



Australian Government

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

Risk Assessment and Risk Management Plan for

DIR 201

Limited and controlled release of wheat and
barley genetically modified for yield
enhancement

Applicant: The University of Adelaide

April 2024

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 201

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 risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Project Title	Limited and controlled release of wheat and barley genetically modified for yield enhancement
Parent organism	Wheat (<i>Triticum aestivum</i> L.) and barley (<i>Hordeum vulgare</i> L.)
Genetic modifications	
Introduced genes and modified traits	<p><u>Wheat:</u></p> <ul style="list-style-type: none"> • Expression of three genes involved in yield enhancement (expressed both individually and in combination) • Expression of five genes involved in yield enhancement and water use efficiency (expressed individually) • Knockout of two endogenous genes involved in yield enhancement • Expression of three selectable marker genes and one reporter gene (expressed both individually and in combination) <p><u>Barley:</u></p> <ul style="list-style-type: none"> • Knockout of eight endogenous genes involved in yield, architecture, and nutrient use efficiency • Expression of one selectable marker gene (expressed individually)
Genetic modification method	Biolistic or <i>Agrobacterium</i> -mediated transformation; gene editing
Number of lines	Up to 103 lines ¹ in total
Principal purpose	To assess agronomic performance of the GM wheat and barley lines under field conditions
Proposed limits	
Proposed use of GM plants	No use in commercial food or animal feed proposed
Proposed location/s	The trial is proposed to take place at one site in South Australia (Light Regional Council)
Proposed release size	Up to a total of 2 ha per year

¹ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

Proposed period of release	From May 2024 to January 2029
<i>Previous releases</i>	<ul style="list-style-type: none"> Wheat lines containing all or some of the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. Wheat lines containing the five genes involved in yield enhancement and water use efficiency have previously been released under DIR 186.

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 risks are considered.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM wheat and barley plants. Potential harms associated with these pathways included toxicity or allergenicity 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 the small trial size and not using GM plant material in 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

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 evaluates and treats identified risks and considers general risk management measures. 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 the GMOs at the end of the trial and to conduct post-harvest monitoring at the trial site to ensure the GMOs are destroyed. In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
CRISPR	Clustered regularly interspaced short palindromic repeats
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GFP	Green fluorescent protein
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
HPH/HPT	Hygromycin phosphotransferase
IBC	Institutional Biosafety Committee
LBO	LATERAL BRANCHING OXIDOREDUCTASE
MAP	Mitogen activated protein
MAPKKK	Mitogen activated protein kinase kinase kinase
MAX1	MORE AUXILLARY GROWTH 1
NHEJ	Non-homologous end joining
NLRD	Notifiable Low Risk Dealings
NPTII	Neomycin phosphotransferase II
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin N-acetyltransferase
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RFP	Red fluorescent protein
SA	South Australia
SARDI	South Australian Research and Development Institute
SDN	Site-directed nuclease
sgRNA	Single guide ribonucleic acid
<i>spp.</i>	species
TGA	Therapeutic Goods Administration
the Act	<i>Gene Technology Act 2000</i>

Chapter 1 Risk assessment context

Section 1 Background

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

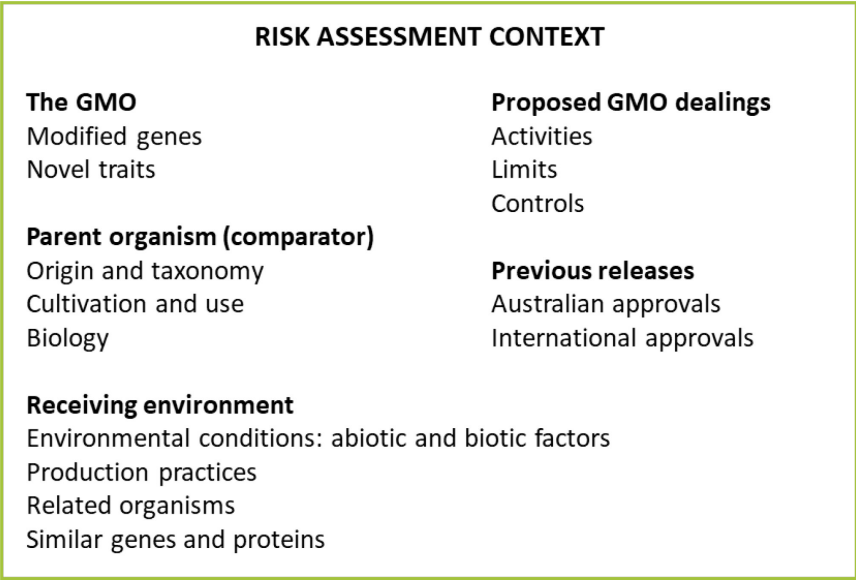


Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework.

- 6. 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. Three public submissions were received and their consideration is summarised in Appendix B.

1.1 Interface with other regulatory schemes

7. 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, the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme 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.

8. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies have not be re-assessed by the Regulator.

Section 2 The proposed dealings

9. The University of Adelaide proposes to release up to 103 lines of wheat and barley genetically modified (GM) for yield enhancement.

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

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- growing or culturing the GMOs
- transporting the GMOs
- disposing of the GMOs

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

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

12. The release is proposed to take place at one site in South Australia (Light Regional Council). The release is proposed to take place between May 2024 and January 2029, on a total of 2 ha in any year.

13. Only trained and authorised staff would be permitted to deal with the GM wheat and barley.

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

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

- locating the proposed trial site at least 50 m away from the nearest natural waterway
- surrounding each planting area with a 2 m buffer zone, within which plant growth and rodent activity will be controlled
- surrounding the buffer zones with a 50 m monitoring zone, in which the 10 m adjacent to the buffer zone will have plant growth controlled

- surrounding the monitoring zone with a 140 m isolation zone in which no sexually compatible crops will be grown during the cultivation of GM wheat and barley
- only permitting trained and authorised staff to access the site
- restricting access by surrounding the trial site with a fence to a height of 1.5 m, with lockable gates
- treating non-GM plants used in the trial as if they were GM
- inspecting all equipment for GM plant material, and cleaning as required prior to equipment leaving the site or being used for any other purpose
- transporting and storing GM plant material in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all plant material from the trial not required for testing or future trials
- post-harvest monitoring of the trial site at least once every 35 days for 2 years, with any wheat or barley volunteers or related species destroyed prior to flowering
- promoting germination of any residual seed post-harvest by tillage and irrigation.

15. **Figure 2** shows the layout proposed by the applicant, including some of the proposed controls. The figure shows a trial site with multiple planting areas (with associated buffer zones). The trial site would be surrounded by a monitoring zone and an isolation zone.

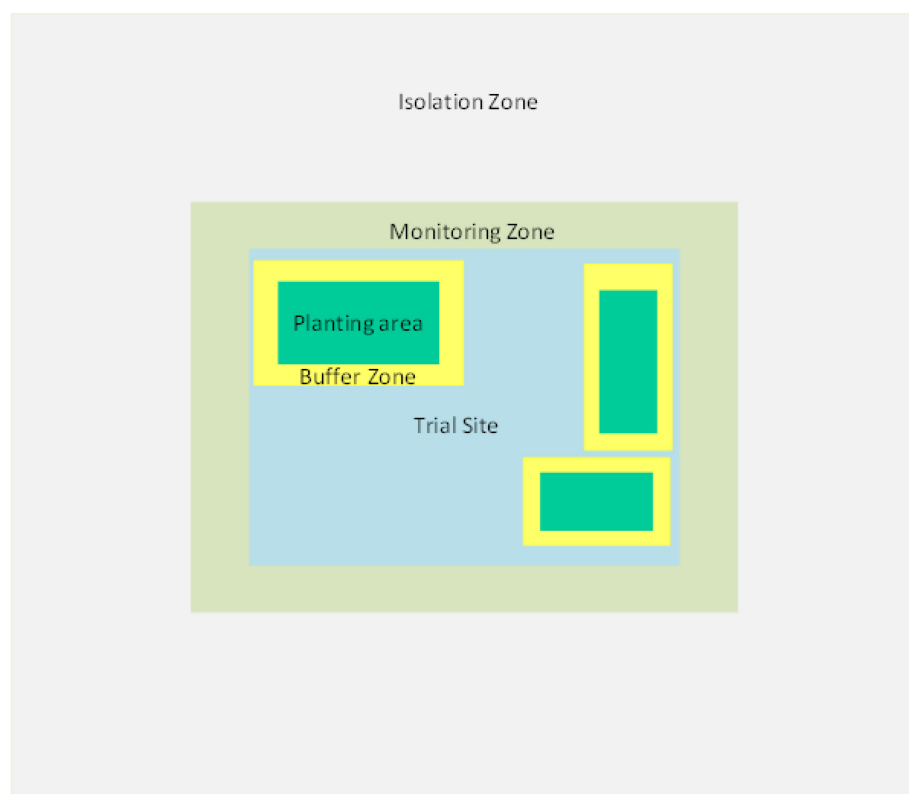


Figure 2. Schematic diagram (not to scale) of trial setup proposed by applicant: Trial site with multiple planting areas.

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

Section 3 The parent organisms

17. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), which are exotic to Australia. Commercial wheat and barley are cultivated in the 'wheat belt' from south-eastern Queensland through New South Wales (NSW), Victoria, Tasmania, southern South Australia (SA) and southern Western Australia (WA).

18. Detailed information about the parent organisms is contained in the reference documents produced to inform the risk analysis process for licence applications involving GM crops: *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR, 2021a). Both documents are available from the [Resources page](#) on the OGTR website. Baseline information from these documents will be used and referred to throughout the RARMP.

19. There are several biotic and abiotic factors, which limit the growth and survival of wheat and barley, with both species grown in similar areas and conditions. Water stress (drought or waterlogging), heat and cold stress as well as nutrient deficiencies are limiting factors for both species. However, barley is generally regarded as being better adapted to salinity and to drought stress than wheat. Both are affected by pests and diseases.

20. Neither wheat nor barley is regarded as a weed of national significance ([National Weeds List](#), accessed 11 December 2023), and both are regarded as naturalised non-native species present in all Australian states and territories with the exception of the Northern Territory (Groves et al., 2003).

21. Weed risk assessments are included in the biology documents for wheat and barley. Wheat possesses few attributes which would make it weedy and this is supported by the observation that there are few weedy populations of wheat in the Australian environment. Cultivated barley is highly domesticated, so has reduced fitness outside of agricultural environments. However, it does have a medium weed rating in parts of WA and in Victoria as it is more tolerant to drought and salinity. However, it has no weed rating in the other States where it occurs and does not cause major or significant harm anywhere in Australia.

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

4.1 Introduction to the GMOs

22. The applicant proposes to release up to 103 lines of wheat and barley lines genetically modified for yield enhancement. Some of the lines contain introduced genes, while other lines have had the function of endogenous genes ‘knocked out’ using SDN-1 gene editing techniques. The introduced or knocked-out genes are divided into four groups based on how they alter yield (Table 1). Field trials of GM wheat containing the introduced genes for direct yield enhancement (Group 1) have previously been evaluated and licensed under DIR 102, DIR 128, DIR 152 and DIR 186. A field trial of GM wheat containing the introduced genes for yield enhancement via water use efficiency (Group 2) has also been evaluated and licensed previously under DIR 186. The applicant has indicated that the GM wheat lines with Group 1 and 2 genes proposed for release include some of the same plants released under DIR 152 and DIR 186.

Table 1. Groups of introduced or knockout genes in the GM wheat and barley

Group	Altered trait	Parent organism	Type of genetic modification	Previous DIRs
1	Direct yield enhancement	Wheat	Gene introduction	DIRs 102, 128, 152, & 186
2	Yield enhancement via water use efficiency	Wheat	Gene introduction	DIR 186
3	Yield enhancement via altered spikelet development and flowering time	Wheat	Gene knockout	-
4	Yield enhancement via altered plant architecture and nutrient use efficiency	Barley	Gene knockout	-

23. The genes *AtAVP1*, *OsNAS2*, *OsPSTOL1*, *TaMUTE*, *TaYDA1*, *TaYDA2*, *TaOST1* or *TaSLAC1* will be introduced into wheat (Table 2). For the lines containing the *AtAVP1*, *OsNAS2*, *OsPSTOL1* genes (Group 1), the applicant intends to release lines containing one of these introduced genes, or combinations of two or all three of the genes.

24. The knockout GM wheat and barley would have small insertions or deletions in endogenous wheat or barley genes making these genetic sequences non-functional (Table 2). These knockout lines have been generated using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing. The *Cas9* gene and sgRNAs used in CRISPR/Cas9 gene editing may also be included in these lines.

Table 2. List of introduced or knocked out genes in the GM wheat and barley

Group	Element	Source organism	Function
1	<i>AtAVP1</i>	<i>Arabidopsis thaliana</i>	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance
	<i>OsNas2</i>	<i>O. sativa</i>	Increase in shoot biomass, higher numbers of tillers and grain
	<i>OsPSTOL1</i>	<i>O. sativa</i>	Enhanced growth vigour and earlier heading, high yield
2	<i>TaMUTE</i>	<i>T. aestivum</i>	Stomatal development, symmetrical division of guard mother cells
	<i>TaYDA1</i>	<i>T. aestivum</i>	Negatively regulates stomatal development
	<i>TaYDA2</i>	<i>T. aestivum</i>	Negatively regulates stomatal development
	<i>TaOST1</i>	<i>T. aestivum</i>	Regulates stomatal aperture
	<i>TaSLAC1</i>	<i>T. aestivum</i>	Guard cell anion channel
3	<i>ALOG-1</i>	<i>T. aestivum</i>	Spikelet development and flowering time
	<i>PDB-1</i>	<i>T. aestivum</i>	Spikelet development and flowering time

Group	Element	Source organism	Function
4	<i>HvLBO</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvMAX1a</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvMAX1b</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvMAX1c</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvMAX1d</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvMAX1e</i>	<i>H. vulgare</i>	Strigolactone biosynthesis
	<i>HvD53a</i>	<i>H. vulgare</i>	Strigolactone signalling
	<i>HvD53b</i>	<i>H. vulgare</i>	Strigolactone signalling
Marker	<i>hptII</i>	<i>Escherichia coli</i>	Hygromycin resistance gene encoding hygromycin phosphotransferase
	<i>nptII</i>	<i>E. coli</i> K12	Neomycin phosphotransferase gene for resistance against geneticin or kanamycin
	<i>bar</i>	<i>Streptomyces hygroscopicus</i>	Bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate
	<i>pporRFP</i>	<i>Porites porites</i>	Red fluorescent protein
CRISPR/Cas9 genetic element (Group 3 and 4 GMOs)	<i>Cas9</i>	<i>Streptococcus pyogenes</i>	RNA-guided nuclease
Single guide RNA (Group 3 and 4 GMOs)	sgRNA	<i>T. aestivum</i> <i>H. vulgare</i>	RNA-guide for genes in Group 3 and 4

*Note: *Triticum aestivum* is a hexaploid plant with three genomes, known as the A, B and D genomes. The genes for Groups 1 and 2 from each of the three *T. aestivum* genomes may be used to modify the GM wheat lines. The genes have the same name except for the final letter (A, B or D), and are homologs derived from the different wheat genomes.

25. The GM wheat and barley plants may also contain selectable marker genes that confer resistance to antibiotics (*hptII* and *nptII*) or to a herbicide (*bar*). The GM wheat lines modified for yield enhancement via water use efficiency may also contain the introduced *pporRFP* gene, which encodes a red fluorescent protein (RFP) used to visually identify GM plant cells. The selectable marker genes and reporter gene are listed in Table 2.

Short regulatory sequences that control expression of the genes are also present in the GM wheat and barley proposed for release. CRISPR/Cas9 regulatory elements are also listed. All of the promoters used to drive expression of the introduced genes are constitutive promoters. Information on the introduced regulatory elements is shown in Table 3.

