	*Agrobacterium tumefaciens*
Green tissue specific promoter	*RbcS2B*	*Arabidopsis thaliana*
Terminator	*octopine synthase (ocs)*	*Agrobacterium tumefaciens*
	nos	*Agrobacterium tumefaciens*
	mas	*Agrobacterium tumefaciens*
Epitope tag	Myc	Synthetic peptide from the human C-Myc protein

4.2 Method of genetic modification

36. The GM canola lines were generated by Agrobacterium–mediated transformation. Information about this method can be found in the document Methods of plant genetic modification, available from the OGTR Risk Assessment References page.

37. After transformation, canola explants were exposed to various selection and regeneration media, prior to being transferred to soil and the greenhouse. Hygromycin and glufosinate were used to select for transgenic material. In addition, cefotaxime and timentin were used during these stages to eliminate bacteria, including *Agrobacterium*.

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

38. As the GMOs are at an early stage of development, no toxicity or allergenicity studies have been conducted on the GM canola plants or purified proteins produced by the 3 introduced genes of interest. These genes of interest were isolated from common sources, thus people and other organisms have a long history of exposure to them. A comprehensive search of the scientific literature yielded no information to suggest that the introduced genes themselves, their protein products, or any associated products or effects were toxic or allergenic to people, or toxic to other organisms.

39. There is no evidence that the *hptII* gene or the proteins it encodes are toxic or allergenic (OGTR Risk Assessment Reference document on marker genes and references therein). Food derived from GM cotton containing the *hptII* gene has been assessed and approved for sale in Australia (FSANZ, 2006).

40. The *bar* gene and the protein it encodes (phosphinothricin N-acetyl transferase or PAT) has been extensively assessed in other RARMPs, and in scientific literature. The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans. Further details are available in the DIR 186 RARMP. FSANZ has approved food derived from a number of GM crops expressing the PAT protein as safe for human consumption. This includes GM canola (ANZFA, 2001; FSANZ, 2017), cotton (FSANZ, 2005b, 2010, 2013), corn (FSANZ, 2005a) and rice (FSANZ, 2008).

4.4 Characterisation of the GMOs

41. The GMOs are at an early stage of development. The applicant has stated that they selected the 3 genes of interest for further development following observations of improved photosynthetic performance of GM canola plants grown in pots in the glasshouse.

42. Except in improving photosynthetic performance, the applicant has not observed any characteristics in the GM canola grown in the glasshouse which may affect the efficiency of gene transfer into any sexually compatible species. This includes anther extrusion, modified pollen shape, modified pollen production or altered pollen viability. The applicant has advised they have insufficient data at this time to determine if the GM canola has increased seed numbers.

43. The applicant has stated that staff working with the GMOs in the greenhouse have not reported any adverse effects.

Section 5 The receiving environment

44. 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).

45. Detailed information about the commercial cultivation and distribution of canola in Australia is presented in the document *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2024a).

5.1 Relevant abiotic factors

46. The geographical distribution of commercial canola cultivation in Australia is limited by several abiotic factors, the most important being water availability. Canola is generally grown as a winter crop in winter-dominant medium and high rainfall environments that receive more than 350 mm rainfall per year (GRDC, 2009; OGTR, 2024a). Germination of seed will only occur if there is sufficient soil moisture, and drought stress after anthesis can significantly reduce yield due to abortion of seed and reduced pod numbers. Canola is also sensitive to waterlogging (GRDC, 2009; OGTR, 2024a). Other abiotic stresses that can reduce canola yields include frost, particularly during early pod development, and heat stress (GRDC, 2009).

47. The proposed release would occur at a field trial facility at Rosedale in SA. The proposed site is located in Light Regional Council, a local government area (LGA) north of Adelaide. The proposed site is on land leased by The University of Adelaide from the South Australian Research and Development Institute (SARDI). Based on information discussed in the OGTR Biology document for canola, Light Regional Council is located in commercial canola growing regions of SA. The proposed site at Rosedale has a climate typical of rain-fed canola production areas for SA based on Bureau of Meteorology climate data (accessed 22 January 2025), which shows a concentration of rainfall during the winter months and drier summer months.

5.2 Relevant biotic factors

48. Diseases have the potential to significantly reduce the yield of canola. Blackleg disease caused by the fungal pathogen *Leptosphaeria maculans* is the most serious disease affecting commercial canola production in Australia (GRDC, 2009; OGTR, 2024a). Other damaging diseases of canola include stem rot caused by the fungus *Sclerotinia sclerotiorum* and damping-off, caused mainly by the fungus *Rhizoctonia solani* (GRDC, 2009, 2015).

49. Canola is most susceptible to insect pests during establishment of the crop, particularly from redlegged earth mite (*Halotydeus destructor*), blue oat mites (*Penthaleus major*, *P. falcatus* and *P. tectus* sp. n.), lucerne fleas (*Sminthurus viridis*), cutworms (*Agrotis* spp.) and aphids (*Brevicoryne brassicae*, *Myzus persicae*, *Lipaphis pseudobrassicae* and *Aphis craccivora*, also as viral vectors) (GRDC, 2009). From flowering

to crop maturity, severe damage can be caused by aphids, Rutherglen bugs (*Nysius vinitor*), diamondback moth caterpillars (*Plutella xylostella*) and heliothis caterpillars (family *Noctuidae*).

50. Canola is highly susceptible to weed competition during the early stages of growth (GRDC, 2009, 2015). Hybrid canola have greater seedling vigour than open-pollinated canola and so are more competitive with weeds (GRDC, 2015, 2017). Common weeds of Australian canola crops include grassy weeds (such as rigid ryegrass, vulpia and wild oat), volunteer cereals, and weeds from the Brassicaceae family including wild radish (*Raphanus raphanistrum*), Indian hedge mustard (*Sisymbrium orientale*), shepherds purse (*Capsella bursa-pastoris*), Asian mustard (*Brassica tournefortii*), charlock (*Sinapis arvensis*), turnip weed (*Rapistrum rugosum*) and Buchan weed (*Hirschfeldia incana*) (GRDC, 2015, 2017).

5.3 Relevant agricultural practices

51. Agronomic and crop management practices for the cultivation of the GM canola by the applicant would be similar to that for commercial canola crops, except that the applicant proposes controls to restrict the dispersal and persistence of the GM canola (see Section 2.2). Standard cultivation practices for canola in Australia are discussed elsewhere (GRDC, 2015, 2017). The applicant proposes to only use the glufosinate tolerance conferred by the introduced *bar* gene as a selectable marker during transformation. Glufosinate herbicide is not intended to be applied to plants growing in the field trial. Glufosinate is not routinely used to control volunteer canola (AOF, 2019).

52. Seeds will be hand planted in rows or by use of a small-scale dedicated GMO seeder for larger plots. Plants will be harvested by hand or using a dedicated GMO plot harvester, then taken to PC2 facilities for analysis and storage.