Table 3. Introduced regulatory sequences in the GM wheat and barley

Element function	Genetic element	Source organism
Constitutive promoter	<i>CaMV35S</i> <i>Ubi</i> <i>OsAct1</i> <i>PvUbi1+3</i> <i>OsUbi1</i>	Cauliflower mosaic virus <i>Zea mays</i> <i>Oryza sativa</i> <i>Panicum virgatum</i> <i>O. sativa</i>
RNA promoter	<i>TaU6a</i> <i>OsU6a</i> <i>OsU6b</i> <i>OsU6c</i> <i>OsU3</i>	<i>T. aestivum</i> <i>O. sativa</i> <i>O. sativa</i> <i>O. sativa</i> <i>O. sativa</i>
Amplification promoting sequence	<i>Ubi1 Intron</i> <i>Ubi 5' UTR</i> <i>CAT-1 intron</i>	<i>Z. mays</i> <i>Z. mays</i> <i>Ricinus communis</i>
Guide RNA scaffold		<i>S. pyogenes</i>
Termination sequence	<i>CaMV35S</i> <i>nos</i> <i>OCS</i>	Cauliflower mosaic virus <i>Agrobacterium tumefaciens</i> <i>A. tumefaciens</i>

4.2 Methods of genetic modification

GM wheat

26. The GM wheat lines with introduced genes for yield enhancement (*AtAVP1*, *OsNAS2* and *OsPSTOL1*) are expressed on their own, or as combinations of genes. Wheat plants with single genes were transformed either with biolistic transformation or *Agrobacterium*-mediated transformation. Information about these methods can be found in the document [Methods of plant genetic modification](#), available from the OGTR Risk Assessment References page. Lines containing more than one introduced gene were generated using either controlled crossing of the GM plants containing single gene insertions, or by direct transformation of GM plants with single gene insertions.

Gene edited wheat and barley

27. The GM wheat and barley lines with endogenous gene knockout have been generated by CRISPR/Cas9 gene editing as follows: a plasmid DNA is generated encoding the Cas9 protein and a single guide RNA (sgRNA) designed to target a specific endogenous gene. Once wheat and barley are transformed with the plasmid, the expressed Cas9-sgRNA complex creates a double-stranded break in the target DNA sequence. Imperfect natural repair of these breaks most often leads to short insertions or deletions (one or a few base pairs) in the target plant DNA sequence, although it can sometimes produce larger deletions (Soyars et al., 2018). The result of this imperfect repair is gene knockout as the target genetic sequences are non-functional.

28. Schedule 1 of the Regulations lists organisms that are not GMOs for the purposes of the Act. Items on this list exclude organisms modified through unguided repair of site-directed nuclease (SDN) activity as no nucleic acid template was added to cells to guide genome repair following SDN application. These organisms are also known as SDN-1 organisms and include CRISPR/Cas9 gene editing. However, some of these methods generate GMOs in the intermediate steps due to the presence of a transgene or expressed products. The gene edited wheat and barley proposed for release are SDN-1 organisms, but may still contain CRISPR/Cas9 genetic elements so they are still classed as GMOs under the Act (for more details see the [Overview of the status of organisms modified using gene editing and other new technologies](#) document, which is available on the OGTR website). The applicant has advised that the gene edited barley has been backcrossed to a wildtype parent,

resulting in the segregation of the CRISPR/Cas9 cassette, which includes the *Cas9* gene, sgRNAs and the marker gene, from the barley knockout lines proposed for release. This was confirmed by PCR but not genome sequencing so the CRISPR/Cas9 genetic elements may still be present. The applicant has also stated that residual T-DNA may still be present in the gene edited barley. The gene edited wheat has not been backcrossed to parental lines, so the introduced CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release.

29. The plasmid DNA used to transform the gene edited wheat and barley proposed for release were produced using a hierarchical cloning system, where sgRNAs are first inserted into plasmids, which are then assembled into a multigene binary vector construct along with *Cas9* and a selection cassette (*hptII*). Gene edited wheat was produced using the pGGG vector system described in Smedley et al. (2021), while gene edited barley was produced using the pYLCRISPR/*Cas9* binary vectors described in Ma et al. (2015). The resulting *Cas9*-sgRNA constructs were transformed into wheat and barley using *Agrobacterium*-mediated transformation (Tingay et al., 1997; Matthews et al., 2001; Hayta et al., 2021). The sgRNA for the target endogenous wheat genes (Table 2) were designed to knock out or modify all three copies of the genes within each of the wheat genomes (A, B and D).

30. The gene edited wheat and barley would be grown in the glasshouse and seed from these plants will be used for planting in the field.

4.3 The introduced or knockout genes for yield enhancement, encoded proteins and associated effects

31. The genes and their encoded proteins are summarised in Table 2, with a description of their expected function in the GM wheat and gene edited wheat and barley. Yield, water use efficiency, plant architecture, and nutrient use efficiency are multigenic traits, involving the interaction of genes where the protein products constitute different biochemical pathways.

4.3.1 Direct yield enhancement

32. The yield enhancement genes proposed for release are *AtAVP1*, *OsNAS2* and *OsPSTOL1*. As mentioned in Section 4.1, field trials of GM wheat with these genes have been evaluated and licensed previously for DIR 102, DIR 128, DIR 152 and DIR 186, so only a summary regarding these genes is presented here.

AtAVP1

33. The *Arabidopsis thaliana* vacuolar H⁺-pyrophosphatase (*AtAVP1*) gene encodes an H⁺-translocating pyrophosphatase (H⁺-PPase) (Gaxiola et al., 1999; Khadilkar et al., 2016). H⁺-PPases in wheat have been shown to be localised in sink tissues (e.g. roots, leaves, kernels) and transport phloem (Regmi et al., 2020).

34. Overexpression of genes encoding the protein significantly increases photosynthetic capacity, yield and nutrient use efficiencies in a number of crops grown under normal or stress conditions (Gaxiola et al., 2001; Park et al., 2005; Yang et al., 2007; Li et al., 2008; Lv et al., 2008). Overexpression of *AtAVP1* in *A. thaliana* increased tolerance of the plants to both drought and salt stress (Gaxiola et al., 2001), and overexpression of *AtAVP1* and its homologs in plants increased proliferation of roots and shoots (Li et al., 2005; Lv et al., 2008; Pei et al., 2012). Overexpression of the gene in wheat also resulted in a significant yield improvement, by producing higher grain yield, an increase in the number of seeds per plant, and an increase in root biomass compared to null segregants (Regmi et al., 2020).

OsNAS2

35. The *OsNAS2* gene encodes a rice nicotianamine synthase (NAS), an enzyme that catalyses the last step in the production of nicotianamine (NA). Nicotianamine is a molecule made by all higher plants that chelates and transports transition metals including iron and zinc (von Wieren et al., 1999). In grasses, nicotianamine is also a precursor for biosynthesis of phytosiderophores, which are molecules

that are secreted from roots to facilitate solubilisation and uptake of iron from the soil (Inoue et al., 2003).

36. Constitutive overexpression of *OsNAS2* in GM wheat increased iron, zinc and copper levels in grain for all GM lines, and increased manganese and magnesium levels for most GM lines, compared to control non-GM wheat (Singh et al., 2017). Constitutive overexpression of *OsNAS2* in GM bread wheat resulted in increased concentrations of iron and zinc in wholemeal flour, white flour and white bread and higher bioavailability of iron in white flour milled from the GM wheat (Beasley et al., 2019; Beasley et al., 2022). Unpublished results from field trials conducted under licence DIR 152 found that several of the GM wheat lines overexpressing *OsNAS2* showed a 20 – 30% increase in shoot biomass due to a higher tiller number. These GM lines also produced approximately 20 – 30% more grain compared to control non-GM wheat.

OsPSTOL1

37. The rice Phosphorous Starvation Tolerance 1 (*OsPSTOL1*) gene encodes a functional serine/threonine protein kinase (Gamuyao et al., 2012). The gene has been shown to improve tolerance to low phosphorous growth conditions in rice (Milner et al., 2023). Overexpression of *OsPSTOL1* in phosphorus-starvation-intolerant rice varieties enhanced grain yield in phosphorus deficient soil, putatively by promoting early crown root development and root growth, which facilitates the uptake of phosphorus and other nutrients like nitrogen and potassium (Gamuyao et al., 2012). GM wheat lines overexpressing *OsPSTOL1* had enhanced growth, crown root number, and overall root plasticity under low phosphorus conditions, while shoot biomass and grain yield were increased when phosphorous was well supplied (Kettenburg et al., 2023).

4.3.2 Yield enhancement via water use efficiency

38. The applicant has stated that the genetic modifications involving MUTE, YDA1, YDA2, OST1 and SLAC1 aim to alter stomatal distribution, density, size and/or regulation. Genetic modification of stomatal development and aperture may result in a number of changes, including:

- protecting plants against drought, allowing them to continue to grow in water-limited environments (Franks et al., 2015; Hepworth et al., 2018)
- reduce carbon dioxide assimilation, which in turn may reduce sugar production by photosynthesis, resulting in a negative impact on plant yield (Dunn et al., 2019)
- influence flowering time (Kinoshita et al., 2011).

39. Field trials of GM wheat involving all of these genes have been evaluated and licensed previously for DIR 186. Preliminary data indicates that GM wheat lines grown in the glasshouse have either improved or poorer water use efficiency compared to control non-GM wheat. GM wheat plants with altered stomata aperture and number have also been observed (unpublished data). More detailed information regarding the genes can be found in the [DIR 186 RARMP](#).

TaMUTE

40. MUTE is one of three transcription factors that have been shown to positively regulate stomatal development in *Arabidopsis* (Liu et al., 2019).

41. Knockout of the MUTE gene in *Arabidopsis* resulted in the complete absence of stomata, while overexpression of MUTE led to the entire epidermis covered in stomata (Pillitteri et al., 2007). Orthologs of these transcription factors are found in other flowering plants, as well as in grasses and other monocots (Liu et al., 2009; Peterson et al., 2010; Raissig et al., 2017).

TaYDA1 and TaYDA2

42. The YDA gene encodes a mitogen activated protein (MAP) kinase kinase kinase (MAPKKK) known as YODA, which is an important negative regulator of stomatal development (Gray and Hetherington, 2004). The MAPKKK signal transduction pathway controls the activity of MUTE and the

two basic helix-loop-helix domain transcription factors involved in stomatal development (Qi and Torii, 2018; Dunn et al., 2019).

43. In *Arabidopsis*, loss-of-function mutations in the YDA gene lead to the massive over-proliferation of stomata in the epidermis (Le et al., 2014). A mutant copy of the YDA gene in *Brachypodium* leaves produced excess stomata arranged in clusters, along with a stunted growth phenotype (Abrash et al., 2018).

TaSLAC1 and TaOST1

44. The SLAC1 gene encodes a guard cell anion channel protein (SLOW ANION CHANNEL-ASSOCIATED 1) that is essential for stomatal closure in response to a number of environmental factors, including carbon dioxide, light/dark transitions, humidity and ozone (Vahisalu et al., 2008; Hedrich and Geiger, 2017).

4.3.3 Yield enhancement via altered spikelet development and flowering time

45. The applicant has stated that the knockout of the *ALOG-1* and *PDB-1* endogenous genes in wheat aims to alter spikelet development and flowering time. This may result in a change to the amount of grain in the spikelet, thereby altering yield. Both genes have been shown to regulate the Photoperiod-1 (*Ppd-1*) gene, which is an important regulator of flowering time in wheat (Gaughley, 2020). Wheat lines that contain *Ppd-1* photoperiod insensitive alleles have been shown to promote flowering, regardless of the amount of light available (Flohr, 2018). The ability of wheat to flower independently of available light could help to adapt wheat to more varied growth conditions, allowing further optimisation of yield (Hunt, 2015).

46. The *Arabidopsis* LSH1 and *Oryza* G1 (ALOG) protein is a transcription factor that is plant-specific and highly conserved among land plants (Yoshida et al., 2009). It has been shown to regulate reproductive growth in flowering plants, including floral and spikelet development, and also the transition from indeterminate to determinate growth in flowering plants (Takeda et al., 2011; Nan et al., 2018; Naramoto et al., 2020). Expression profiles of *ALOG-1* shows that it is negatively regulated during the floral transition (Gaughley, 2020).

47. *PDB-1* is a bZIP transcription factor involved in wheat spike development, including flowering time and spikelet architecture (Gaughley, 2020; Cao et al., 2021). A recent study in rice proposes that complexes of bZIP proteins function together to regulate inflorescence development by forming a florigen repressor or activation complexes (Kaneko-Suzuki et al., 2018; Cerise et al., 2021).

48. The applicant has indicated that the activity of *ALOG-1* and *PDB-1* is disrupted in wheat lines that contain null or overexpression of the *Ppd-1* gene (unpublished data). The applicant has predicted that the absence of the *ALOG-1* and *PDB-1* proteins will modify spikelet architecture and flowering time, leading to changes in the number of grains produced and therefore yield.

4.3.4 Yield enhancement via altered plant architecture and nutrient use efficiency

49. The gene edited barley lines proposed for release have knockout of endogenous genes involved in the strigolactone (SL) biosynthesis pathway. SLs are a class of phytohormones that control plant architecture by modulating shoot and root branching (Brewer et al., 2016; Yoneyama and Brewer, 2021). SLs negatively regulate branching, allowing them to alter plant architecture to optimise growth depending on the conditions (Kelly et al., 2023). When growing conditions are poor, the production of SL increases, which reduces the number of branches a plant can make. Conversely, SL production decreases when growing conditions are optimal, which then increases the number of branches a plant can make (Figure 3)(Kelly et al., 2023). Fewer branches result in less plant biomass, which is a desired phenotype in low rainfall or nutrient conditions, while more branching will result in more biomass and grain heads which is desirable when water and nutrient supply is plentiful.

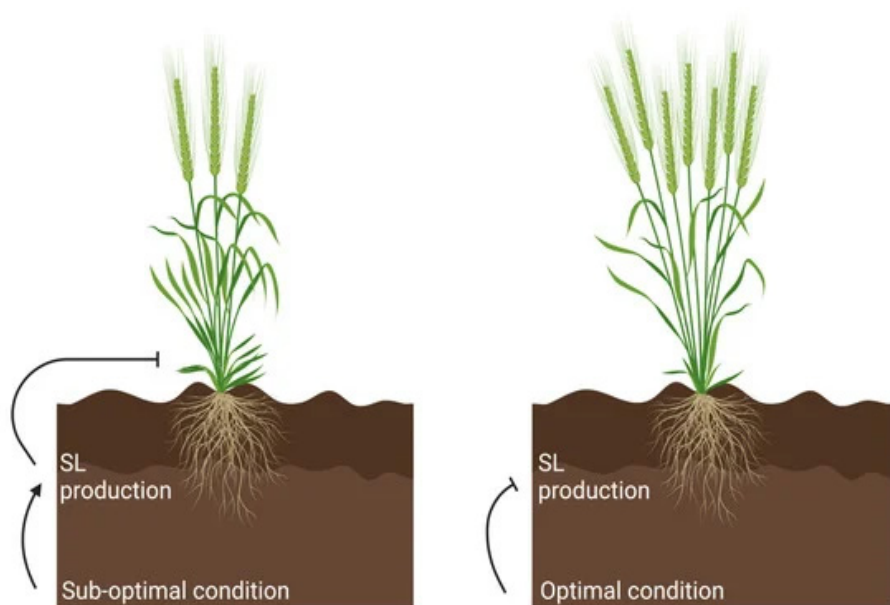


Figure 3. Strigolactone production in optimal and sub-optimal conditions (Kelly et al., 2023)

50. The genes involved in SL biosynthesis that have been targeted in the proposed release are *LBO*, *MAX1* and *D53*. The *LBO* gene encodes LATERAL BRANCHING OXIDOREDUCTASE (LBO), a 2-oxoglutarate and Fe (II)-dependent dioxygenase that has been shown to act in the final stages of strigolactone biosynthesis in *Arabidopsis* (Brewer et al., 2016). The *MAX1* gene encodes MORE AUXILLARY GROWTH 1 (MAX1), a cytochrome P450 enzyme that produces most of the structural diversity of SLs during the final stages of their biosynthesis in rice (Marzec et al., 2020). The DWARF53 (*D53*) protein was first identified in rice, where it acted as a negative regulator in the SL signalling pathway to promote shoot branching (Jiang et al., 2013). The applicant predicts that the *D53* gene in barley encodes key transcriptional repressors that are degraded during SL signalling.

51. The applicant has stated that the knockout of endogenous *LBO*, *MAX1* and *D53* barley genes may be a way to maintain plant growth under low nutrient conditions and therefore maintain or enhance yield.

4.3.5 Marker genes

52. The GM wheat and barley plants contain selectable marker genes that confer resistance to different classes of antibiotics or to a herbicide (Table 2). Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. The selectable marker genes are *hptII*, which codes for hygromycin phosphotransferase enzymes (HPH or HPT; confers resistance to hygromycin; (Stogios et al., 2011); *nptII* (neomycin phosphotransferase II) which encodes an aminoglycoside 3'-phosphotransferase II enzyme that is also known as neomycin phosphotransferase II (NPTII; confers resistance to kanamycin and related antibiotics) and the *bar* gene which encodes the phosphinothricin N-acetyltransferase (PAT) protein (confers tolerance to glufosinate herbicides).

53. The *nptII* and *hptII* genes are derived from *Escherichia coli*, a common gut bacterium that is widespread in human and animal digestive systems and in the environment. The *bar* gene is derived from *Streptomyces hygroscopicus* (Thompson et al., 1987), a common saprophytic, soil-borne microorganism that is not considered to be a pathogen of plants, humans, or other animals (OECD, 2002). More information on marker genes in general may be found in the document [*Marker Genes in GM Plants*](#), available on the OGTR website.

54. Some of the GM wheat plants that have introduced genes for yield enhancement via water use efficiency may contain the introduced *pporRFP* gene as a visual marker. This gene encodes a DsRed-type RFP derived from the coral *Porites porites* (Alieva et al., 2008). Coral fluorescent proteins like *pporRFP* and DsRed are homologous to green fluorescent proteins (GFP) from the jellyfish *Aequorea*

victoria, which have been widely used as reporter genes in GM plants (Jach et al., 2001; Alieva et al., 2008; Mann et al., 2012). More information on the *pporRFP* gene can be found in the [DIR 186 RARMP](#) on the OGTR website. General information on the use of reporter genes may be found in the document [Marker Genes in GM Plants](#), also available on the OGTR website.

4.4 Toxicity/allergenicity of the proteins associated with the introduced and knockout genes

55. Non-GM wheat and barley contain a number of anti-nutritional factors and allergens that, in extreme cases, may have a toxic effect (OGTR, 2021b, a).

56. The applicant has not yet performed any toxicity or allergenicity studies on the GM wheat and barley plants proposed for release.

57. Apart from *pporRFP*, all of the genes introduced into the GM wheat 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, except for *OsNAS2* as discussed below. This includes homologues isolated from other species, apart from the *pporRFP* homologue *DsRed*. However, toxicity/allergenicity tests have only been performed on the introduced HPH, NPTII and PAT proteins.

58. The CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release and may also be present in the knockout barley lines (Chapter 1, Section 4.2). Cas9 is an RNA-guided nuclease for genome editing derived from *Streptococcus pyogenes*, a human-specific bacterial pathogen (Ibrahim et al., 2016). Comparisons of amino acid sequences revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are widely exposed to this protein (El-Mounadi et al., 2020). A recent bioinformatic and literature assessment of a human codon-optimized version of the Cas9 protein derived from *S. pyogenes* Cas9 found that, while Cas9 nuclease activity can be toxic to some cell types *in vitro*, there was no evidence from previous studies of a risk of toxicity to humans and other animals from the *Cas9* gene. Also, the full amino acid sequence of this Cas9 protein was not homologous to any known allergens (Qureshi and Connolly, 2023).

59. In the current application, the introduction of the *OsNAS2* gene is being examined for its role in yield enhancement as a result of increased iron uptake. This gene has also been studied by other research groups with the aim of increasing levels of iron in plant tissues and biofortification. Excessive iron in the diet can result in toxicity (Balmadrid and Bono, 2009). Studies have indicated that susceptibility to excess iron toxicity is similar in people and other mammals (Albretsen, 2006). *OsNAS2* introduction or overexpression could lead to accumulation of metals other than iron, such as cadmium, that are also toxic to humans and animals (Flora et al., 2008; Jaishankar et al., 2014; Clemens and Ma, 2016). However, GM rice overexpressing *OsNAS2* and soybean ferritin genes had grain cadmium, lead and arsenic levels below detection limits when grown in normal soil, and when grown in cadmium-contaminated soil there was no difference between grain cadmium levels in the GM and non-GM rice (Trijatmiko et al., 2016). There have been no adverse effects reported from similar GM lines overexpressing *OsNAS2* planted under DIR 102, DIR 128, DIR 152 and DIR 186. It should be noted that none of these licences permitted use of the GM lines in human food or animal feed, and this use is also not proposed in the current application. Further details on the potential toxicity of the *OsNAS2* gene is detailed in the [DIR 186 RARMP](#).

60. There is no evidence that the *nptII* or *hptII* genes or the proteins they encode are toxic or allergenic (OGTR Risk Assessment documents and references therein). GM foods containing the *nptII* and *hptII* genes have been assessed and approved for sale in Australia ([FSANZ website](#), accessed 17 December 2023).

61. 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, 2010a, b, 2013), corn (FSANZ, 2005a) and rice (FSANZ, 2008).

62. The *pporRFP* gene and its encoded protein has been recently assessed by the OGTR in the DIR 186 RARMP. Like other red fluorescent proteins, pporRFP is a tetramer, which can lead to cytotoxicity when expressed as a fusion protein in GM plants (Campbell et al., 2002; Shemiakina et al., 2012). However, the GM wheat and barley do not contain pporRFP as a fusion protein, and there is no information in the literature to suggest that this introduced gene or its product is toxic or allergenic to people or toxic to other organisms.

4.5 Characterisation of the GMOs

63. Although the GM wheat lines are at an early stage of development, the applicant has provided some preliminary information on expected phenotypes for some of the genes introduced into the GM wheat. There is no characterisation data available for the gene edited wheat and barley proposed for release.

64. Some GM wheat lines constitutively overexpressing *OsNAS2* have increased iron concentration in grains (Beasley et al., 2019; Beasley et al., 2022). The applicant stated that the lines also show a 20 - 30% increase in shoot biomass due to a higher tiller number and produce approximately 20 - 30% more grain than wild-type plants (data not provided).

65. The applicant also claims that overexpression of *OsPSTOL1* in GM wheat resulted in enhanced plant vigour and earlier heading. Data from DIR 152 and DIR 186 shows field grown GM wheat expressing *OsPSTOL1* has enhanced grain yield (Kettenburg et al., 2023). In GM rice, *OsPSTOL1* conferred enhanced root growth, thus increasing uptake of phosphorous as well as nitrogen and potassium (data not supplied). Six genes with sequence similarity to *OsPSTOL1* have been identified in sorghum. Two of these genes were associated with an increased root surface and grain yield under low phosphorous conditions in the field (Hufnagel et al., 2014).

66. The modification of *MUTE*, *YDA1*, *YDA2*, *OST1* and *SLAC1* aims to alter stomatal distribution, density, size and/or regulation. As discussed in Section 4.3.2, published data indicates that overexpression, or mutant copies of these genes, do alter the presence and function of stomata in studied plants. The applicant anticipates the same phenotypes in the GM wheat proposed for release. GM wheat lines expressing these genes were released under DIR 186. The applicant has stated that preliminary data from glasshouse trials indicate that GM wheat lines expressing these genes have either improved or reduced water use efficiency compared to non-GM controls. GM wheat lines with altered stomata aperture and number have also been observed.

67. The applicant has stated that one of the unintended changes in the GM wheat lines is reduced time to flowering. Some of the GM wheat lines approved for release under DIR 152 and DIR 186 have been shown to flower 5-10 days earlier than non-GM plants within the same cultivar in the glasshouse and under field conditions.

68. Genetic modification of the *OST1* and *SLAC1* genes in the GM wheat and barley may also alter the abiotic stress tolerance of the plant, as *SLAC1* (under the control of *OST1*) has been shown to be involved with anion transport (Geiger et al., 2009).

69. The GM wheat lines for yield enhancement (*AtAVP1*, *OsNAS2* or *OsPSTOL1*, individually and in combination) were grown under DIR 152 and DIR 186. According to the applicant, these lines had up to 60% increased biomass under greenhouse conditions, however this phenotype was not observed in the field under increased nutrient and water-limited conditions. The applicant stated that only yield was successfully increased by up to 30% under these field conditions (no data or further information supplied).

Section 5 The receiving environment

70. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

71. Detailed information about non-GM wheat in the Australian environment is presented in the document *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021b). Detailed information relevant to the commercial cultivation and distribution of non-GM barley in Australia is available in *The Biology of Hordeum vulgare L. (barley)* (OGTR, 2021a).

5.1 Relevant biotic factors

72. A number of biotic factors are important in the cultivation of both wheat and barley. There are several weeds that impact on wheat production, while barley is generally regarded as being more competitive with weeds. A number of vertebrate pests, which are discussed further in Chapters 2 and 3, affect both wheat and barley. Insect pests are generally regarded as more of a concern for wheat than for barley, although barley can also be damaged under conditions where insect populations build up. Both wheat and barley are affected by a number of invertebrate pests and pathogens including nematodes, fungal diseases, bacteria and viruses. Both species also interact with potentially beneficial endophytic bacteria and fungi.

5.2 Relevant abiotic factors

73. It is proposed that the GMOs will be grown at a field trial facility at Rosedale in SA. The applicant intends to plant the GMOs in more than one planting area at the site, which allows for the analysis of seasonal and environmental stress variation. The total planting area would be up to 2 ha per year. GM plants approved under other DIR licences, including DIR 186 and future DIR licences, if approved, would also be grown at the site. The applicant has indicated that although DIR 186 allows planting of GM barley, only GM wheat has been planted at the site to date.

74. The site in Rosedale is located in Light Regional Council, a local government area (LGA) north of Adelaide. The proposed trial site is on land leased by The University of Adelaide from the South Australian Research and Development Institute (SARDI). Light Regional Council is located in commercial wheat and barley growing regions of SA, based on information discussed in the [OGTR Biology documents](#) for these plants. The proposed Rosedale site has a climate typical of rain-fed wheat production areas for SA based on [Bureau of Meteorology climate data](#), which shows a concentration of rainfall during the winter months and drier summer months.

75. Nutrient stress, particularly nitrogen, potassium and phosphorus, affects both species. Both crop species are affected by drought, although barley is generally regarded as more tolerant to drought than wheat due to better water use efficiency. However, barley is susceptible to waterlogging. Heat stress impacts on wheat and barley production, and barley is generally regarded as less cold tolerant than wheat, although both can be affected by frost. Wheat is susceptible to salinity, while barley is generally regarded as the most salinity tolerant cereal crop. Barley is also sensitive to acidic soils and to aluminium and boron toxicity.

5.3 Relevant agricultural practices

76. The limits and controls of the proposed release are outlined in Section 2.1 and Section 2.2 of this Chapter. It is anticipated that the agronomic practices for the cultivation of the GM wheat and barley by the applicant will not differ significantly from industry best practices used in Australia.

77. Seeds would be harvested either by hand or with a machine (e.g. plot harvester) that can be cleaned within the planting area. Threshing would occur within the same planting area or heads transported to approved facilities for threshing, analysis or other processing.

78. Waste material derived from the harvest would be left on the trial area and ploughed back into the soil along with any stubble remaining after harvest. Cultivation would be to the depth of seeding so that grain is not transferred any deeper into the soil profile. If not ploughed back into the soil, the waste may be burnt or buried elsewhere on site.

5.4 Presence of related plants in the receiving environment

79. The proposed location is within a cereal-producing region.

80. The Rosedale site has previously been used for sheep grazing for over 10 years. No wheat or barley has been sown in surrounding fields. However, planting of GM wheat and barley can occur at the site until (and including) the 2026/2027 growing season under the DIR 186 licence, so planting could occur under DIR 186 concurrently with that proposed under DIR 201.

81. Cultivated wheat and barley are not known to hybridise with one another naturally, but each can hybridise with other species. Bread wheat (*Triticum aestivum* L.) is sexually compatible with other bread wheat or durum plants. Bread wheat is cultivated in the LGA where proposed field trial site may be located. There are strict isolation barriers to gene flow between *Hordeum* species. It is therefore highly unlikely that barley would outcross to other species to produce fertile progeny and *H. vulgare* ssp. *spontaneum*, with which it may outcross, is not known to be present in Australia. Further details are given in the biology documents for these species and briefly summarised in the RARMP for [DIR 186](#).

5.5 Presence of similar genes and encoded proteins in the environment

82. The introduced genes listed in Table 2 were originally isolated from naturally occurring organisms, most of which are already widespread and prevalent in the environment. The edited genes listed in Table 2 are endogenous wheat and barley genes. Thus, humans and animals have been exposed to the introduced genes and their encoded proteins, and the edited genes, either through consumption of the parent organisms or through other exposures in the environment. In addition, homologues of the genes and encoded proteins occur naturally in animals, plants, yeast and bacteria.

83. The CRISPR/Cas9 mechanism is an adaptive immune system that occurs naturally in many bacteria and archaea, where it provides protection against invading pathogens and any toxic molecules (Ran et al., 2013; Modrzejewski et al., 2020). A recent review found that CRISPR and genes coding for their associated proteins were present in a diverse range of bacteria, including those used in food production (El-Mounadi et al., 2020). The *Cas9* gene used in the gene edited wheat and barley proposed for release has been isolated from *Streptococcus pyogenes*, a human-specific bacterial pathogen that causes a wide array of infections ranging from mild to life-threatening (Ibrahim et al., 2016). Comparisons of amino acid sequences revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are widely exposed to this protein (El-Mounadi et al., 2020).

84. The *hptII* and *nptII* genes are derived from *E. coli*, a common gut bacterium that is widespread in human and animal digestive systems and in the environment. Both humans and animals are routinely exposed to the genes and their encoded proteins through contact with plants or food.

85. The *bar* gene was isolated from the common bacterium *S. hygrosopicus*, which is a saprophytic, soilborne microorganism that is not considered a pathogen of plants, humans or other animals (OECD, 1999). Genes encoding PAT and similar acetyltransferase enzymes are present in a range of common soil bacteria, and are not known to be toxic or allergenic (H  rouet et al., 2005).

86. The *pporRFP* gene was isolated from *P. porites*, a finger-like coral that is distributed in a variety of coral reefs environments across the Caribbean, in the western Atlantic Ocean and also along the

coast of West Africa (Aronson et al., 2008). *Porites spp.*, including *P. porites*, are collected and traded for use as decorative objects (e.g. ornaments, jewellery and aquarium decoration) (Kinch et al., 2010; Taylor, 2016). Sources of the pporRFP protein in the terrestrial environment would be minimal.

87. All promoters used to drive expression of the introduced genes, including the CRISPR/Cas9 genetic elements, are derived from plant species (maize, rice and switchgrass), with the exception of the *CaMV35S* promoter from a plant virus. Humans and animals have been exposed to these plants and the plant virus for centuries. Other regulatory sequences are from common organisms including maize (*Z. mays*), castor bean (*Ricinus communis*) and *A. tumefaciens*, a common bacterium that can cause galls in various plants.

88. While some of the source organisms can cause toxicity and/or allergies (e.g. castor beans, wheat), the introduced proteins are not known to cause harm.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

89. Wheat and barley lines containing the three genes for yield enhancement (*AtAVP1*, *OsNAS2* and *OsPSTOL1*) proposed for release under the current application have been approved in Australia for limited and controlled release under licences including [DIR 102](#) (*AtAVP1*), [DIR 128](#) (*AtAVP1* and *OsNAS2*, individually), [DIR 152](#) and [DIR 186](#) (*AtAVP1*, *OsNAS2* and *OsPSTOL1*, individually and in combination). There have been no reports of adverse effects on human health and safety or the environment resulting from these releases.

90. Wheat and barley lines containing the five genes for water use efficiency (*MUTE*, *YDA1*, *YDA2*, *OST1* and *SLAC1*) proposed for release under the current application have been approved in Australia for limited and controlled release under licence [DIR 186](#). There have been no reports of adverse effects on human health and safety or the environment resulting from this release.

91. The GM wheat and barley knock-out lines (Table 1, Groups 3 and 4) have not been grown in the field.

92. Information on previous DIR licences for GM wheat and barley is available from the [OGTR GMO Record](#). The Regulator has previously approved 23 field trial releases of GM wheat, of which eleven are licences for both wheat and barley. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

93. There have been no approvals for the commercial release of GM wheat or barley in Australia.

6.2 International approvals

94. Field trials of other GM wheat and barley have been approved in a number of countries including the United States, Canada, the United Kingdom and a number of European countries, for a range of modified traits, including improved yield and tolerance to abiotic stresses ([USDA APHIS Biotechnology Permits](#), [EU GMO Register](#); accessed 17 December 2023).

95. On a commercial scale, drought tolerant HB4 GM wheat has been approved for cultivation in Argentina, Paraguay and Brazil, and for food and feed in a number of countries including Australia and New Zealand ([ISAAA website](#); accessed 17 December 2023; [FSANZ website](#); accessed 17 December 2023, [BioTrack Product database](#); accessed 14 January 2024).

96. None of the GM wheat and barley in the current application have been approved for release in any other country.

Chapter 2 Risk assessment

Section 1 Introduction

97. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the 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.