53. The waste material derived from harvest will be left on the trial area and will be ploughed back into the soil along with any stubble remaining after harvest. This cultivation will be only to the depth of seeding so as not to transfer grain any deeper into the soil profile. If not ploughed back into the soil, the waste may be burnt or buried elsewhere on site.

54. After leaving the location fallow during the off-season, it may be re-planted with the GM canola in the following growing season.

55. As the proposed site is limited in area and also being used for other DIR licences (DIR 186 and DIR 201 GM wheat and barley), the applicant has requested to plant GMOs from DIR 201 over areas under this licence that are undergoing post-harvest monitoring and vice versa. The applicant has reasoned that it would be straight-forward to identify volunteers of the other species amongst the GMOs due to their different visual appearance.

5.4 Presence of related species in the receiving environment

56. In recent years the site has been used for field trials of GM wheat and barley (DIR 186 and DIR 201). The site has not previously been used for field trials of GM canola, however the applicant may use the site for future GM field trials and has requested the licence allow concurrent planting of GMOs from multiple field trial licences, where the other licences have compatible licence conditions. As there are currently no field trial licences with compatible conditions, planting of other field trial licences with sexually compatible GMOs at the same site will not be considered further in this RARMP, but would be considered in future RARMPs.

57. Canola is widely grown as an oil seed crop in Australia, and the proposed trial site is located in a canola growing region. The applicant has stated that commercial canola crops are expected to be grown in the LGA of the proposed release site, but not within at least 400 m of the release site. Commercial canola in these areas includes non-GM canola and GM canola authorised for commercial release. Most Australian canola crops are herbicide tolerant, with 4 different herbicide tolerance traits available for commercial cultivation: triazine tolerance (non-GM), imidazolinone tolerance (non-GM), glyphosate tolerance (GM), or glufosinate tolerance (GM) (Brown, 2021; Matthews et al., 2021). Details of all GM canola varieties approved by the Regulator for commercial release in Australia under a licence are available from the OGTR

website. GM canola authorised by the Regulator as safe for anyone to grow in Australia without a licence is listed on the GMO Register.

58. Canola is primarily self-pollinating, but approximately 30% of seeds are produced by cross-pollination (Hüsken and Dietz-Pfeilstetter, 2007). Cross-pollination can be mediated by insects, wind or physical contact (OGTR, 2024a).

59. Canola has been reported to outcross in the field with the following species: *Brassica carinata*, *B. napus, B. juncea, B. oleracea, B. rapa, Hirschfeldia incana* (Buchan weed), *Raphanus raphanistrum* (wild radish) and *Sinapis arvensis* (charlock) (Ford et al., 2006; Warwick et al., 2009). All of these species are known to be present in Australia, with the exception of *B. carinata* (Atlas of Living Australia, accessed 17 January 2025).

60. Of the *Brassica* species in Australia, canola may hybridise under natural conditions with sexually compatible species that include: other *B. napus* groups or subspecies (including vegetables such as swedes, rutabaga and Siberian kale), *B. juncea, B. rapa* (wild turnip; includes vegetables such as turnip, Chinese cabbage and pak choi) and *B. oleracea* (wild cabbage; includes vegetables such as cauliflower, Brussels sprouts, kale and cabbage) (Salisbury, 2002). However, hybrids between *B. napus* and *B. oleracea* have been shown to be difficult to obtain (Ford et al., 2006).

61. Under open pollination conditions, naturally occurring hybrids between *B. napus* and the related weedy species *R. raphanistrum*, *H. incana* and *S. arvensis* have been reported at low frequencies (Darmency and Fleury, 2000; Darmency et al., 1998; Salisbury, 2002), and are generally sterile or predominantly sterile (Salisbury, 2002).

5.5 Presence of similar genes and their products in the environment

62. All of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment (Atlas of Living Australia, accessed 17 January 2025).

63. The *GhPGLP1* gene is derived from *G. hirsutum*, a commercially cultivated cotton species common on roadsides in the cotton growing regions of NSW, southern Queensland (Qld) and northern Vic (OGTR, 2024b).

64. The *AtPetC* and *AtPip1;3* genes are derived from *A. thaliana*, a plant commonly known as thale cress. *A. thaliana* is native to Europe, central Asia and Africa, but is naturalised worldwide (Yim et al., 2024).

65. The *bar* gene was obtained from the common soil bacterium *S. hygroscopicus*. The *bar* gene or the similar *pat* gene from *S. viridochromogenes* are also present in many types of GM canola or cotton authorised for commercial release in Australia (search the OGTR website for GM plants containing the *bar* or *pat* genes).

66. The *hptII* gene is derived from *E.coli*, which is widespread in the environment (reviewed in Jang et al., 2017).

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

6.1.1 Approvals by the Regulator

67. The GM canola lines included in this application have not been previously approved for release in Australia.

6.1.2 Approvals by other government agencies

68. The GM canola lines included in this application have not been previously approved by any other government agencies in Australia.

6.2 International approvals

69. The GM canola lines included in this application have not received any approvals from authorities in other countries.

Chapter 2 Risk assessment

Section 1 Introduction

70. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 3). 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.