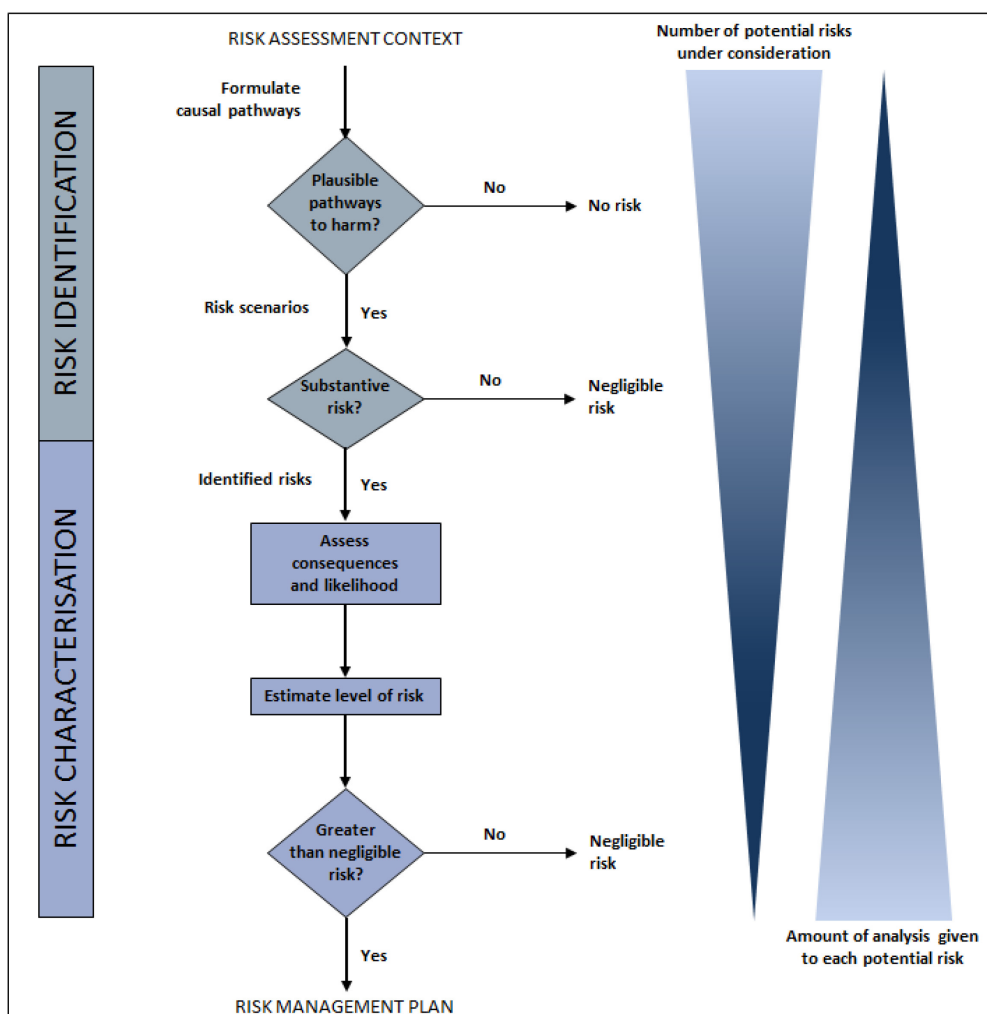


Figure 4. The risk assessment process

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

99. 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 causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

100. 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 4), i.e. the risk is considered to be no greater than negligible.

101. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined 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

102. Postulated risk scenarios are comprised of three components (Figure 5):

- 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.

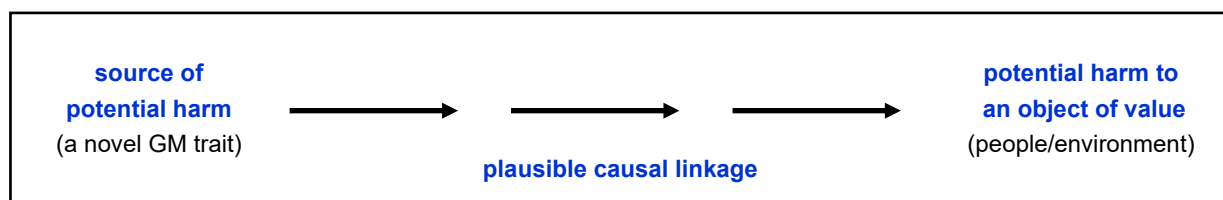


Figure 5. Components of a risk scenario

103. 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 GMO and
- the characteristics of the parent organism(s).

2.1 Risk source

104. 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.

105. As discussed in Chapter 1 (Table 2 and 3), the GM wheat and barley have been modified by introduction (wheat) or knockout of genes (wheat and barley) conferring yield enhancement. These introduced genes will be considered further as a potential source of risk.

106. The GM wheat and barley also contains other marker genes, *nptII* and *hptII* from *E. coli* that confer antibiotic resistance, and the *bar* gene that confers herbicide tolerance. These genes were used as selectable markers during development of the GM plants. While there is concern regarding the overuse of antibiotics and the development of resistance to these antibiotics (see, e.g. [FSANZ website](#), accessed 19 March 2024), the *nptII*, *hptII* and *bar* genes and their products have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas when expressed in GM plants. Further information about the antibiotic resistance genes can be found in the document [Marker genes in GM plants](#) on the OGTR website. The *bar* gene and its protein product, PAT, have been assessed in other RARMPs as well as in scientific literature, as detailed in Chapter 1 (Section 4.3). The environmental safety of the PAT protein present in biotechnology-derived crops has also been

extensively assessed worldwide (CERA, 2011). As the marker genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

107. A red colour marker gene, *pporRFP*, has also been introduced into some of the GM wheat lines. As discussed in Chapter 1 (Section 4.3.5), the gene was isolated from the coral *P. porites* and encodes a novel DsRed-like red fluorescent protein pporRFP (Alieva et al., 2008). The *pporRFP* gene and its encoded protein has been previously assessed in the RARMP for [DIR 186](#) and was not found to pose a substantive risk to either people or the environment. Therefore, this visual marker gene will not be considered further for this application.

108. The introduced genes for yield enhancement, including the genes used for CRISPR/Cas9 gene editing of knockout wheat and barley lines (Chapter 1, Section 4.2), are controlled by introduced regulatory sequences derived from viruses, bacteria and plants. Regulatory sequences, such as promoters, enhancer sequences and terminators, are naturally present in all plants and the introduced sequences are expected to operate in similar ways to endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

109. 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.

110. The genetic modifications involving knockout of genes by CRISPR/Cas9 have the potential to cause two classes of unintended effects. The first class of unintended effects are significant genomic deletions or rearrangements at the intended site of gene editing (Hahn and Nekrasov, 2018), leading to altered expression of endogenous genes. The applicant will use CRISPR/Cas to generate double-stranded breaks in DNA sequences that will be randomly repaired by non-homologous end joining (NHEJ). The conventional plant breeding technique of mutagenesis also generates double-strand breaks repaired by NHEJ and can also produce significant genomic deletions or rearrangements (Shirley et al., 1992). As discussed in the previous paragraph, conventional breeding using mutagenesis has a long history of safe use. The second class of unintended effects is off-target gene editing, leading to inadvertent knockout of additional genes with sequences that closely match the intended site of gene editing. A recent review of CRISPR/Cas off-target edits in plants found that most of the observed off-target changes were small insertions or deletions or nucleotide substitutions, and large deletions were rare (Sturme et al., 2022). Off-target sites have few mismatches with the target sequence and were often located in homologues of the target gene (Modrzejewski et al., 2020; Sturme et al., 2022). Other studies have observed that off-target CRISPR/Cas9 gene editing is rare in plants (Hahn and Nekrasov, 2018; Soyars et al., 2018; Tang et al., 2018; Wang et al., 2021). It is also noted that all DNA breaks generated by conventional mutagenesis are untargeted. CRISPR/Cas9 edited plants show lower off-target mutation frequencies than conventionally bred plants (Sturme et al., 2022). Therefore, unintended effects arising from genome editing will not be further assessed for this application.

2.2 Causal pathway

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

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

112. Although all of these factors are taken into account, some are not included in the risk scenarios because they have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

113. The potential for HGT from GMOs, including GM plants, 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 many previous RARMPs. HGT was most recently considered in the RARMP for [DIR-108](#). Although the DIR-108 RARMP is for GM canola, the HGT considerations are the same for the current RARMP: HGT events rarely occur, and the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

114. 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 a 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

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

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

116. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, 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

117. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 4 and examined in detail in Sections 2.4.1 - 2.4.4 (this Chapter).

118. The CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release and may also be present in the knockout barley lines (Chapter 1, Section 4.2). Therefore, these gene editing elements are included in the Risk scenarios.

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

Table 4. Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	<p>Growing GM wheat and barley at the trial site</p> <p>↓</p> <p>GM wheat and barley composition is different from non-GM wheat and barley</p> <p>↓</p> <p>Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites</p> <p>OR</p> <p>Exposure of animals eating the GM wheat or barley</p>	<p>Increased toxicity or allergenicity to people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p>	No	<ul style="list-style-type: none"> GM plant material would not be used as human food or animal feed. Proposed limits and controls would further minimise the exposure of people and animals to GM plant material. No reasonable expectation that the gene knockouts and presence of the Cas9 protein could lead to increased toxicity or allergenicity in people or to increased toxicity in animals.
2	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	<p>Growing GM wheat and barley at the trial site</p> <p>↓</p> <p>Pollen flow to other GM wheat or barley grown at the trial site</p> <p>↓</p> <p>GM wheat and barley composition is different from non-GM wheat and barley</p> <p>↓</p> <p>Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites</p> <p>OR</p> <p>Exposure of animals eating the GM wheat or barley</p>	<p>Increased toxicity or allergenicity to people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p>	No	<ul style="list-style-type: none"> The limited time, small scale and other proposed limits and controls for this application, and also those imposed by the DIR 186 licence, minimise exposure of people and other desirable organisms to the GM hybrid seeds and other plant material. Wheat and barley are mostly self-pollinating, and outcrossing occurs at low levels. No reasonable expectation that expression of the introduced genes or the knockout of genes could lead to increased toxicity or allergenicity in people or to increased toxicity in animals.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
3	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	<p>Growing GM wheat and barley at the trial site</p> <p>↓</p> <p>Presence of GM wheat and barley outside the trial limits</p> <p>↓</p> <p>Spread and persistence of GM wheat and barley in the environment</p>	<p>Increased toxicity or allergenicity for people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment and yield of desirable plants</p> <p>OR</p> <p>Reduced utility or quality of the environment</p> <p>OR</p> <p>Increased ability to provide a reservoir for pathogens or shelter for pests</p>	No	<ul style="list-style-type: none"> The proposed limits and controls minimise the likelihood of seed dispersal or persistence outside the trial limits. GM wheat and barley have limited ability to spread and persist outside the trial site. There is no expectation the introduced genes confer characteristics in the GM wheat and barley that may lead to environmental harms. The GM wheat and barley is susceptible to most standard weed control measures.
4	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	<p>Pollen flow from GM wheat and barley to sexually compatible plants outside the trial site</p> <p>↓</p> <p>GM hybrid seed grows into volunteer plants</p> <p>↓</p> <p>Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas</p> <p>↓</p> <p>Increased exposure of people and desirable animals by ingestion of, or contact with, the GM hybrid plant material</p> <p>OR</p> <p>Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas</p>	<p>Increased toxicity or allergenicity for people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment and yield of desirable plants</p> <p>OR</p> <p>Reduced utility or quality of the environment</p>	No	<ul style="list-style-type: none"> Wheat and barley have limited ability to outcross because of the limited occurrence of long-distance pollen flow. The proposed limits and controls minimise the likelihood of pollen flow from the trial site to sexually compatible plants. Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.

2.4.1 Risk scenario 1

<i>Risk Source</i>	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
<i>Causal Pathway</i>	<p>Growing GM wheat and barley grows at the trial site</p> <p>↓</p> <p>GM wheat and barley composition is different from non-GM wheat and barley</p> <p>↓</p> <p>Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites</p> <p>OR</p> <p>Exposure of animals eating the GM wheat or barley</p>
<i>Potential Harm</i>	<p>Increased toxicity or allergenicity to people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p>

Risk source

120. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

121. The inserted genes and the genes used for CRISPR/Cas9 gene editing are under the transcriptional control of constitutive promoters and so the encoded proteins could potentially be produced in all plant tissues throughout plant development. However, this has not been determined and therefore the level of exposure is an area of uncertainty for this risk assessment.

Exposure of people to the GM wheat and barley

122. The GM wheat and barley would be grown at the trial site. People could be exposed to the GM plant material through inadvertent ingestion, skin contact or inhalation.

123. The applicant proposes that the GM wheat and barley would not be used as human food. There is little potential for accidental ingestion of wheat and barley grown on the trial site. Therefore, it is not expected that people would be exposed to the GM wheat and barley by consumption.

124. The applicant proposes that only trained and authorised persons would be permitted to deal with the GM wheat and barley, or to access the trial site. Due to the small scale of the proposed trial, few people would handle the GM wheat and barley. These authorised staff could have direct skin contact with GM plant material or could inhale GM pollen during cultivation, transportation or analysis. Transport and storage of the GM plant material would be conducted according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs, thus limiting exposure of people during transport and storage of the GMOs.

125. Wheat pollen is wind dispersed, and although most pollen falls within 3 m of the source plant, some travels up to 60 m (reviewed in (Hegde and Waines, 2004)). Similarly, barley pollen is predominantly dispersed over short distances by wind (Wagner and Allard, 1991), but some has been detected at distances of up to 50 m from the pollinator source (Ritala et al., 2002). Therefore, people who are not involved with the trial but who pass within 60 m of the trial site could be exposed to low levels of GM pollen, if the GM wheat or barley were flowering at the time. However, as the proposed trial is of a small size and limited duration, and the fact that the proposed trial site is located in agricultural areas, only a very limited number of people not involved with the trial could be exposed to small amounts of GM pollen during flowering.

Exposure of animals eating the GM wheat and barley

126. The GM wheat and barley would not be used as animal feed. However, animals, including birds and insects, entering the trial site could consume the GM wheat and barley. A range of animals consume cereals (Hill et al., 1988; AGRI-FACTS, 2002; OGTR, 2021b, a) and may be attracted to the GM plant material. The applicant proposes to surround the trial site with a fence and locked gates that would restrict access to some large animals such as livestock. However, other animals such as insects, birds and small animals could enter the trial site and feed on the GM wheat and barley. The small size and short duration of the proposed field trial and the proposed controls (Chapter 1, Section 2.1 and 2.2) would restrict the numbers of animals that could be exposed to the GM wheat and barley.

Potential harm

127. 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).

128. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for toxicity or allergenicity of the proteins were identified in the respective RARMPs nor have there been any reports of adverse effects from these earlier releases to people or animals. Consistent with the assessment in the DIR 186 RARMP and as no further data has been provided, uncertainty still remains about the potential for increased toxicity or allergenicity of the GM plants expressing *OsNAS2* relative to non-GM wheat and barley, including the potential for uptake of heavy metals. More information can be found in the [DIR 186 RARMP](#) on the OGTR website.

129. No toxicity or allergenicity studies have been performed on the GM plant material with the knockout of endogenous genes and this is an area of uncertainty for this risk assessment. As discussed in Chapter 1 (Section 4.4), a recent review found that CRISPR and genes coding for their associated proteins were present in a diverse range of bacteria (El-Mounadi et al., 2020). Amino acid sequence comparisons revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are already widely exposed to this protein (El-Mounadi et al., 2020). The applicant has indicated that the purpose of this trial is to evaluate the candidate genes for yield enhancement under field conditions. Gene edited lines that are shown to have a beneficial impact on yield will have the CRISPR/Cas9 genetic elements segregated out by backcrossing to a wildtype parent. Segregation will be confirmed by genome sequencing before the wheat and barley lines are progressed to the next stage of assessment by the applicant.