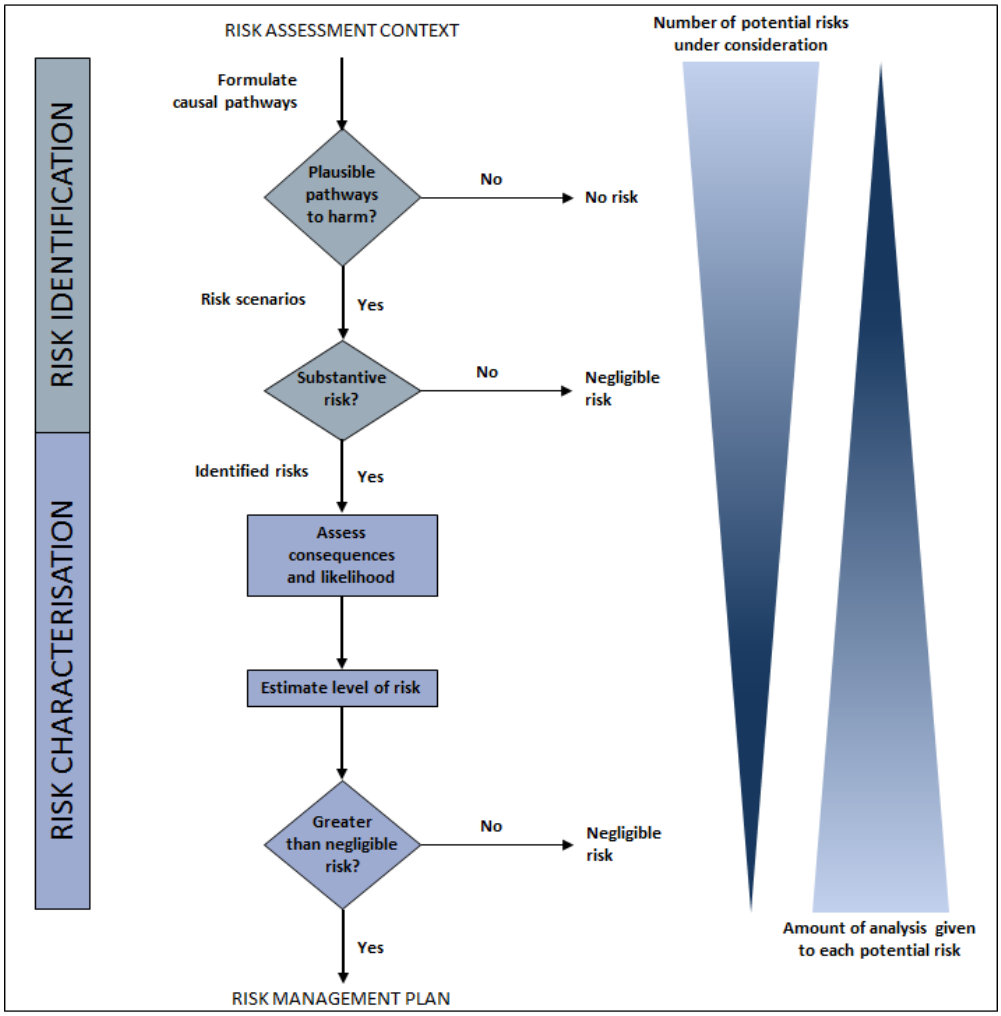

Figure 3. The risk assessment process

71. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

72. 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 3), that is, the risk is considered to be no greater than negligible.

73. 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

74. Postulated risk scenarios are comprised of three components (Figure 4):

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.

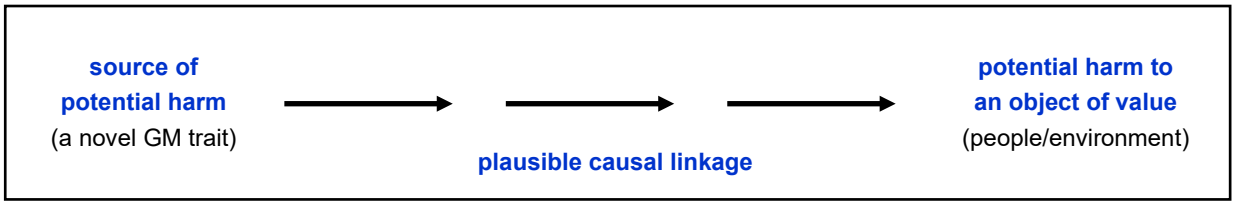

Figure 4. Risk scenario

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

- 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 GMOs
- the characteristics of the parent organism(s).

2.1 Risk source

76. 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.

77. As discussed in Chapter 1, the GM canola lines have been modified by the introduction of 3 genes intended to improve photosynthesis and photorespiration. These genes of interest may also include a Myc epitope tag that will enable detection of the recombinant proteins in the GM plants. As discussed in Chapter 1, Section 4.1, the Myc tag is a widely used small peptide tag that does not generally disturb protein function. However, there is some uncertainty about the immunogenicity of the Myc tag. These 3 introduced genes and the Myc tag will be considered further as a source of potential harm.

78. The GM canola may also contain the introduced *bar* gene that confers glufosinate herbicide tolerance and is used as a selectable marker gene. The *bar* gene has been previously assessed as a herbicide tolerance gene in RARMPs for multiple commercial GM crops (most recently DIR 190), and as a marker gene in RARMPs for multiple GM field trials (most recently DIR 204). These RARMPs are available from the OGTR GMO Record. As the *bar* gene has not been found to pose a substantive risk to either people or the environment in previous assessments, this introduced gene will not be further considered as a source of potential harm.

79. In addition, the GM lines may contain the *hptII* selection marker gene which confers resistance to the antibiotic hygromycin B. This gene and its product have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as other regulatory agencies in Australia and overseas. As this gene has not been found to pose substantive risks to either people or the environment, its potential effects will not be further assessed for this application. More information on selectable marker genes can be obtained from the OGTR document Marker genes in GM plants, available on the OGTR website.

80. The introduced genes are controlled by regulatory sequences. These were originally derived from plants, plant viruses and a bacterium (Table 2). Regulatory sequences are naturally present in all plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Delaney et al., 2018). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

81. 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

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

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

83. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs and are not expected to give rise to substantive risks.

84. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

85. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise

risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

86. Potential harms from GM plants are based on those used to assess risk from weeds (Keese et al., 2014; Virtue, 2004) 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).

87. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

88. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 3 and are examined in Section 2.4.1.

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

Table 3. Summary of risk scenarios from the proposed dealings with the GM canola

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
1	The introduced genetic modifications	Cultivation of GM canola at trial sites ⬇ Exposure of people and desirable animals to products of the introduced genes	Adverse health effects in people OR increased toxicity to desirable animals	No	• The GM canola would not be used as human food or animal feed. • The small size and short duration of the proposed trial would restrict consumption of GM plant material by wild animals. • The limits and controls of the field trial would restrict exposure of people and desirable animals to the GM plants. • The proteins encoded by the introduced genes are not expected to be toxic or allergenic. • The Myc-tag peptide present in GM canola is not expected to elicit

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
					strong immune responses.
2	The introduced genetic modifications	Cultivation of GM canola at trial sites ⬇ Dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment	Adverse health effects in people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants	No	• The limits and controls of the field trial would minimise dispersal or persistence of GM seeds. • GM canola is susceptible to standard weed management measures. • As discussed in Risk Scenario 1, no substantive risk was identified for increased adverse effects in people or toxicity to animals. • Canola has limited ability to compete with other plants and the genetic modifications are not expected to alter the dispersal characteristics of the GM canola.
3	The introduced genetic modifications	Cultivation of GM canola at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	Adverse health effects in people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants	No	• The controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites. • As discussed in Risk Scenario 1, no substantive risk was identified for increased adverse effects in people or toxicity to animals. • As discussed in Risk Scenario 2, the genetic modifications are not expected to alter the dispersal characteristics of the GM canola.

2.4.1 Risk Scenario 1

Risk source	The introduced genetic modifications
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ Exposure of people and desirable animals to products of the introduced genes ⬇
Potential harm	Adverse health effects in people OR increased toxicity to desirable animals

2.4.1.1 Risk source

90. The source of potential harm for this postulated risk scenario is the introduced genetic modifications in the GM canola plants.

2.4.1.2 Causal pathway

91. The GM canola would be grown at the trial sites. As the introduced genes *GhPGLP1* and *AtPip1,3*, are controlled by constitutive promoters, the encoded proteins would be expected to be produced in all tissues of the GM plants. Expression of the *AtPetC* gene is driven by a green tissue-specific promoter, so the encoded protein may only be produced in green tissues. People and desirable animals could be exposed to the GM plants containing the introduced proteins. Exposure could occur via ingestion, inhalation or dermal contact.

92. The GM canola would not be used for human food. Only authorised and trained trial staff would be permitted to deal with the GM plants and their seeds. Therefore, there is little potential for the public to be exposed to the GM plants grown at the trial sites.

93. Trial staff would handle the GM plant material produced by processing of the GM plants. Workers could be exposed to the introduced proteins by dermal contact and inhalation. Due to the small scale of the proposed trial, only a limited number of people would engage in dealings with the GM plant material.

94. The GM canola would not be used for animal feed and livestock would not be permitted to graze the trial sites. Therefore, livestock are not expected to be exposed to GM plants grown at the trial sites.

95. Desirable wild animals, such as native mammals and birds, could enter the trial sites and consume GM plants including seeds. The limited size and duration of the field trial would restrict the number of desirable wild animals exposed to GM plants grown at the trial sites.

2.4.1.3 Potential harm

96. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot, 2000). Allergenicity is the potential of a substance to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al., 2006).

97. The introduced proteins for increased photosynthesis and photorespiration have not been assessed in toxicity and allergenicity studies by the applicant and this is an area of uncertainty for this risk assessment. However, as discussed in Chapter 1, Section 4.3, the introduced genes were isolated from naturally occurring organisms that are widespread and prevalent in the environment. Therefore, people and other organisms are exposed to the same or similar proteins through their diet and/or in the environment. In addition, no information could be found in the literature to suggest that the introduced genes or their products are toxic or allergenic to people or toxic to other desirable organisms.

98. As mentioned in Chapter 1 Section 3, while non-GM canola is not generally regarded as allergenic or toxic to humans or animals, it does produce some allergens, toxins and anti-nutritional factors. As discussed in Chapter 1, Section 4.1, the proteins encoded by the introduced genes for play a role in photosynthesis and photorespiration. Overexpression of the protein in plants has been associated with increased

photosynthetic capabilities in preliminary glasshouse trials. There is no reasonable expectation that the introduced genes for increased photosynthesis and photorespiration expressed in the GM canola would affect the pathways producing endogenous toxins or allergens in canola or lead to the production of novel toxins or allergens.

99. Some of the constructs introduced into the GM canola lines also have the Myc tag sequence from the human c-Myc protein fused to the introduced genes of interest for detection of the tagged proteins. As discussed in Chapter 1 Section 4.1, the Myc tag is a widely used small peptide tag that does not normally disturb protein function and does not have a documented history of eliciting harmful immune responses. The Myc tag is not expected to alter the biological function of the genes of interest. The Myc tag could elicit an immune response under certain conditions, such as in the presence of an appropriate adjuvant. The Myc tag present in the GM canola is unlikely to meet such conditions and is therefore not expected to elicit a strong immune response. However, this is an area of uncertainty.

2.4.1.4 Conclusion

100. Risk scenario 1 is not identified as a substantive risk because the GM plant material would not be used as human food and animal feed, the small size and short duration of the proposed trial would restrict consumption of GM plant material by wild animals, the introduced proteins for increased photosynthesis and photorespiration are not expected to be toxic or allergenic, the Myc tag is not expected to elicit a strong immune response, and the limits and controls of the field trial would restrict exposure of people and desirable animals to the GM plants. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	The introduced genetic modifications
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ Dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment ⬇
Potential harm	Adverse health effects in people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants

2.4.2.1 Risk source

101. The source of potential harm for this postulated risk scenario is the introduced genetic modifications in the GM canola plants.

2.4.2.2 Causal pathway

102. The GM canola would be grown at the trial sites. GM seeds could be physically dispersed outside the trial sites by human activity, animal activity, wind or water. GM seeds could also persist on trial sites after completion of the trial. These GM seeds could grow in the environment and establish populations of volunteer GM plants.

103. Viable GM canola seeds could be dispersed outside the trial sites by human activity, such as transport of seeds and movement of agricultural machinery. To minimise dispersal of GM seeds by human activity, the applicant proposes to clean all equipment used with the GM plants after use, and to transport all GM seed in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

104. GM seeds could be dispersed outside the trial sites by animal activity. Canola seeds have no specific adaptions, such as burrs or hooks, for dispersal by animals (OGTR, 2024a). Dispersal of viable canola seed via endozoochory (consumption and excretion of seed) by birds only occurs at very low levels (Twigg et al., 2008; Woodgate et al., 2011). Canola seeds could be transported short distances by hoarding animals, such as ants and mice. The applicant proposes that monitoring zones around trial sites would be inspected for volunteers.

105. Canola seeds lack specialised structures that would assist their dispersal by wind (OGTR, 2024a). However, the GM canola may be windrowed prior to harvesting, and under strong wind conditions plant material could disperse outside trial sites. The applicant proposes that monitoring zones around trial sites would be inspected for volunteers.

106. GM canola seeds could be dispersed by water during flooding or heavy runoff, although seeds are unlikely to remain viable after prolonged exposure to water (OGTR, 2024a). To minimise the potential for seed dispersal during flooding, the applicant proposes to locate the trial in a site which is not prone to flooding.

107. During harvest of the GM canola, a small percentage of the GM seeds are expected to be lost and to remain on the trial sites. Persistence of GMOs at the trial sites after the field experiment is finished could occur if seeds in the seed bank were dormant. Canola generally does not exhibit primary dormancy, but secondary dormancy has been described (OGTR, 2024a). A study carried out in western Canada revealed that secondary seed dormancy prolonged persistence of volunteer canola plants (Gulden et al., 2003). Persisting canola seed banks have been shown to significantly contribute to the dynamics of feral canola populations (Pivard et al., 2008). A long-term monitoring study in Germany detected GM canola volunteers in arable fields for up to 15 years after the field trial concluded, but did not detect spatial dispersion (Belter, 2016). In Australia, volunteers can be found for up to 3 years after growing canola due to persistence in seed banks, though the majority of volunteer seedlings emerge the year following a canola crop (AOF, 2019).

108. To minimise persistence of GM seeds on the trial sites, the applicant proposes to promote seed germination by light post-harvest tillage and irrigation. During a post-harvest monitoring period, the applicant would regularly inspect the trial sites and destroy any canola volunteers, until volunteers cease to emerge. The suitability of the proposed controls to manage GM seed dispersal and persistence is discussed in detail in Chapter 3, Section 3.1. These control measures are expected to minimise persistence of viable GM canola seeds on the trial sites.

109. If GM canola seeds were dispersed outside trial limits, it is unlikely that they would establish ongoing volunteer populations. Even in environments without active weed management, volunteer canola populations along transportation routes rely on recurrent spillages to persist (Yoshimura et al., 2006) and volunteer canola dispersed into natural areas was reported to rapidly become extinct (Busi and Powles, 2016). It is uncertain whether the introduced genetic modifications may affect the overall ability of volunteers to survive in the environment, as abiotic factors, usually temperature and water availability, are the main factors restricting the potential distribution of canola, and some of the genetic modifications could confer increased abiotic stress tolerance in the GMOs (see Chapter 1, Section 4.1). However, the degree of improvement for the various abiotic stress tolerances is unknown. This is an area of uncertainty for this risk assessment.