130. Non-GM wheat and barley are not regarded as toxic to humans or animals. However, both can produce allergic responses in susceptible individuals via inhalation of pollen or inhalation of flour (Astwood et al., 1995; Pahr et al., 2012). Common symptoms of respiratory allergy to wheat include rhinitis, conjunctivitis and asthma (Houba et al., 1998). Both wheat and barley can produce allergic and autoimmune responses in susceptible individuals by inhalation of flour (for example baker's asthma) or ingestion (coeliac disease). Barley pollen may also cause allergic reactions in susceptible individuals (OGTR, 2021b, a). The genes knocked-out in the GM plants proposed for release are involved in spikelet development and flowering time (wheat) and altered plant architecture and nutrient use efficiency (barley) (Chapter 1, Section 4.3.3 and 4.3.4). Altering spikelet development, flowering and plant architecture may lead to an increase in the amount of pollen produced per plant compared to non-GM wheat and barley, but there is no information to suggest that pollen characteristics that facilitate dispersal would be changed. Furthermore, there is no reasonable expectation that the knockout of the endogenous wheat and barley genes proposed for this trial would influence the pathways producing known allergens in wheat or barley or lead to the production of a novel toxin.

Conclusion

131. Risk scenario 1 is not identified as a substantive risk because the GM wheat and barley would not be used for human food or animal feed, and other proposed limits and controls would minimise exposure of people and animals to the GM wheat and barley. In addition, there is no reasonable expectation that the gene knockouts and the presence of the Cas9 protein could lead to increased toxicity or allergenicity in people or to increased toxicity in animals. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

<i>Risk Source</i>	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
<i>Causal Pathway</i>	<p>Growing GM wheat and barley at the trial site</p> <p>↓</p> <p>Pollen flow to other GM wheat or barley grown at the trial site</p> <p>↓</p> <p>GM wheat and barley composition is different from non-GM wheat and barley</p> <p>↓</p> <p>Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites OR Exposure of animals eating the GM wheat or barley</p>
<i>Potential Harm</i>	<p>Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals</p>

Risk source

132. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

133. The GM wheat and barley would be grown at the trial site and would produce pollen. When the GM wheat and barley flowers, GM pollen could be carried by wind to other GM wheat and barley plants nearby. If these are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM plant material. People or animals could be exposed to the hybrid GM plant material, if it is used for human food or animal feed, or by coming into contact with the hybrid GM plant material at the trial site.

134. It is possible that the different lines proposed for release would be planted close to one another during the trial. In addition, the GM wheat and barley may be grown in close proximity to other GM wheat or barley planted under licence DIR 186. Given that the different GM lines are sexually compatible and that they may have similar flowering times, pollen flow between plants with different introduced or knocked-out genes may occur. This may result in hybrid GM wheat and barley seeds with additional – ‘stacked’ – introduced or knocked-out genes for yield enhancement. If the CRISPR/Cas9 genetic element is present in the GM plant pollen, then this would also lead to an SDN-1 type change in the target locus of the genome of the recipient egg cell. This would result in an increase in the frequency of inheritance of the knocked-out genes.

135. 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.

136. Wheat and barley are mainly self-pollinating and where pollen dispersal does occur, the main method is wind. The chances of natural hybridisation occurring with sexually compatible plants are

low and decline significantly over distance, with most pollen falling within the first few metres (OGTR, 2021b, a).

137. Outcrossing rates in both wheat and barley are very low, and decrease as distance from the pollen source increases (DIR 186 RARMP). Outcrossing rates are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity (OGTR, 2021b, a). The GM wheat containing the introduced genes for yield enhancement and water use efficiency have been previously released under DIR 186. The RARMP for DIR 186 did not identify any information that would indicate an effect of these introduced genes on pollen characteristics leading to an increase in the likelihood of outcrossing. There have also been no reports of changes to phenotypic characteristics that may influence outcrossing rates from this previous release. Similarly, there is no current information to indicate that knockout of genes involved in spikelet development and flowering time (wheat) and altered plant architecture and nutrient use efficiency (barley) would influence pollen characteristics leading to an increase in the likelihood of outcrossing.

138. The low likelihood of cross pollination between GM wheat and barley grown under different licences is further reduced by the limits and controls imposed under the DIR 186 licence as well as those proposed for the current application. These would reduce the likelihood of presence and persistence of any hybrid GM wheat and barley at the trial site. For example, site monitoring and post-harvest monitoring requirements have been imposed under licence DIR 186. In addition, seeds, including any possible hybrid seeds, obtained from the trial authorised under DIR 186 must not be used for breeding or propagation to produce cultivars for future commercial release. Taken together, exposure of people or animals to any hybrid GM wheat and barley would be highly unlikely.

Potential harm

139. If pollen flow occurred between the GM wheat or the GM barley grown under DIR 201, or between lines from DIR 186 and DIR 201, it is possible that some GM hybrid seed may be produced. If this occurs, hybrid seeds and any resulting plants with new combinations of introduced or knocked-out genes which may be harmful to people or other organisms. It is noted that all of the GM wheat lines with introduced genes are the same as those released under DIR 186. Therefore, if hybridisation were to occur between these GM wheat lines and those released under DIR 186, then the same hybrid plants with the same gene combinations could occur. Therefore, only hybridisation between GM barley lines of DIR 201 and DIR 186 would result in new combinations of genetic modifications.

140. It is unlikely that any plants grown from the hybrid GM seeds would persist, due to post-harvest control measures to ensure removal of GM volunteers (Chapter 1, Section 2.2). Thus, exposure of people or other desirable animals to hybrid GM wheat and barley would be highly unlikely.

141. No substantive risks for toxicity or allergenicity as a result of introduction or knock-out of genes were identified in Risk Scenario 1 (above) and the RARMP for DIR 186. Likewise, there is no expectation that combinations of introduced and knocked-out genes will result in the production of novel proteins, or that their expression will be altered in a hybrid background, thus production of novel allergens or toxins is highly unlikely.

Conclusion

142. Risk scenario 2 is not identified as a substantive risk because of the proposed limits and controls for this application and also those imposed by the DIR 186 licence, and because wheat and barley are mainly self-pollinating with low levels of outcrossing. There is also no reasonable expectation that expression of the introduced genes or the knockout of genes could lead to increased toxicity or allergenicity in people or to increased toxicity in 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	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
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<i>Causal Pathway</i>	<p>Growing GM wheat and barley at the trial site</p> <p>↓</p> <p>Presence of GM wheat and barley outside the trial limits</p> <p>↓</p> <p>Spread and persistence of GM wheat and barley in the environment</p>
<i>Potential Harm</i>	<p>Increased toxicity or allergenicity for people</p> <p>OR</p> <p>Increased toxicity to desirable animals</p> <p>OR</p> <p>Reduced establishment and yield of desirable plants</p> <p>OR</p> <p>Reduced utility or quality of the environment</p> <p>OR</p> <p>Increased ability to provide a reservoir for pathogens or shelter for pests</p>

Risk source

143. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

144. GM wheat and barley would be grown at the trial site and would produce seed. If viable GM wheat and barley seeds remained at the trial site after completion of the trial, or if GM seed dispersed outside the trial site, volunteer GM wheat and barley may establish populations in the environment. These hybrids could then spread further and persist in the environment. This could increase the likelihood of exposure of people or desirable animals to the GM wheat and barley.

145. As discussed in Risk scenario 2, the different GM lines proposed for release would be planted close to one another, and to other GM wheat or barley planted under licence DIR 186. Pollen flow between these GM plants may result in hybrid GM wheat and barley seed with stacked traits for yield enhancement, including CRISPR/Cas9 genetic elements, which could also be dispersed from the trial site.

146. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for spread and persistence of the proteins were identified (See the respective RARMPs for details). There have not been any reports of changes in dispersal, establishment and survival from these earlier releases and the same containment measures as required under the DIR 186 licence have been proposed for this release. Therefore, the likelihood of dispersal of Group 1 and 2 GM lines will not be discussed further here.

Persistence of GM wheat and barley on the trial site

147. For GM wheat and barley seeds to be available to persist at the proposed trial site, seeds from any GM wheat or barley would need to drop to the ground during sowing and/or near maturity and harvest. During domestication, both non-GM wheat and barley have been selected for reduced shattering of seed heads – a mechanism for seed dispersal in ancestral wheat and barley plants (OGTR,

2021b, a). The knocked-out genes have not been linked to alterations in this trait in the GM wheat and barley proposed for release under the current application.

148. The applicant has proposed hand harvesting of seeds or use of a plot harvester, which would reduce the likelihood of seeds ending up on, or in the ground, when compared to the use of commercial harvesting equipment.

149. GM wheat or barley at the trial site could persist through dormant seeds in the seed bank. This could increase the number of volunteers at the site after the trial and provide seeds for spread to other areas. Although a range of factors in the environment can influence seed dormancy in both wheat and barley, neither species shows a high degree of dormancy or a persistent seed bank under Australian conditions (for details, see the [biology documents](#)). Importantly, both wheat and barley seeds germinate easily under favourable conditions which includes appropriate temperature while sufficient soil moisture is present. The knocked-out genes are not expected to alter seed dormancy in the GM wheat and barley.

150. The applicant proposes to remove or destroy all GM wheat and barley plants at the trial site after each harvest, but some seeds may remain. The applicant also proposes post-harvest monitoring for at least two years after the final harvest, as well as tillage and irrigation to encourage seed germination. Any wheat and barley volunteers found would be destroyed prior to flowering. In previous GM wheat and barley field trials in Australia, these control measures to minimise the persistence of GM wheat and barley at trial sites were considered appropriate.

Dispersal of GM seed outside the trial site

151. Seeds of the GM wheat and barley could be dispersed outside the trial site through the activity of people or through natural means, such as animals, wind and water. There is no reasonable expectation that the knocked-out genes would affect any of the seed characteristics important for dispersal.

152. Human activity is the most important dispersal pathway for non-GM wheat and barley seed (OGTR, 2021b, a). Important mechanisms for inadvertent seed dispersal by people include dispersal via equipment such as harvesters, and grain loss during transport. The applicant has proposed controls to reduce the likelihood of this occurring during the trial, including restricting access to the site; using trained staff; cleaning all equipment before removing it from the site or using it for any other purpose; and transporting and storing all GM wheat and barley in accordance with the [Regulator's Transport, Storage and Disposal of GMOs guidelines](#). These control measures would minimise dispersal of GM wheat and barley seed outside the trial site by human activity.

153. Animals can potentially spread plant seed by movement of seeds adhering to fur, feathers or feet, consumption and excretion of whole seeds, or by removing and hoarding seed (Chambers and MacMahon, 1994). Ingestion and excretion can affect seed viability and reduce the likelihood of germination (Cummings et al., 2008; Oveisi et al., 2021). Further details are available in the [DIR 186 RARMP](#) (Risk scenario 3).

154. The applicant proposes controls that would reduce the likelihood of seed dispersal by animals, including: fencing the site to limit access by large animals; a 10 metre wide monitoring zone where the vegetation is controlled which would also deter rodent activity; and using rodent bait or traps. The limited time frame during which viable seed would be available in each growing season and the small size of the trial would further reduce the likelihood of seed dispersal by animals.

155. Wheat and barley seeds are not usually dispersed by wind as domesticated wheat and barley have non-shattering seed heads, the seeds are heavy and they lack specialised structures to aid windborne dispersal (OGTR, 2021b, a). It is possible that some viable GM wheat or barley seeds could be dispersed by high winds if a severe storm occurred while mature seed was present on plants or the soil surface. Wheat and barley seeds on the soil surface could also be transported by water during heavy runoff or flooding. Proposed controls, including locating the trial site at least 50 m from any

natural waterway in areas not prone to flooding, would minimise the potential for seed dispersal through flooding.

Ability of the GM wheat and barley to establish populations in the environment

156. Wheat and barley are domesticated plants that have limited ability to survive outside cultivation (OGTR, 2021b, a). During domestication, both wheat and barley lost their natural seed dispersal mechanism of seed shattering and lost seed dormancy traits that allow seeds to delay germination until environmental conditions are favourable (OGTR, 2021b, a). This limited ability to survive outside cultivation is reflected in the weed risk ratings for wheat and barley (OGTR, 2021b, a). Although both crops have a long history of cultivation in Australia, neither is listed as a weed of national significance (National Weeds List, accessed 11 December 2023), nor as a significant weed in Australian ecosystems (Groves et al., 2003). Large weedy populations of wheat and barley are not observed in the agricultural or natural environment. There is no reasonable expectation that any of the knocked-out genes will alter characteristics such as seed shattering, other seed dispersal characteristics or seed dormancy which would alter the GMOs' ability to disperse and establish outside an agricultural setting.

157. The knocked-out genes are likely to be pleiotropic (that is, they have effects on several traits) thus potentially enhancing their ability to thrive in sub-optimal conditions. For example, a gene involved in abiotic stress tolerance may impart tolerance to a number of abiotic stresses or to biotic stresses (Howles and Smith, 2013). This may increase the ability of the GM wheat and barley to establish in agricultural, natural and intensive use areas, and may provide the GM wheat and barley with an advantage over non-GM wheat and barley. No studies have been conducted and this is an area of uncertainty for this risk assessment. However, tolerance to abiotic stress(es) or enhanced yield in an agricultural setting will not in isolation increase the invasiveness and persistence of the plants, due to the complexity of environmental conditions.

158. If the wheat and barley knockout plants have improved abiotic stress tolerance compared to non-GM wheat and barley, this could increase their ability to spread and persist in the environment. Knockout of the *ALOG-1* and *PDB-1* genes in the GM wheat is predicted to alter spikelet development and flowering time (Chapter 1, Section 4.3.3). The applicant has stated that these gene knockouts could increase or decrease the amount of grain from the wheat spikelet. Increased seed production is a factor that contributes to the invasiveness of plants (Keese et al., 2014). A recent laboratory study has shown that *ALOG-1* wheat knockout lines flowered faster and produced fewer spikelets compared to non-GM controls, but there is no information on the length of flowering or the number of grains produced (Gaughley, 2020). Knockout of genes involved in the strigolactone biosynthesis pathway in the GM barley may improve yield under nutrient limited conditions (Chapter 1, Section 4.3.4). Therefore, it might be expected that their competitive ability may be increased under poor nutrient conditions compared to non-GM barley. However, in order to increase weediness these characteristics would need to be coupled with other mechanisms that increase invasiveness through increased spread and persistence in the environment, through changes in dispersal, establishment and survival. Furthermore, proposed controls, including site monitoring and post-harvest requirements, would reduce the likelihood of spread and persistence of any GM wheat and barley seed outside the trial site.

Potential harm

159. If GM plants were able to establish outside the trial site, they could cause increased toxicity to people or animals, or increased allergenicity for people through increased exposure. As discussed in risk scenarios 1 and 2, no substantive risk was identified for increased toxicity or allergenicity of the GM wheat and barley, or any of their hybrids with other GM wheat or barley.

160. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No

substantive risks for spread and persistence of the GM wheat containing the expressed proteins were identified (see the respective RARMPs for details) and therefore will not be discussed further here.

161. If the GM wheat or barley with the knocked-out genes were able to establish outside the trial site, the quality of the biotic environment could be potentially reduced. This could occur through reduced establishment or yield of desirable plants in agricultural or natural land uses; reduced utility of intensive use areas, such as roadsides, drains or channels; or increased ability to provide a reservoir for pathogens or shelter for pests. However, none of the knocked-out genes have been reported to affect characteristics that would lead to an increase in these harms in the GM wheat or barley. For example, none of the knocked-out genes are known to make the GM wheat or barley susceptible to pathogens non-GM wheat or barley are resistant to; or to enable the GM wheat or barley to produce allelopathic substances which would negatively affect plant establishment around them. As none of the knocked-out genes are involved in relevant pathways, there is no reasonable expectation this may occur.

162. The ability of volunteer GM wheat and barley to compete with desirable plants is restricted because the genetic modifications are not expected to change the susceptibility of the GM wheat and barley to conventional weed management. Thus, GM wheat and barley volunteers could be controlled by standard weed management measures, such as cultivation or the use of appropriate herbicides, if required.

163. As discussed in risk scenarios 1 and 2, knocking out the genes are unlikely to change the GM wheat or barley composition such that they would more toxic or allergenic than the non-GM parents.

Conclusion

164. Risk scenario 3 is not identified as a substantive risk due to the proposed limits and controls designed to restrict dispersal, the limited ability of the GM wheat or barley to spread and persist outside the trial site and their susceptibility to standard weed control measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

<i>Risk Source</i>	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
<i>Causal Pathway</i>	<p>Pollen flow from GM wheat and barley to sexually compatible plants outside the trial site</p> <p>↓</p> <p>GM hybrid seed grows into volunteer plants</p> <p>↓</p> <p>Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas</p> <p>↓</p> <p>Increased exposure of people and desirable animals by ingestion of, or contact with, the GM hybrid plant material</p> <p>OR</p> <p>Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas</p>
<i>Potential Harm</i>	<p>Increased toxicity or allergenicity to people or toxicity to desirable animals</p> <p>OR</p> <p>Other environmental harms (see risk scenario 3)</p>

Risk source

165. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

166. The GM wheat and barley would be grown at the trial site and would produce pollen. When these plants flower, their pollen could be carried by wind to sexually compatible crops growing in the vicinity of the trial site. If these related crops are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM seed. If the introduced CRISPR/Cas9 genetic element is present in the GM pollen fertilising a non-GM flower, then this would lead to the expressed proteins causing SDN-1 changes in the target locus in the non-GM egg cell. The resulting GM plants could form the basis for establishment, spread and dispersal of the knocked-out genes in other varieties of wheat or barley, or other sexually compatible plant species. This could increase the likelihood of exposure of people or animals to the GM wheat and barley.

167. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the wheat and barley [biology documents](#). Relevant details have also been provided in the [DIR 186 RARMP](#) and Risk scenario 2.

168. Interspecific cross-pollination from bread wheat to durum wheat occurs at lower levels than intraspecific cross-pollination between bread wheat plants (Matus-Cádiz et al., 2004). Crossing of bread wheat to *Hordeum marinum* and other close relatives rarely occurs (OGTR, 2021b). Barley has a primary gene pool containing only one *H. vulgare* subspecies – which is not known to be present in Australia. Interspecific crosses within the *Hordeum* genus and intergeneric crosses have not been observed under natural conditions (OGTR, 2021a).

169. The proposed limits and controls for this trial would minimise the likelihood of pollen flow from the trial to related species. For example, no wheat or barley crops may be planted within at least 200 m of a planting area while GM wheat or barley are being cultivated, any sexually compatible species would be controlled within at least 50 m of a planting area during flowering, and GM wheat and barley volunteers would be destroyed before flowering during post-harvest monitoring.

170. Any hybrid seed resulting from vertical gene flow would need a suitable environment for germination, plant establishment and persistence (see Risk scenario 3). Volunteers can be controlled with integrated weed management practices.

Potential harm

171. If GM hybrid plants spread and persisted in the environment, this may lead to increased toxicity to people or other desirable animals, or allergenicity to people. Any offspring containing the knocked-out genes could also reduce the establishment and yield of desired plants and cause other environmental harms as per Risk scenario 3.

172. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for hybrid GM wheat containing the expressed proteins were identified (see the respective RARMPs for details) and therefore will not be discussed further here.

173. The knocked-out genes could be introduced, via vertical gene transfer, into other non-GM wheat, barley or other sexually compatible species. The properties the knocked-out genes confer are not expected to differ in a hybrid background. Therefore, in the event of vertical transfer from the GM wheat or barley lines to non-GM wheat or barley plants or sexually compatible species, it is expected that the knocked-out genes in any subsequent hybrids would confer the same properties as the GM parent.

174. As discussed in Risk scenarios 1-3, knocking out the genes are unlikely to change the GM wheat or barley characteristics such that they would more toxic or allergenic than the non-GM parents.

Conclusion

175. Risk scenario 4 is not identified as a substantive risk due to the limited occurrence of long-distance pollen flow for wheat and barley, and the proposed controls. In addition, Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs or their hybrids as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

176. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's [Risk Analysis Framework](#) document.

177. 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.

178. 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.

179. For DIR-201, uncertainty is noted particularly in relation to:

- expression patterns of the introduced genes in the GM plants
- potential for increased toxicity or allergenicity of the GM plants
- potential for the introduced or knockout genes to increase weediness of the GM plants.

180. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

181. 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.

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

Section 4 Risk evaluation

183. 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.

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

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

185. Four 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 4 and include:

- none of the GM plant material would be used for human food or animal feed
- 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 wheat and barley plants and their genetic material
- GM wheat and barley have limited ability to survive outside of cultivation
- GM wheat and barley volunteers could be controlled by various standard weed management methods.

186. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley 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

187. 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.

188. 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.

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

190. 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

191. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM wheat and barley. 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

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

3.1 Limits and controls on the release

193. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by the University of Adelaide. Many of these are discussed in the four risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections. Furthermore, many of the control measures replicate licence conditions as issued for [DIR 186](#).

3.1.1 Consideration of limits and controls proposed by The University of Adelaide

194. The applicant proposes that the release would take place at one site in Rosedale (SA). The field trial would run between May 2024 and January 2029, inclusive. A total of 2 hectares in any year can be used for planting of the GM plants. The applicant has stated that more than one planting area may be used at the site. The small size and short duration of the trial restricts the potential exposure of people and animals to the GMOs (Risk Scenario 1) and limits the opportunity for presence of the GMOs outside or after the trial (remaining risk scenarios).

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

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

196. The applicant states that the GM wheat and barley and its material would not be used for human food or animal feed. A licence condition prohibits the use of GM plant material in human food or animal feed. This measure would minimise exposure of people or animals to the GM wheat and barley by consumption (Risk scenario 1).

197. The applicant has indicated that the Rosedale property will have lockable gates on perimeter fences. Since restricting the dealings to only authorised personnel is considered appropriate for limiting exposure of humans to the GMOs, it is not considered necessary to have fences with lockable gates and hence this is not a licence condition. In addition, there is no evidence that the GM wheat and GM barley lines or hybrid GM wheat or barley lines would be more harmful to people or animals than the non-GM parental wheat or barley lines (Risk Scenarios 1 and 2).

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

198. Figure 2 in Chapter 1 shows a schematic diagram of the trial setup proposed by applicant. Each GM wheat and barley planting area is proposed to be surrounded with a 2 m buffer zone, where plant growth will be controlled. A 10 - 20 cm border of non-GM wheat will be planted as a pollen trap around each planting area, inside the buffer zone. The buffer zone is surrounded by a 10 m monitoring zone and a 50 m inspection zone. The monitoring and inspection zones would be inspected while the GMOs are flowering to destroy any wheat, barley, or sexually compatible plants. The inspection zone would be surrounded by a 140 m isolation zone where no wheat, barley, or sexually compatible plants would be deliberately grown. The combination of a 10 m monitoring zone, the 50 m inspection zone and a 140 m isolation zone were considered in Risk Scenarios 3 and 4 and in previous RARMPs (e.g. [DIR 186](#)). These isolation distances are expected to minimise pollen flow from the GMOs to non-GM plants outside the trial site, so are included in the licence. The 2 m buffer zone and pollen trap are not required to minimise pollen flow outside the trial site, so they have not been imposed under the conditions of the licence.

199. The applicant proposes that the monitoring and inspection zones would be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until all GMOs in the planting area have finished flowering. It is desirable to have one inspection after the completion of flowering of the GMOs, in case any plants were missed in the previous inspection, but no further inspections are necessary. Therefore, a licence condition requires the monitoring and inspection zones to be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering.

200. The applicant has stated that, under field conditions, the GM wheat lines expressing *PSTOL1* and *AVP1* (direct yield enhancement) flower 5-10 days earlier than non-GM plants within the same cultivar. The introduced genes for direct yield enhancement may also influence tillering in the GM wheat lines (Chapter 1, Section 4.5). Genetic modification of stomatal development and aperture and gene editing of *ALOG-1* and *PDB-1* may also reduce time to flowering in the GM wheat and barley plants (Chapter 1, Section 4.3 and 4.5). Earlier flowering in the GM lines could potentially alter the flowering period for the different GM lines, such that pollen would be present for a longer period, thus increasing the time during which gene

flow could occur. A monitoring zone of at least 10 m, kept free of volunteers and related species and maintained in a manner that facilitates the detection of such plants, would help to minimise the likelihood of gene flow from the planting area (Risk Scenarios 2 and 4). Gene flow is further minimised by licence conditions requiring the monitoring and inspection zones to be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering. Any volunteers or related species are to be destroyed or prevented from flowering.

201. The applicant proposes that more than one planting area 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 10 m monitoring zone surrounding the whole trial site (see Figure 1 in licence). Any land between planting areas is also considered part of the monitoring zone and would need to be maintained and inspected as such.

3.1.4 Consideration of proposed controls regarding persistence of the GMOs

202. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or future plantings. It is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed, to limit persistence of the GMOs. Licence conditions require that the trial site must be cleaned (which would destroy any surviving GM plants) within 35 days after harvest, and that harvested GM seed not required to conduct experiments or for future planting must be destroyed as soon as practicable. In addition, to deal with the case of failed crops that are not harvested, licence conditions require that GMOs must be harvested or destroyed within ten months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been cleaned.

203. Consistent with the DIR 186 licence, the licence for DIR 201 does not require the monitoring zone to be cleaned post-harvest. Experience of both the applicant and the OGTR is that there has been negligible dispersal of GMOs into the monitoring zone for similar previous trials of this type and size. If any GMOs are dispersed into areas within the monitoring zone, the licence specifies that the area would need to be cleaned and inspected for any volunteers.

204. The applicant has proposed that all waste material generated from harvest of the GM wheat and barley would be left in the planting area and either ploughed into the soil with crop stubble to the depth of seeding or burned/buried on site. They have also proposed that any waste material collected during cleaning would be destroyed using a method approved by the Regulator. These methods may include, but are not limited to, autoclaving, hammer-milling, incineration or burial to a depth of 1 m. Autoclaving, crushing and milling are considered effective for destruction, as they render seed non-viable, therefore minimising the likelihood of germination and/or spread. Deep burial of seed is also considered an effective method of destruction, therefore conditions allowing deep burial 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.

205. The applicant has proposed that areas used for destruction of plant material by burial, burning or incineration would take place in a clearly marked area, immediately adjacent to the trial site. The applicant proposes to inspect these areas for the presence of volunteers at least once every 35 days for two years, and until the site is free of volunteer plants for at least 6 months. A licence condition has been included where the burial site must not be intentionally disturbed for 12 months from the date of burial. If seed is dispersed during burial, this area would be considered an area in which the GMOs have been dispersed in the course of dealings under the licence, and post-cleaning conditions would apply.

206. The applicant has proposed that any non-GM wheat or barley planted as part of the field trial would be treated as if it were GM. Non-GM wheat or barley grown at the trial site may be cross-pollinated by the GM wheat and barley, resulting in hybrid seeds. It is therefore appropriate to require non-GM wheat and barley to be destroyed in the same manner as GM wheat and barley, to manage persistence of the GMOs, and this measure is included in the licence.

207. The Rosedale site is currently approved for planting of GM wheat and barley under licence DIR 186. The licence for DIR 186 permits planting until the end of the 2026/27 growing season (inclusive), so GMOs

from both DIR 186 and DIR 201 (if approved) could be grown concurrently at the same site in different planting areas. The applicant has proposed buffer zones and pollen traps that may reduce the amount of pollen flow between different planting areas at the site, but these measures will not eliminate crossing and so a buffer zone and pollen trap have not been imposed in the licence. The applicant has also indicated that, if needed, they may sow DIR 201 GMOs over planting areas that have been previously planted with DIR 186 GMOs that are in post-harvest monitoring. Plots may be sown directly over previous DIR 186 plots or offset to previous DIR 186 planting areas. As discussed in Chapter 1 (Section 4.1) and assessed in Risk scenario 2, the GM wheat lines released under DIR 186 contain the same introduced genes as those proposed for release under DIR 201. Therefore, no new gene combinations would occur if there was hybridisation between DIR 186 and DIR 201 GM wheat lines, as the same hybridisation could occur between DIR 201 GM lines. Hybridisation between DIR 186 and DIR 201 GM barley lines could result in new combinations of genes. Therefore, if a DIR 186 site is overplanted or if DIR 201 and DIR 186 sexually compatible lines are grown concurrently, a licence condition has been imposed whereby seed produced from the GMOs grown under DIR 201 must not be used for development of cultivars for potential future commercial release, unless it has been determined that the GM seed only contains the expected genetic modifications. The licence conditions also include requirements to notify the OGTR of planting area details, including the GPS coordinates, identity of the GMOs planted, and a history of how the area has been used for the previous two years. These conditions are expected to manage unintended mixing of seed or production of unexpected hybrids.

208. The applicant has proposed that any equipment used during the trials, including for seeding, harvesting, and threshing on site, will be inspected for seeds and cleaned as soon as practical after use and before it is used for other purposes. Cleaning would take place either in the trial area or in dedicated washdown facilities at the exit point for each trial site. Dedicated equipment would be used for the GM trial, where possible. A licence condition requires that any area used to clean equipment in connection with the GMOs, and any area where GMOs have dispersed in the course of dealings under this licence, must be cleaned as soon as practicable, and then monitored in the same way as the planting areas after cleaning.

209. After harvest, the applicant proposes to inspect the planting areas and monitoring zone at least once every 35 days for two years, until the site is free of volunteer plants for the last 6 months of the post-harvest inspection period. Any wheat or barley volunteers found would be destroyed prior to flowering, to prevent pollen flow to non-GM plants outside the trial site. Wheat typically requires 1275 degree-days² to grow from emergence to flowering (Bowden et al., 2008), which in hot weather (average daily temperature 26°C), would be about 49 days. Flowering in many barley varieties responds to day length as well as temperature, so development patterns can vary with latitude. Many varieties of barley pollinate while still in the head, so no physical flowering occurs. Sowing of most barley varieties grown in Australia occurs between early May and early June, depending on variety and location, so that flowering occurs from September to early October (OGTR, 2021a). Allowing for variation between cultivars and between individual plants, as well as early flowering of the GM wheat lines (Chapter 1, Section 4.5), monitoring the trial site at least every 35 days would be sufficient to detect volunteers before flowering. The total monitoring period of at least two years, with at least the last six months volunteer-free is expected to minimise persistence of GM wheat and barley at the trial site, so is included in a licence condition.

210. The applicant proposes at least one tillage to the depth of seeding within the planting areas, and three irrigations for each trial site during the post-harvest monitoring period. This will encourage germination of any remaining seed. There is a difference in germination rates between buried grain and grain lying on the surface; grains remaining near the surface, e.g. following shallow tillage after harvest, can generally easily germinate and become established (Ogg and Parker, 2000). An Australian field trial found that wheat seed banks were most persistent during dry seasons in no-tillage plots (Wicks et al., 2000). Shallow tillage after harvest, combined with irrigation, will germinate much of the seed lying on the surface

² The physiological development of a plant can be measured in degree-days, which is a means of combining time and temperature into a single number. Degree-days in wheat have been calculated as the sum of the average daily temperature, minus the minimum temperature at which the plant grows, over consecutive days (Bowden et al., 2008).

(Ogg and Parker, 2000). However, deep cultivation in certain soil types can reduce seed viability, but can also encourage prolonged dormancy in seeds as a result of a cool, moist low oxygen environment (Pickett, 1989; Ogg and Parker, 2000).

211. The Regulator considers that under Australian conditions, a post-harvest monitoring period of at least two years, with monthly inspections, and with no volunteers detected for a minimum of 6 months prior to the end of the time period, would effectively manage survival and persistence of viable wheat and barley seeds in the soil. Therefore, these measures are included in the licence. The licence contains conditions requiring that after harvest, the trial site should receive at least three irrigations, at intervals of at least 28 days, with the last required irrigation occurring at a time that would promote germination of volunteers within the final volunteer-free period. These measures will minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4).

212. The applicant proposes that rainfall events of greater than 10 mm in a 24 h period would be deemed to be equivalent to an irrigation event. A licence condition states that a period of natural rainfall may be taken as irrigation if it meets specified rainfall totals or is agreed to by the Regulator. Evidence (such as rainfall measurements, photos etc.) that the rainfall has been sufficient to promote germination may need to be provided. Additionally, prior to the last irrigation, the area must be tilled to a depth no greater than the depth of sowing. These treatments would ensure that seeds are exposed to sufficient moisture and placed at an appropriate depth for germination, as well as encouraging the microbial decomposition of any residual seed (Risk Scenarios 3 and 4).

213. The applicant has proposed that a 2 m buffer zone, kept free of vegetation, surround each planting area with specific inspection and cleaning requirements. A 2 m buffer zone has not been imposed under the conditions of the licence, however licence conditions do require any other areas where GM material has been dispersed, including during planting, harvest or threshing, must be inspected and volunteers and related species must be destroyed or prevented from flowering. The licence also requires harvest of GM wheat and barley to be conducted separately from other crops. These conditions are imposed to manage the likelihood for spread and persistence of the GMOs due to mechanical dispersal of grain during sowing and harvesting (Risk Scenario 3).

3.1.5 Consideration of proposed controls to limit dispersal of the GMOs

214. The applicant proposes to conduct harvest by hand or a dedicated plot harvester, and that all equipment used in connection with cultivating and harvesting the GMOs, such as harvesters, seeders, storage equipment, transport equipment (bags, container, trucks etc.), tools, shoes and other clothing, would be inspected for seeds and cleaned after use on site. The Rosedale site has a dedicated washdown facilities at its exit point, which allows for cleaning to occur prior to re-use or removal from the area. The applicant has stated that, where possible, dedicated equipment would be used for the GM trials. These measures would minimise human-mediated dispersal of GM plant material (Risk Scenario 3).