110. In agricultural areas of Australia where canola is grown, volunteer populations are controlled by a range of weed management measures. Effective methods for control of canola volunteers include grazing, mowing, cultivation and application of a range of knockdown or selective herbicides (AOF, 2019). The introduced genetic modifications are not expected to affect the susceptibility of GM volunteers to standard weed management measures. Although some of the canola lines will contain the *bar* gene and be tolerant to glufosinate herbicide, as discussed in Chapter 1 Section 5.3, glufosinate herbicide is not routinely used for controlling volunteer canola (AOF, 2019).

111. In preliminary glasshouse trials, the applicant states that the only trait observed in the GMOs has been increased photosynthesis.

2.4.2.3 Potential harm

112. If the GM canola entered the Australian environment, the potential harms are adverse health effects to people, increased toxicity to desirable animals, and reduced establishment or yield of desirable plants.

113. As discussed in risk scenario 1, no substantive risk was identified for increased adverse effects of the GM canola for people or increased toxicity to desirable animals.

114. The genetic modifications for increased photosynthesis and photorespiration may confer increased yield (seed number) and tolerance to certain abiotic stressors (Chapter 1, Sections 4.1 and 4.4). Therefore, the GM canola volunteers could have increased persistence in the environment under certain abiotic stress conditions compared to non-GM canola volunteers.

115. Populations of volunteer GM canola could reduce establishment or yield of desirable plants. GM volunteers could directly compete with agricultural crops, pastures or native vegetation. GM volunteers could also reduce the yield of commercial canola crops by providing a reservoir for pathogens, such as the important fungal diseases blackleg and stem rot (see Chapter 1, Section 5.2). No information could be found to suggest that the introduced genetic modifications are likely to make the GM canola more susceptible to pathogens.

116. Canola is considered a less competitive crop species than wheat or barley (GRDC, 2011), which are the main crops grown in South Australia (ABARES, 2024). All domesticated crop plant species are expected to be poor competitors with pasture species or established native vegetation. Therefore, canola volunteers have limited ability to compete with desirable plants. As discussed in Chapter 1, Section 4, the applicant expects that the GM canola plants might have better yield, including better agronomic performance under drought conditions, and plans to examine these traits in the field. However, even if the GM canola plants display increased seed numbers and abiotic stress tolerance compared to non-GM canola, in order to increase weediness, this characteristic would need to be coupled with other mechanisms that increase spread and persistence in the environment, through changes in dispersal, establishment and survival. While dispersal characteristics are not reasonably expected to be altered by the introduced genetic modifications, it is uncertain if establishment and survival characteristics may be altered. It is therefore uncertain whether the introduced genetic modifications would increase the overall competitiveness of the GM plants. No information could be found to suggest that the introduced genetic modifications would enable the GM canola to produce allelopathic substances which would negatively affect plant establishment around them.

2.4.2.4 Conclusion

117. Risk scenario 2 is not identified as a substantive risk because the proposed limits and controls of the field trial would minimise dispersal and persistence of the GM canola, GM canola is susceptible to standard weed management measures, and the genetic modifications are not expected to increase adverse effects to people or toxicity to animals. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

Risk source	The introduced genetic modifications
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment ⬇
Potential harm	Adverse health effects in people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants

2.4.3.1 Risk source

118. The source of potential harm for this postulated risk scenario is the introduced genetic modifications in the GM canola plants.

2.4.3.2 Causal pathway

119. The GM canola would be grown at the trial sites. Pollen from the GM plants could be transported out of the trial sites by wind or insect vectors and fertilise sexually compatible plants. Hybrid seeds containing the introduced genes could be harvested by farmers or could grow as volunteers.

120. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome.

121. Canola is primarily self-pollinating, but approximately 30% of seeds are produced by cross pollination. Outcrossing decreases rapidly with distance, with the majority of cross-pollination occurring over distances less than 10 m (OGTR, 2024a). The introduced genetic modifications are not expected to affect the pollen dispersal characteristics of the GM canola. In the case of *AtPip1;3*, it is uncertain if overexpression will affect the pollination process through pollen hydration (Chapter 1, Section 4.1), however this would only be relevant if the genetic modification is in the female parent in a cross.

122. The GM canola could outcross with nearby canola crops or volunteers, if there is synchronicity of flowering. As discussed in Chapter 1, Section 5.4, canola can also occasionally hybridise with the related horticultural crops *B. juncea, B. oleracea* and *B. rapa* and the related weeds *H. incana, R. raphanistrum* and *S. arvensis*.

123. 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). During flowering of the GM plants, each planting area would be either surrounded by a pollen trap or the GMOs covered in insect-proof tents, and then surrounded by monitoring zone and isolation zone. In addition, any GM volunteers growing on the trial sites after harvest would be destroyed prior to flowering. The suitability of the proposed controls to manage pollen flow is discussed in detail in Chapter 3, Section 3.1. These controls are expected to minimise pollen flow from the GM canola to sexually compatible non-GM plants outside the trial sites.

124. If pollen from GM plants fertilised plants in a commercial canola crop, hybrid GM seeds could be harvested for human food and animal feed, or be replanted in a crop. However, even in the complete absence of measures to restrict pollen flow, outcrossing rates between neighbouring commercial canola fields are less than 0.1% under Australian conditions (Rieger et al., 2002). Therefore, the planting seed

described in this risk pathway could only contain a very low proportion of hybrid GM seed, so people and desirable animals could only be exposed to very low levels of the hybrid GMOs.

125. If pollen from GM plants fertilised sexually compatible plants growing as crops, volunteers or weeds, the hybrid GM seeds could grow as volunteers. Populations of hybrid GM volunteers could be consumed by desirable animals or could reduce the establishment or yield of desirable plants.

2.4.3.3 Potential harm

126. As discussed in risk scenario 1, no substantive risk was identified for adverse health effects of the GM canola for people or toxicity to desirable animals than non-GM canola. Similarly, in hybrids between the GM plants and sexually compatible plants, the same considerations as discussed in Risk Scenario 1 would apply.

127. As discussed in risk scenario 2, while dispersal characteristics are not reasonably expected to be altered by the introduced genetic modifications, it is uncertain if establishment and survival characteristics may be altered. It is therefore uncertain whether the introduced genetic modifications would increase in overall competitiveness when compared to non-GM canola. Similarly, in hybrids between the GM plants and sexually compatible plants, it is uncertain whether the genetic modifications would contribute to the overall competitiveness.

2.4.3.4 Conclusion

128. Risk scenario 3 is not identified as a substantive risk because the controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites. GM hybrids are not likely to differ from the GM canola, for which Risk scenarios 1 and 2 did not identify adverse health effects in people, toxicity in animals or weediness as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

129. 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 Risk Analysis Framework document.

130. 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.

131. 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.

132. For DIR 212, uncertainty is noted particularly in relation to:

- the potential for increased toxicity of the GM canola to people or animals
- the potential for increased allergenicity of the GM canola to people
- the potential for immunogenic reactions to the Myc tag
- the potential for the genetic modifications to increase plant persistence and survival, or overall competitiveness.

133. 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.

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

Section 4 Risk evaluation

135. 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.

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

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

137. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- none of the GM plant material would enter human food or animal feed
- no adverse health effects were observed in people handling the GM plants in glasshouse
- limits on the size and duration of the proposed release
- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM canola plants and their genetic material.

138. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM canola plants into the environment are considered to be negligible. The Risk Analysis Framework (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no additional controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

139. 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.

140. 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.

141. All licences are subject to 3 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.

142. 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

143. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM canola. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

144. 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 licence.

3.1 Limits and controls on the release

145. Sections 2.1 and 2.2 of Chapter 1 list the limits and controls proposed by the applicant. Many of these are discussed in the three risk scenarios considered in Chapter 2. The appropriateness of these limits and controls is considered further in the following sections.

3.1.1 Consideration of the limits proposed by the applicant

146. The applicant proposes that the field trial would take place between April 2025 and January 2030. This would limit the duration of the trial to less than 5 years. As a decision on the licence has not been made by April 2025, the licence specifies that dealings may begin 'from issue of licence' rather than April 2025. The GM canola would be grown at one site, with a planting area of up to 2 ha per year. The applicant has stated that more than one planting area may be used at the site, however, the total planting area will be no more than 2 ha per year. The small size and short duration of the trial would restrict the exposure of people and desirable animals to the GMOs and reduce the potential for dispersal from the trial site and for outcrossing to compatible species outside trial limits (Risk scenarios 1-3).

147. The applicant proposes that only authorised and trained people would be permitted to deal with the GMOs. Standard licence conditions included in the licence state that only people authorised by the licence holder are covered by the licence and permitted to deal with the GMOs. In addition, the licence holder must inform all people dealing with the GMOs of relevant licence conditions. These measures would ensure that the field trial is conducted in accordance with the specified limits and controls (important for all risk scenarios).

148. The licence limits the plants that can be intentionally grown in the planting area to the GMOs, non-GM canola, and any plants approved in writing by the Regulator. The applicant proposes to treat any non-GM canola plants grown in planting areas or pollen traps like the GMOs. These non-GM plants may be mingled with or fertilised by the GM plants, and it is necessary to handle the non-GM plants in the same way as the GMOs to manage the dispersal or persistence of GM seed. This measure is therefore included in the licence.

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

149. The applicant proposes that GM plants or products from the GM plants would not be used in human food or animal feed. The licence requires that GM plant material must not be used as food for humans or feed for animals. This condition would maintain the risk context by restricting the exposure of people and desirable animals to the GM canola by consumption (Risk scenario 1).

150. The applicant has proposed that the site be surrounded by livestock proof fencing. This has not been mandated in the licence, but is one method of preventing livestock from accessing and consuming the GMOs.

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

151. The applicant proposed 2 different options to control pollen flow from the trial sites while the GMOs are flowering.

152. The first option proposed by the applicant to control pollen flow is to surround the planting area with a 15 m pollen trap of non-GM canola plants, a 35 m monitoring zone and a 390 m isolation zone (Chapter 1, Figure 2b). The GM canola plants would not be planted at a trial site if any plants that are sexually compatible with canola were being grown in the monitoring or isolation zones. The pollen trap would be managed to flower at the same time as the GM canola plants. Pollen trap plants may provide sufficient forage for incoming pollinating insects so that they do not visit the GM plants, and any insects that reach the GM plants are expected to deposit most GM pollen on pollen trap plants while exiting the trial site. Pollen trap plants may also absorb some pollen dispersed by wind. As a pollen trap serves the same function as an unplanted monitoring zone (Hüsken and Dietz-Pfeilstetter, 2007), it is considered unnecessary to surround the trial site with both a pollen trap and a full-sized 50 m monitoring zone. Therefore, the licence condition requires a 15 m pollen trap and a 35 m monitoring zone. The applicant has proposed an isolation zone of 390 m. As discussed in previous RARMPs for GM canola field trials (e.g. DIR 164, DIR 188 and DIR 205), the use of a pollen trap justifies a reduced isolation zone of 350 m and thus an overall distance of 400 m between the GMOs and any crops of related species. If multiple planting areas are established at the site, they may be surrounded

by a single pollen trap only if the pollen trap is expected to flower at the same time as the GMOs in all planting areas (i.e. the planting areas are established at a similar time). Although it is considered that a pollen trap is most effective when it immediately surrounds a planting area, given that the site is relatively small (2 ha), a single pollen trap surrounding multiple planting areas is still considered effective at minimising GM pollen transfer by pollinating insects.

153. The other option proposed by the applicant to control pollen flow is to cover the planting area with an insect proof tent, and to surround the planting area with a 10 m monitoring zone and a 390 m isolation zone (Chapter 1, Figure 2a). The tents would be in place from at least 7 days before flowering until the GMOs complete flowering, and would be inspected for damage fortnightly and after any extreme weather event. The tents are expected to prevent all insect mediated pollen flow. The tents may also reduce wind-mediated pollen flow as it is expected that surrounding the GMOs with a tent would lessen air flow across the GMOs. Therefore, the use of an insect-proof tent justifies a reduced monitoring zone of 10 m and an isolation zone of 390 m. This option was proposed for previous GM canola field trials and was considered an effective means of restricting pollen flow from canola (e.g. DIR 188).

154. Considering that there may be circumstances when a pollen trap may fail to function (e.g. failure to grow to a required density, or to form a continuous barrier, or to flower at the same time as the GM plants) or the insect proof tent fails, the licence also includes an alternate option to control pollen flow by surrounding the planting area with a 50 m monitoring zone and a 950 m isolation zone, a combined isolation distance of 1 km from related species. This option was proposed for previous GM canola field trials and was considered an effective means of restricting pollen flow from canola (e.g. DIR 164 and DIR 188).

155. If there is a mixture of tented and non-tented plots within a planting area, either the use of a pollen trap (plus a 350 m isolation zone) or a 950 m isolation zone, in combination with a monitoring zone as required, is considered suitable for pollen flow management.

156. The applicant proposes that more than one planting area at a time could be established at the trial site. Under the conditions in the licence, where more than one planting area is established at a field trial site, all planting areas must be inside a shared monitoring zone of 10, 35 or 50 m (depending on the pollen control option) surrounding the whole trial site. Any land between planting areas is also considered part of the monitoring zone and would need to be maintained and inspected as such.