215. Threshing of wheat or barley after harvest would take place in the planting area or seed heads would be packaged and transported to approved facilities for threshing, analysis or other processing. As required for previous wheat and barley field trial licences issued by the Regulator, a licence condition states that GM wheat and barley must be threshed separately from any other crop, and threshing must take place on the planting areas, monitoring zones or in a facility approved by the Regulator. Any seed heads or grain for analysis would be bagged on site and transported to approved facilities for analysis according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. Any grain remaining after analysis would be stored in an approved facility for subsequent use or destroyed by autoclaving (or another method approved by the Regulator). These are standard conditions for the handling of GM plants to minimise exposure of people and other organisms to the GMOs (Risk Scenario 1 and 2), dispersal into the environment and gene flow/transfer (Risk Scenario 3 and 4).

216. The applicant has proposed to fence the trial site. Whilst animals will consume wheat or barley plant material, there is negligible risk of seed spread via livestock and there is no evidence that the GM wheat and barley would be more toxic to livestock than non-GM wheat or barley. A standard licence condition has been included in the licence which prohibits the use of plant material in this trial for food or feed, thus

livestock would not be allowed to feed on the GM wheat or barley (Risk Scenarios 1, 2 and 3). The applicant may achieve this requirement in a number of ways, not limited to fencing the trial site, so a fence would not be a requirement.

217. A variety of birds may feed on cereal crops, including wheat and barley, however a search of the literature found little evidence of extensive spread of seed via birds. Birds such as cockatoos do most damage to wheat during germination (Temby and Marshall, 2003). Emus may feed on wheat seed, but generally prefer other foods (Davies, 1978) and it is likely that germination rates of seed after digestion are low, although experimental evidence is sparse. Corellas and galahs will feed on wheat seed, but even under controlled conditions germination rates of seed were very low, ranging from 0.8% to 2% (Woodgate et al., 2011). The majority of wheat varieties grown in Australia are white wheat varieties (Blakeney et al., 2009) which have thin seed coats and are easily broken down during digestion (Temby and Marshall, 2003; Yasar, 2003). Viable barley seeds were not excreted by birds fed barley grain (Cummings et al., 2008; Woodgate et al., 2011), thus spread of barley by this route is highly unlikely. For these reasons, it is considered unnecessary to impose measures to control access of birds to the planting areas (Risk Scenario 3).

218. In addition, there is no evidence that the GM wheat and barley or hybrid GM wheat or barley lines would be more toxic to birds than the non-GM parental wheat or barley lines. Hence, there is no requirement to control access of birds to the GM wheat and barley lines with respect to Risk Scenarios 1 and 2.

219. Both wheat and barley seed may be spread through animal fur, feathers or muddy feet or hooves and barley seeds do have some structures which increase their ability to do so. However, the limited duration and size of the trial and the limited time in which viable seed is available reduces opportunities for contact with and spread of viable seed by large animals or birds. In addition, the requirement that livestock not be allowed to access viable grain further limits the likelihood of spread of wheat or barley seed via these routes (Risk Scenario 3).

220. Small animals including rodents may remove seed from the planting area, providing a potential means of dispersal (Risk Scenario 3). Although the applicant has not discussed the incidence of rodent activity at the site, they have proposed rodent control by use of traps and/or baits in the planting areas and surrounding areas and keeping the 2 m buffer zone surrounding each planting area where vegetation is heavily controlled. The applicant also proposes a 10 m monitoring zone, with vegetation kept mown at a maximum height of 10 cm. It has been a requirement of previous GM wheat and barley licences that the monitoring zone is maintained in a manner that does not attract or harbour rodents, such as keeping the area either free of vegetation or planted with vegetation mown to a height of less than 10 cm. This is expected to deter rodents from transporting seed through the monitoring zone, as well as facilitate the detection of GM plant material that has been dispersed during sowing and harvesting (Risk scenario 3).

221. As discussed in Risk Scenario 3, a combination of rodent baits and/or traps in the planting area in conjunction with a monitoring zone of at least 10 m, maintained in a manner that would deter rodents, would be adequate to minimise rodent activity, thus a 2 m buffer zone would not be required. Rodent control measures such as traps and/or baits in the planting area are a requirement under the conditions of the licence.

222. Both licence conditions (keeping vegetation short and rodent controls) apply while the GMOs are being grown and until the planting area is cleaned. Cleaning of a planting area, as defined in the licence, includes removal of most of the GM seeds from the soil surface where they could be readily accessed by rodents or dispersed by other means.

223. The applicant has proposed that the trial site would be located at least 50 m from any natural waterway and in areas that are not prone to flooding. This would reduce the likelihood of plant material being washed away from the planting areas (Risk Scenario 3). It is a standard licence condition that trial sites be located at least 50 m from waterways to limit the dispersal of viable plant material in the event of flooding. There is also a condition in the licence requiring immediate notification of any extreme weather event affecting the properties during the release to allow assessment and management of any risks.

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

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

- limit the duration of the release to the period from May 2024 and January 2029
- limit the release to one site in SA (Rosedale)
- limit the release to a combined total of 2 ha in any year
- locate trial site at least 50 m from any natural waterways
- surround the planting area(s) with a monitoring zone of at least 10 m, maintained in a manner that does not attract or harbour rodents, and in which related species must be prevented from flowering
- surround the monitoring zone with a 50 m inspection zone in which no wheat or barley may be planted and which must be inspected for volunteers and related species during flowering
- surround the inspection zone with a 140 m isolation zone in which no wheat, barley or related species may be grown
- implement measures including rodent baits and/or traps to control rodents within the planting areas
- harvest the GM wheat and barley separately from other crops
- harvest the GM wheat and barley by hand or with a dedicated plot harvester
- clean the areas after use including the planting area and any area in which seed has been dispersed
- clean any equipment used on site after use
- apply measures to promote the germination of any wheat or barley seeds that may be present in the soil after harvest, including irrigation and shallow tillage
- monitor for at least 24 months after harvest and destroy any wheat or barley plants that may grow, until no volunteers have been detected for a continuous six-months period
- destroy all GMOs not required for further analysis or future trials
- transport and store the GMOs in accordance with the Regulator's guidelines
- not use GM seeds to develop future wheat and barley cultivars if there is the potential of hybridisation/mixing with GMOs authorised under other licences
- not allow the GM plant material to be used for human food or animal feed.

3.2 Other risk management considerations

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

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

3.2.1 Applicant suitability

226. 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
- the capacity of the applicant to meet the conditions of the licence.

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

228. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 Contingency plans

229. If a licence were issued, The University of Adelaide would be required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM wheat and barley outside permitted areas.

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

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

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

3.2.4 Reporting requirements

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

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

233. 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 with the GMOs
- expected dates of flowering
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

234. 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 would continue until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

235. 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.

236. 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

237. Additional information has been identified that may be required to assess an application for a commercial release of the GM wheat and barley or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM wheat and barley lines, particularly with respect to potential for increased toxicity and allergenicity
- additional phenotypic characterisation of the GM wheat and barley lines, particularly with respect to traits that may contribute to weediness
- additional sequencing data to confirm that CRISPR/Cas9 genetic elements and residual T-DNA have been segregated from the gene edited wheat and barley lines.

Section 5 Conclusions of the consultation RARMP

238. The risk assessment concludes that the proposed limited and controlled release of GM wheat and barley 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.

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

References

- Abrash, E., Anleu Gil, M.X., Matos, J.L., and Bergmann, D.C. (2018). Conservation and divergence of YODA MAPKKK function in regulation of grass epidermal patterning. *Development* **145**.
- AGRI-FACTS (2002). Mice and their control. Report No. Agdex 683. (Alberta Agriculture, Food and Rural Development).
- Albretsen, J. (2006). The toxicity of iron, an essential element. *Veterinary Medicine Feb 2006*, 82-90.
- Alieva, N.O., Konzen, K.A., Field, S.F., Meleshkevitch, E.A., Hunt, M.E., Beltran-Ramirez, V., Miller, D.J., *et al.* (2008). Diversity and evolution of coral fluorescent proteins. *PLoS One* **3**, e2680.
- ANZFA (2001). Draft risk analysis report - Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and oil derived from glufosinate-ammonium tolerant and pollination controlled lines MS1, MS8, RF2 and RF3. Report No. 13/01. (Canberra, Australia: Australia New Zealand Food Authority).
- Aronson, R., Bruckner, A., Moore, J., Precht, B., E., W., and porites, P. (2008). *Porites porites*. Accessed: 15 November 2021.
- Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical Reviews in Toxicology* **36**, 219-251.
- Astwood, J.D., Mohapatra, S.S., Ni, H., and Hill, R.D. (1995). Pollen allergen homologues in barley and other crop species. *Clinical and Experimental Allergy* **25**, 66-72.
- Balmadrid, C., and Bono, M. (2009). Recognizing and managing iron toxicity. *Emergency Medicine* **41**, 36-41.
- Beasley, J.T., Bonneau, J.P., Moreno-Moyano, L.T., Callahan, D.L., Howell, K.S., Tako, E., Taylor, J., *et al.* (2022). Multi-year field evaluation of nicotianamine biofortified bread wheat. *The Plant Journal* **109**, 1168-1182.
- Beasley, J.T., Bonneau, J.P., Sánchez-Palacios, J.T., Moreno-Moyano, L.T., Callahan, D.L., Tako, E., Glahn, R.P., *et al.* (2019). Metabolic engineering of bread wheat improves grain iron concentration and bioavailability. *Plant Biotechnology Journal* **17**, 1514-1526.
- Blakeney, A.B., Cracknell, R.L., Crosbie, G.B., Jefferies, S.P., Miskelly, D.M., O'Brien, L., Panozzo, J.F., *et al.* (2009). Understanding Australian wheat quality. A basic introduction to Australian wheat quality. (Grains Research and Development Corporation).
- Bowden, P., Edwards, J., Ferguson, N., McNee, T., Manning, B., Roberts, K., Schipp, A., *et al.* (2008). Wheat growth and development. (NSW DPI).
- Brewer, P.B., Yoneyama, K., Filardo, F., Meyers, E., Scaffidi, A., Frickey, T., Akiyama, K., *et al.* (2016). LATERAL BRANCHING OXIDOREDUCTASE acts in the final stages of strigolactone biosynthesis in Arabidopsis. *Proceedings of the National Academy of Sciences* **113**, 6301-6306.
- Campbell, R.E., Tour, O., Palmer, A.E., Steinbach, P.A., Baird, G.S., Zacharias, D.A., and Tsien, R.Y. (2002). A monomeric red fluorescent protein. *Proceedings of the National Academy of Sciences* **99**, 7877.
- Cao, P., Fan, W., Li, P., and Hu, Y. (2021). Genome-wide profiling of long noncoding RNAs involved in wheat spike development. *BMC genomics* **22**, 1-14.

CERA (2011). A Review of the Environmental Safety of the PAT Protein. (Center for Environmental Risk Assessment, ILSI Research Foundation).

Cerise, M., Giaume, F., Galli, M., Khahani, B., Lucas, J., Podico, F., Tavakol, E., *et al.* (2021). OsFD4 promotes the rice floral transition via florigen activation complex formation in the shoot apical meristem. *New Phytol* 229, 429-443.

Chambers, J.C., and MacMahon, J.A. (1994). A Day in the Life of a Seed: Movements and Fates of Seeds and Their Implications for Natural and Managed Systems. *Annual Review of Ecology and Systematics* 25, 263-292.

Clemens, S., and Ma, J.F. (2016). Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu Rev Plant Biol* 67, 489-512.

Cummings, J.L., Handley, L.W., MacBryde, B., Tupper, S.K., Werner, S.J., and Byram, Z.J. (2008). Dispersal of viable row-crop seeds of commercial agriculture by farmland birds: implication for genetically modified crops. *Environmental Biosafety Research* 7, 241-252.

Davies, S.J.J.F. (1978). The food of emus. *Australian Journal of Ecology* 3, 411-422.

Dunn, J., Hunt, L., Afsharinafar, M., Meselmani, M.A., Mitchell, A., Howells, R., Wallington, E., *et al.* (2019). Reduced stomatal density in bread wheat leads to increased water-use efficiency. *Journal of Experimental Botany* 70, 4737-4748.

El-Mounadi, K., Morales-Florian, M.L., and Garcia-Ruiz, H. (2020). Principles, applications, and biosafety of plant genome editing using CRISPR-Cas9. *Frontiers in plant science* 11, 56.

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

Flohr, B. (2018). Stabilising the flowering time of wheat in response to autumn rainfall decline in southern Australia. Doctor of Philosophy Thesis (The Australian National University).

Flora, S.J., Mittal, M., and Mehta, A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J Med Res* 128, 501-523.

Franks, P.J., Doheny-Adams, T.W., Britton-Harper, Z.J., and Gray, J.E. (2015). Increasing water-use efficiency directly through genetic manipulation of stomatal density. *New Phytologist* 207, 188-195.

FSANZ (2005a). Final assessment report - Application A543: Food derived from Insect-protected, glufosinate ammonium-tolerant corn line 59122-7. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2005b). Final assessment report - Application A553: Food derived from glyphosate-tolerant cotton line MON 88913. (Canberra: Food Standards Australia New Zealand).

FSANZ (2008). Final assessment report - Application A589: Food derived from glufosinate ammonium tolerant rice line LLRICE62. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2010a). Application A1028: Food derived from insect-protected & herbicide-tolerant cotton line T304-40 - Approval report. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2010b). Application A1040: Food derived from insect-protected and herbicide-tolerant cotton line GHB119 - Approval report. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2013). Approval report - Application A1080. Food derived from herbicide-tolerant cotton line MON 88701. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2017). A1140 – Food derived from Herbicide-tolerant Canola Line MS11: Supporting document 1 - Safety Assessment (at Approval). (Canberra, Australia: Food Standards Australia New Zealand).

Gamuyao, R., Chin, J.H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., *et al.* (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature* 488, 535-539.

Gaughley, A. (2020). Connecting the dots for flowering time genes in wheat. Doctor of Philosophy Thesis (University of East Anglia).

Gaxiola, R.A., Li, J., Undurraga, S., Dang, L.M., Allen, G.J., Alper, S.L., and Fink, G.R. (2001). Drought- and salt-tolerant plants result from overexpression of the AVP1 H⁺-pump. *PNAS* 98, 11444-11449.

Gaxiola, R.A., Rao, R., Sherman, A., Grisafi, P., Alper, S.L., and Fink, G.R. (1999). The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. *Proceedings of the National Academies of Sciences* 96, 1480-1485.

Geiger, D., Scherzer, S., Mumm, P., Stange, A., Marten, I., Bauer, H., Ache, P., *et al.* (2009). Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *PNAS* 106, 21425-21430.

Gray, J.E., and Hetherington, A.M. (2004). Plant development: YODA the stomatal switch. *Current Biology* 14, R488-490.

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

Hahn, F., and Nekrasov, V. (2018). CRISPR/Cas precision: do we need to worry about off-targeting in plants? *Plant Cell Rep preprint*.

Hayta, S., Smedley, M.A., Clarke, M., Forner, M., and Harwood, W.A. (2021). An efficient *Agrobacterium*-mediated transformation protocol for hexaploid and tetraploid wheat. *Current Protocols* 1, e58.

Hedrich, R., and Geiger, D. (2017). Biology of SLAC1-type anion channels - from nutrient uptake to stomatal closure. *New Phytologist* 216, 46-61.

Hegde, S.G., and Waines, J.G. (2004). Hybridization and introgression between bread wheat and wild and weedy relatives in North America. *Crop Science* 44, 1145-1155.

Hepworth, C., Caine, R.S., Harrison, E.L., Sloan, J., and Gray, J.E. (2018). Stomatal development: focusing on the grasses. *Current Opinion in Plant Biology* 41, 1-7.

Hérouet, C., Esdaile, D.J., Mallyon, B.A., Debruyne, E., Schulz, A., Currier, T., Hendrickx, K., *et al.* (2005). Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. *Regulatory Toxicology and Pharmacology* 41, 134-149.

Hill, G.J.E., Barnes, A., and Wilson, G.R. (1988). The use of wheat crops by grey kangaroos, *Macropus giganteus*, in southern Queensland. *Wildlife Research* 15, 111-117.