157. For all 3 of these options, licence conditions require that the monitoring zone would be inspected at least once every 35 days from 14 days prior to flowering of the GMOs until the GMOs are harvested, to ensure that it is free from any sexually compatible plants. The isolation zone would be inspected at least once every 35 days from 14 days prior to flowering of the GMOs until the GMOs complete flowering, to ensure that it is free from intentionally planted sexually compatible plants.

158. The proposed measures to control pollen flow would minimise outcrossing between the GMOs grown on the trial sites and sexually compatible plants growing outside the trial sites (Risk scenario 3).

159. After harvest of the trial sites, the applicant proposes to monitor the sites for volunteers (see Section 3.1.5). The applicant proposes to inspect at least once every 2 months, in order to find and destroy volunteers before they flower. The licence has specified that these post-harvest inspections must be conducted at least once every 35 days, ensuring that volunteers are detected and destroyed before flowering. These post-harvest inspections are required in the licences for other GM canola field trials and are considered an effective means of restricting pollen flow from GM canola volunteers to plants outside the trial sites (e.g. DIR 164 and DIR 188).

3.1.4 Consideration of proposed controls regarding dispersal of the GMOs

160. The applicant proposes that any equipment used with the GMOs would be cleaned as soon as practicable and before use for any other purpose, to avoid movement of viable plant material together with equipment. The applicant would contain the GM seeds during transport and storage in

accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. The licence also includes a condition that the GM canola must be harvested separately from other crops, to avoid inadvertent seed mixing. These measures would minimise human-mediated dispersal of GM seeds (Risk scenario 2).

161. The applicant proposes to not locate the trial site in a flood-prone area in order to minimise the chance of viable plant material being washed away from the sites. This has been included as a condition in the licence. The licence also requires the trial sites to be located at least 50 m away from waterways as a standard licence condition for canola licences and that any extreme weather events must be reported to the Regulator. These measures would minimise dispersal of GM seeds by flooding (Risk scenario 2).

162. GM canola seeds could be dispersed short distances from the trial sites during sowing, windrowing or harvest activities; by pod shattering, by seed-hoarding behaviours of animals such as ants or rodents; or by strong winds or runoff after heavy rain. 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 growing from dispersed GM seeds during this period would be detected and destroyed. Specific conditions to minimise dispersal of GM plant material from windrowed plants by wind or rain have also been included in the licence. The applicant also proposes to inspect the monitoring zones after harvest to destroy any volunteers growing from dispersed GM seeds. As the short-distance seed dispersal mechanisms listed above are unlikely to transport seeds further than 10 m from the trial sites, the licence only requires post-harvest inspections of the innermost 10 m of the monitoring zone.

163. The 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 2).

3.1.5 Consideration of proposed controls regarding persistence of the GMOs

164. After harvest of each trial site, the applicant proposes to destroy GMOs not required for further evaluation or future trials. This would involve both cleaning the trial site within 14 days after harvest in a manner that destroys any surviving GMOs, and destroying any harvested GM seed that is not required for experimentation or future planting.

165. The applicant has proposed that the GMOs would be destroyed by herbicide application, root cutting and mulching, uprooting, burning/incineration, autoclaving, seed grinding, or seed burial to a depth of at least 1 m. These methods are considered effective for rendering canola plants and/or seeds non-viable, and have been included in the licence. To ensure the effectiveness of destruction by seed burial, a licence condition specifies how this must be carried out, including a requirement that seeds must be sufficiently irrigated at time of burial to encourage decomposition.

166. To deal with the case of failed crops that are not harvested, licence conditions require that GMOs must be harvested or destroyed within 9 months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been harvested and cleaned.

167. The applicant proposes to monitor trial sites after harvest and destroy any volunteers that emerge. The areas that would be monitored are the planting area, the pollen trap, and other areas where GM seed may have dispersed, as discussed in Section 3.1.4. The frequency of inspections of the trial sites are discussed in Section 3.1.3. The proposed duration of monitoring by the applicant is at least 24 months, and until the site is free of volunteer canola plants for at least 12 months. In minimum-tillage Australian farms, the canola seedbank is reported to decline rapidly, and no viable seed was recovered from the seedbank by 2.5 years after canola harvest (Baker and Preston, 2008). Similarly, OGTR monitoring data for 9 GM canola trial sites planted in 2015 found that in most sites no

canola volunteers emerged more than 1 year after harvest and no volunteers emerged at any site more than 2.5 years after harvest. Therefore, the proposed duration for monitoring of least 24 months, and until the site is free of volunteer canola plants for at least 12 months is considered to be appropriate. This monitoring duration was also required for previous GM canola field trials and is considered effective for managing persistence of canola seed (e.g. DIR 164, DIR 188 and DIR 205).

168. The applicant proposes shallow cultivation of the trial sites to encourage seed germination. The licence conditions require that tillage depth would be no greater than 5 cm, to avoid deep burial of seed that could induce dormancy. The first tillage would occur within 60 days after harvest and the final tillage would occur during the volunteer-free period prior to sign-off. To ensure that the final tillage produces conditions that are conducive to germination of volunteers, the licence requires this tillage to be followed by specified levels of rainfall or irrigation that provide sufficient moisture to the seedbank.

169. While the applicant has not currently proposed to plant post-harvest crops during the post-harvest monitoring period for each trial site, licence conditions are included to allow planting of plant crops permitted on GM brassica trial sites by the Regulator's Policy on Post-Harvest Crops. This will help to maintain the area in a manner appropriate to allow identification of volunteers.

170. The combination of control measures described in this section would minimise the persistence of GM seeds leading to establishment of GM volunteer populations in the environment (Risk scenario 2).

3.1.6 Consideration of managing multiple field trials at the same trial site

171. As outlined in Chapter 1 Section 5.3, the applicant may use the single field trial site for multiple future GM field trials and has requested the licence allow concurrent planting of GMOs from multiple field trial licences, where the other licences have compatible licence conditions, and also to overplant areas that are in post-harvest monitoring. However, an assessment can only be done on GM plants that are currently allowed to be planted at the field trial site. Any future GMOs proposed for the site would be assessed in a new RARMP.

172. In recent years, the field trial site has been used to plant GM wheat and barley under licences DIR 186 and DIR 201. As the field trial site is limited in area, the applicant has requested to plant GMOs from this licence over areas that are undergoing post-harvest monitoring for DIR-201 and vice versa. As canola is not sexually compatible with wheat or barley, consideration of gene transfer between the species does not apply, however the ability to easily detect and destroy volunteers post-harvest is critical. The applicant has reasoned that any GM wheat and barley volunteers could be easily identified amongst the GM canola due to their different visual appearance. This rationale also applies for GM canola volunteers amongst GM wheat and barley. The licence specifies that after the planting area has been cleaned, no plants may be intentionally grown in the area unless the area is planted as a new planting area, the plants are listed as post-harvest crops permitted for GM Brassica field trial sites in the OGTR Policy on Post Harvest Crops or the plants are agreed to in writing by the Regulator. The OGTR policy specifies that non-GM cereals (not including corn/maize or sorghum) are permitted as post-harvest crops on GM brassica field sites due to distinct morphology and relative ease of volunteer detection. As such, the licence states that GMOs from DIR 201 can be planted in a DIR 212 area that is in post-harvest monitoring. If the applicant wishes to overplant GMOs from DIR 212 over an area that is undergoing post-harvest monitoring for DIR 201, they must seek approval from the Regulator as the DIR 201 licence does not currently allow this.

173. As discussed in Section 3.1.1, the licence limits the plants that can be intentionally grown in the planting area to the GMOs, non-GM canola, and any plants approved in writing by the Regulator. Volunteers are defined as GM canola plants that have not been intentionally grown. Therefore, if volunteer GM wheat and barley were to emerge in a planting area that has been intentionally planted with GM canola, this would not be in contravention of the GM canola licence, nor the post-harvest

requirements of the GM wheat and barley licence as long as the volunteers can be identified and destroyed before flowering.

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

174. A number of licence conditions are imposed 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 issue of licence to January 2030
- limit the size of the release to a maximum of one site per year, with a maximum area of 2 ha per year
- limit the location of the release to the nominated local government area of Light Regional Council (South Australia)
- not allow GM plant material to be used in human food or animal feed
- control pollen flow from the trial sites using one of the following options:
    a. surround the planting area with a pollen trap of 15 m, a monitoring zone of 35 m and an isolation zone of a further 350 m, or
    b. cover the planting area with an insect proof tent, and surround the planting area with a monitoring zone of 10 m and an isolation zone of a further 390 m
    c. surround the planting area with a monitoring zone of 50 m and an isolation zone of a further 950 m
- treat any non-GM canola grown in planting areas or pollen traps like the GMOs
- harvest the GM canola separately from other crops
- 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 trial sites at least 50 m from any natural waterways
- destroy all GMOs not required for further evaluation or future trials
- conduct post-harvest monitoring of the planting area and other areas where GM seeds may have been dispersed and destroy any volunteers that emerge
- post-harvest monitoring of the trial sites at least once every 35 days for at least 24 months after harvest and until the site is free of volunteers for at least 12 consecutive months
- conduct post-harvest tillage and irrigation of trial sites to encourage seed germination.

3.2 Other risk management considerations

175. 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 Application suitability

176. 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 include:

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

177. Licence conditions include a requirement for The University of Adelaide to inform the Regulator of any information that would affect their suitability.

178. In addition, any applicant organisation must have access to an Institutional Biosafety Committee (IBC) and be an accredited organisation under the Act.

3.2.2 Contingency plan

179. The University of Adelaide is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM canola outside permitted areas.

180. Before planting the GMOs, The University of Adelaide is also required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

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

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

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

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

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

3.2.5 Monitoring for compliance

184. 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.

185. 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.

186. 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 the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

187. Additional information has been identified that may be required to assess an application for a commercial release of the GM canola, or to justify a reduction in limits and controls.

188. This includes:

- molecular and biochemical characterisation of the GM canola lines, particularly with respect to potential for increased toxicity, allergenicity and immunogenicity
- phenotypic characterisation of the GM canola lines, particularly with respect to abiotic stress tolerance, changes in flowering and seed production or other characteristics that may contribute to increased weediness.

Section 5 Conclusions of the RARMP

189. The risk assessment concludes that the proposed limited and controlled release of GM canola poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

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

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Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received several submissions from prescribed experts, agencies and authorities [1] on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Submission	Summary of issues raised	Comment
1	Does not have any advice or comments on the RARMP for DIR 212.	Noted.
2	Are satisfied that there is no risk to the human population or environment.	Noted.
3	Noted that the trial proposed is similar to many canola trials and approvals already carried out and that, based on the information contained and the risk mitigation processes, the risk of the GMO being released into the food chain or to agricultural animals is negligible.	Noted.
4	Agreed that the proposed release poses negligible risk to human health and safety and the environment as a result of gene technology and that the proposed limits and controls will effectively minimise exposure to the GMOs. Noted the uncertainties documented in the RARMP and that there may be requirement for additional data and information to address these uncertainties where future applications involve reduced limits and controls, larger scale trials or commercial release of the GMO. Noted that licence conditions: • limit the size, location and duration of the release • restrict pollen flow, seed dispersal, harvesting and persistence at the trial sites • prohibit the use of GM plant material in human food or animal feed • address transport, storage and disposal of the GMOs.	Noted.
5	• Agrees that the risk assessment identifies all plausible risk scenarios by which the proposed release could give rise to risks relating to the health and safety of people or the environment. • Agrees that the limits and controls proposed in the draft licence are appropriate for the field trial. • Agrees with the overall conclusion of the RARMP. • Advises that the Regulator should consider clarifying the degree of hydration with Pip1;3 overexpression in pollen.	The effect that overexpression of Pip1;3 may have on pollen characteristics, including pollen hydration, is noted as an uncertainty in the RARMP. Further discussion about the role of other aquaporins in pollen hydration, specifically in the female parent in a cross, has been added to Chapter 1 Section 4.1.3 of the RARMP.

[1] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

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

The Regulator received 2 submissions from members of the public on the consultation RARMP. The issues raised in the submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Comment
1	Totally opposes all genetically modified methods as they are unnatural and artificial and puts profits before people's health.	Noted.
2	Questioned whether heavy winds carry pollen past the 400-450m zone and, therefore, whether a 400-450m monitoring/isolation zone is sufficient. Cited a literature reference doi:10.1016/j.ecolmodel.2009.01.013 (Hoyle & Cresswell, 2009).	As detailed in Chapter 3 Section 3.1.3 of the RARMP, and the licence, 3 site layout options have been considered appropriate for managing gene flow resulting from GM pollen dispersal outside the site. In the absence of other measures, a combined isolation distance of 1 km from related species is considered to be appropriate. If either a pollen trap or insect proof tents are used, a combined isolation distance of 400 m is considered to be appropriate. In windy conditions, pollen trap plants may absorb some pollen dispersed by wind and insect proof tents may also reduce wind-mediated pollen flow through reducing air flow across the GMOs. The combination of these controls is considered sufficient to manage pollen flow with a reduced 400 m combined isolation distance. In the event of extreme weather events, the licence requires the licence holder to notify the Regulator and to have a contingency plan.
	Cited page 8 of the RARMP which states that "The applicant has advised they have insufficient data at this time to determine if the GM canola has increased seed numbers." and questioned whether this data should be obtained before proceeding with a field trial.	Licence application DIR 212 is for a small-scale field trial. As discussed in Section 3 of Chapter 2 of the RARMP, field trials are part of the process of gathering data about a GMO. Therefore, at the point when a field trial application is evaluated, generally the applicant does not have complete data about the GMO. Field trials are permitted under stringent limits and controls that manage spread and persistence of the GMO.