Houba, R., Heederik, D., and Doekes, G. (1998). Wheat sensitization and work-related symptoms in the baking industry are preventable. An epidemiologic study. *Am J Respir Crit Care Med* **158**, 1499-1503.

Howles, P., and Smith, J. (2013). Risk assessment of abiotic stress tolerant GM crops. In *Improving Crop Productivity in Sustainable Agriculture*, N. Tuteja, S.S. Gill, and R. Tuteja, eds. (Wiley-Blackwell), pp. 163-181.

Hufnagel, B., de Sousa, S.M., Assis, L., Guimaraes, C.T., Leiser, W., Azevedo, G.C., Negri, B., *et al.* (2014). Duplicate and conquer: Multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils. *Plant Physiology* **166**, 659-677.

Hunt, J. (2015). Novel wheat genotypes for early sowing across Australian wheat production environments. *Building Productive, Diverse and Sustainable Landscapes*.

Ibrahim, J., Eisen, J.A., Jospin, G., Coil, D.A., Khazen, G., and Tokajian, S. (2016). Genome analysis of *Streptococcus pyogenes* associated with pharyngitis and skin infections. *PLoS One* **11**, e0168177.

Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S., and Nishizawa, N.K. (2003). Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *The Plant Journal* **36**, 366-381.

Jach, G., Binot, E., Frings, S., Luxa, K., and Schell, J. (2001). Use of red fluorescent protein from *Discosoma* sp. (dsRED) as a reporter for plant gene expression. *Plant J* **28**, 483-491.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., and Beeregowda, K.N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol* **7**, 60-72.

Jiang, L., Liu, X., Xiong, G., Liu, H., Chen, F., Wang, L., Meng, X., *et al.* (2013). DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature* **504**, 401-405.

Kaneko-Suzuki, M., Kurihara-Ishikawa, R., Okushita-Terakawa, C., Kojima, C., Nagano-Fujiwara, M., Ohki, I., Tsuji, H., *et al.* (2018). TFL1-Like Proteins in Rice Antagonize Rice FT-Like Protein in Inflorescence Development by Competition for Complex Formation with 14-3-3 and FD. *Plant Cell Physiol* **59**, 458-468.

Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* **7**, 123-149.

Keese, P.K., Robold, A.V., Myers, R.C., Weisman, S., and Smith, J. (2014). Applying a weed risk assessment approach to GM crops. *Transgenic Research* **23**, 957-969.

Kelly, J.H., Tucker, M.R., and Brewer, P.B. (2023). The strigolactone pathway is a target for modifying crop shoot architecture and yield. *Biology* **12**, 95.

Kettenburg, A.T., Lopez, M.A., Yogendra, K., Prior, M.J., Rose, T., Bimson, S., Heuer, S., *et al.* (2023). PHOSPHORUS-STARVATION TOLERANCE 1 (*OsPSTOL1*) is prevalent in upland rice and enhances root growth and hastens low phosphate signaling in wheat. *Plant, Cell & Environment* **46**, 2187-2205.

Khadilkar, A.S., Yadav, U.P., Salazar, C., Shulaev, V., Paez-Valencia, J., Pizzio, G.A., Gaxiola, R.A., *et al.* (2016). Constitutive and companion cell-specific overexpression of AVP1, Encoding a proton-pumping pyrophosphatase enhances biomass accumulation, phloem loading, and long-distance transport. *Plant Physiology* **170**, 401-414.

Kinch, J., Teitelbaum, A., and Pippard, H. (2010). *Proceedings of the Regional Workshop on Trade in Corals and Determining Non-detrimental Findings. (Secretariat of the Pacific Community Coastal Fisheries Programme).*

- Kinoshita, T., Ono, N., Hayashi, Y., Morimoto, S., Nakamura, S., Soda, M., Kato, Y., *et al.* (2011). FLOWERING LOCUS T Regulates Stomatal Opening. *Current Biology* 21, 1232-1238.
- Le, J., Zou, J., Yang, K., and Wang, M. (2014). Signaling to stomatal initiation and cell division. *Frontiers in Plant Science* 5, 1-6.
- Li, B., Wei, A., Song, C., Li, N., and Zhang, J. (2008). Heterologous expression of the *TsVP* gene improves the drought resistance of maize. *Plant Biotechnology Journal* 6, 146-159.
- Li, J., Yang, H., Peer, W.A., Richter, G., Blakeslee, J., Bandyopadhyay, A., Titapiwantakun, B., *et al.* (2005). *Arabidopsis* H⁺-PPase AVP1 regulates auxin-mediated organ development. *Science* 310, 121-125.
- Liu, H., Song, S., and Xing, Y. (2019). Beyond heading time: FT-like genes and spike development in cereals. *J Exp Bot* 70, 1-3.
- Liu, T., Ohashi-Ito, K., and Bergmann, D.C. (2009). Orthologs of *Arabidopsis thaliana* stomatal bHLH genes and regulation of stomatal development in grasses. *Development* 136, 2265-2276.
- Lv, S., Zhang, K., Gao, Q., Lian, L., Song, Y., and Zhang, J. (2008). Overexpression of an H⁺-PPase gene from *Thellungiella halophila* in cotton enhances salt tolerance and improves growth and photosynthetic performance. *Plant and Cell Physiology* 49, 1150-1164.
- Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R., Wang, B., *et al.* (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Molecular plant* 8, 1274-1284.
- Mann, D.G., Abercrombie, L.L., Rudis, M.R., Millwood, R.J., Dunlap, J.R., and Stewart, C.N., Jr. (2012). Very bright orange fluorescent plants: endoplasmic reticulum targeting of orange fluorescent proteins as visual reporters in transgenic plants. *BMC Biotechnol* 12, 17.
- Marzec, M., Situmorang, A., Brewer, P.B., and Braszewska, A. (2020). Diverse Roles of MAX1 Homologues in Rice. *Genes (Basel)* 11.
- Matthews, P.R., Wang, M.B., Waterhouse, P.M., Thornton, S., Fieg, S.J., Gubler, F., and Jacobsen, J.V. (2001). Marker gene elimination from transgenic barley, using co-transformation with adjacent 'twin T-DNAs' on a standard *Agrobacterium* transformation vector. *Molecular Breeding* 7, 195-202.
- Matus-Cádiz, M.A., Hucl, P., Horak, M.J., and Blomquist, L.K. (2004). Gene flow in wheat at the field scale. *Crop Science* 44, 718-727.
- Milner, M.J., Bowden, S., Craze, M., and Wallington, E.J. (2023). OsPSTOL but not TaPSTOL can play a role in nutrient use efficiency and works through conserved pathways in both wheat and rice. *Frontiers in Plant Science* 14, 1098175.
- Modrzejewski, D., Hartung, F., Lehnert, H., Sprink, T., Kohl, C., Keilwagen, J., and Wilhelm, R. (2020). Which factors affect the occurrence of off-target effects caused by the use of CRISPR/Cas: a systematic review in plants. *Frontiers in plant science* 11, 574959.
- Nan, W., Shi, S., Jeewani, D.C., Quan, L., Shi, X., and Wang, Z. (2018). Genome-wide identification and characterization of wALOG family genes involved in branch meristem development of branching head wheat. *Genes* 9, 510.
- Naramoto, S., Hata, Y., and Kyojuka, J. (2020). The origin and evolution of the ALOG proteins, members of a plant-specific transcription factor family, in land plants. *Journal of plant research* 133, 323-329.

OECD (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Report No. ENV/JM/MONO(99)13. (Organisation for Economic Cooperation and Development).

OECD (2002). Series on Harmonization of Regulatory Oversight in Biotechnology, No 25. Module II: Phosphinothricin. Report No. ENV/JM/MONO(2002)14. (Organisation for Economic Cooperation and Development).

Ogg, A.G., and Parker, R. (2000). Control of volunteer crop plants. Report No. EB 1523. (Washington State University Cooperative Extension).

OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator).

OGTR (2021a). The Biology of *Hordeum vulgare* L. (barley) Version 2.1. (Office of the Gene Technology Regulator).

OGTR (2021b). The Biology of *Triticum aestivum* L. (Bread Wheat) Version 3.2. (Office of the Gene Technology Regulator).

Oveisi, M., Ojaghi, A., Rahimian Mashhadi, H., Müller-Schärer, H., Reza Yazdi, K., Pourmorad Kaleibar, B., and Soltani, E. (2021). Potential for endozoochorous seed dispersal by sheep and goats: Risk of weed seed transport via animal faeces. *Weed Research* 61, 1-12.

Pahr, S., Constantin, C., Mari, A., Scheiblhofer, S., Thalhamer, J., Ebner, C., Vrtala, S., *et al.* (2012). Molecular characterization of wheat allergens specifically recognized by patients suffering from wheat-induced respiratory allergy. *Clin Exp Allergy* 42, 597-609.

Park, S., Li, J., Pittman, J.K., Berkowitz, G.A., Yang, H., Undurraga, S., Morris, J., *et al.* (2005). Up-regulation of a H⁺-pyrophosphatase (H⁺-PPase) as a strategy to engineer drought-resistant crop plants. *PNAS* 102, 18830-18835.

Pei, L., Wang, J., Li, K., Li, Y., Li, B., Gao, F., and Yang, A. (2012). Overexpression of *Thellungiella halophila* H⁺-pyrophosphatase gene improves low phosphate tolerance in maize. *PLoS ONE* 7, e43501. doi:43510.41371/journal.pone.0043501.

Peterson, K.M., Rychel, A.L., and Torii, K.U. (2010). Out of the mouths of plants: the molecular basis of the evolution and diversity of stomatal development. *Plant Cell* 22, 296-306.

Philips, J.G., Martin-Avila, E., and Robold, A.V. (2022). Horizontal gene transfer from genetically modified plants - Regulatory considerations. *Front Bioeng Biotechnol* 10, 971402.

Pickett, A.A. (1989). A review of seed dormancy in self-sown wheat and barley. *Plant Varieties and Seeds* 2, 131-146.

Pillitteri, L.J., Sloan, D.B., Bogenschutz, N.L., and Torii, K.U. (2007). Termination of asymmetric cell division and differentiation of stomata. *Nature* 445, 501-505.

Qi, X., and Torii, K.U. (2018). Hormonal and environmental signals guiding stomatal development. *BMC Biology* 16, 1-21.

Qureshi, A., and Connolly, J.B. (2023). Bioinformatic and literature assessment of toxicity and allergenicity of a CRISPR-Cas9 engineered gene drive to control *Anopheles gambiae* the mosquito vector of human malaria. *Malaria Journal* 22, 234.

- Raissig, M., Matos, J.L., Ximena Anleu Gil, M., Kornfield, A., Bettadapur, A., Abrash, E., Allison, H.R., *et al.* (2017). Mobile MUTE specifies subsidiary cells to build physiologically improved grass stomata. *Science* 355, 1215-1218.
- Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature protocols* 8, 2281-2308.
- Regmi, K.C., Yogendra, K., Farias, J.G., Li, L., Kandel, R., Yadav, U.P., Sha, S., *et al.* (2020). Improved yield and photosynthate partitioning in AVP1 expressing wheat (*Triticum aestivum*) plants. *Frontiers in Plant Science* 11, 273.
- Ritala, A., Nuutila, A.M., Aikasalo, R., Kauppinen, V., and Tammisola, J. (2002). Measuring gene flow in the cultivation of transgenic barley. *Crop Science* 42, 278-285.
- Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C., *et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research* 24, 1-17.
- Shemiakina, I.I., Ermakova, G.V., Cranfill, P.J., Baird, M.A., Evans, R.A., Souslova, E.A., Staroverov, D.B., *et al.* (2012). A monomeric red fluorescent protein with low cytotoxicity. *Nat Commun* 3, 1204.
- Shirley, B.W., Hanley, S., and Goodman, H.M. (1992). Effects of ionizing radiation on a plant genome: analysis of two *Arabidopsis* *transparent testa* mutations. *Plant Cell* 4, 333-347.
- Singh, S.P., Keller, B., Gruissem, W., and Bhullar, N.K. (2017). Rice *NICOTIANAMINE SYNTHASE 2* expression improves dietary iron and zinc levels in wheat. *Theor Appl Genet* 130, 283-292.
- Smedley, M.A., Hayta, S., Clarke, M., and Harwood, W.A. (2021). CRISPR-Cas9 based genome editing in wheat. *Current Protocols* 1, e65.
- Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. *Toxicological Sciences* 71, 2-8.
- Soyars, C.L., Peterson, B.A., Burr, C.A., and Nimchuk, Z.L. (2018). Cutting edge genetics: CRISPR/Cas9 editing of plant genomes. *Plant Cell Physiol* 59, 1608-1620.
- Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., *et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. *Plant Physiology* 161, 1587-1594.
- Stogios, P.J., Shakya, T., Evdokimova, E., Savchenko, A., and Wright, G.D. (2011). Structure and function of APH(4)-Ia, a hygromycin B resistance enzyme. *The Journal of Biological Chemistry* 286, 1966-1975.
- Sturme, M.H., van der Berg, J.P., Bouwman, L.M., De Schrijver, A., de Maagd, R.A., Kleter, G.A., and Battaglia-de Wilde, E. (2022). Occurrence and nature of off-target modifications by CRISPR-Cas genome editing in plants. *ACS Agricultural Science & Technology* 2, 192-201.
- Takeda, S., Hanano, K., Kariya, A., Shimizu, S., Zhao, L., Matsui, M., Tasaka, M., *et al.* (2011). CUP-SHAPED COTYLEDON1 transcription factor activates the expression of LSH4 and LSH3, two members of the ALOG gene family, in shoot organ boundary cells. *Plant J* 66, 1066-1077.
- Tang, X., Liu, G., Zhou, J., Ren, Q., You, Q., Tian, L., Xin, X., *et al.* (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. *Genome Biol* 19, 84.

- Taylor, T. (2016). *Porites porites* (Finger Coral). (UWI St. Augustine) Accessed: 3 November 2021.
- Temby, I., and Marshall, D. (2003). Reducing cockatoo damage to crops. Landcare Notes. Report No. LC0009. (State of Victoria, Department of Sustainability and Environment).
- Thompson, C.J., Movva, N.R., Tizard, R., Cramer, R., Davies, J., Lauwereys, M., and Botterman, J. (1987). Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. *EMBO Journal* 6, 2519-2523.
- Tingay, S., McElroy, D., Kalla, R., Feig, S., Wang, M., and Thornton, S. (1997). *Agrobacterium tumefaciens*-mediated barley transformation. *Plant Journal* 11, 1369-1376.
- Trijatmiko, K.R., Duenas, C., Tsakirpaloglou, N., Torrizo, L., Arines, F.M., Adeva, C., Balindong, J., *et al.* (2016). Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Sci Rep* 6, 19792.
- Vahisalu, T., Kollist, H., Wang, Y.F., Nishimura, N., Chan, W.Y., Valerio, G., Lamminmaki, A., *et al.* (2008). SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. *Nature* 452, 487-491.
- Virtue, J.G. (2008). SA weed risk management guide. (Adelaide: Government of South Australia: Department of Water, Land and Biodiversity Conservation).
- von Wiren, N., Klair, S., Bansal, S., Briat, J.F., Khodr, H., Shioiri, T., Leigh, R.A., *et al.* (1999). Nicotianamine chelates both FeIII and FeII. Implications for metal transport in plants. *Plant Physiol* 119, 1107-1114.
- Wagner, D.B., and Allard, R.W. (1991). Pollen migration in predominantly self-fertilizing plants: barley. *Journal of Heredity* 82, 302-304.
- Wang, X., Tu, M., Wang, Y., Yin, W., Zhang, Y., Wu, H., Gu, Y., *et al.* (2021). Whole-genome sequencing reveals rare off-target mutations in CRISPR/Cas9-edited grapevine. *Horticulture research* 8.
- Wicks, G.A., Felton, W.L., Murison, R.D., and Martin, R.J. (2000). Changes in fallow weed species in continuous wheat in northern New South Wales, 1981-90. *Australian Journal of Experimental Agriculture* 40, 831-842.
- Woodgate, J.L., Steadman, K.J., and Buchanan, K.L. (2011). A study of seed viability following consumption by birds. (Unpublished final report submitted to the OGTR).
- Yang, H., Knapp, J., Koirala, P., Rajagopal, D., Peer, W.A., Silbart, L.K., Murphy, A., *et al.* (2007). Enhanced phosphorus nutrition in monocots and dicots over-expressing a phosphorus-responsive type I H⁺-pyrophosphatase. *Plant Biotechnology Journal* 5, 735-737.
- Yasar, S. (2003). Performance of broiler chickens on commercial diets mixed with whole or ground wheat of different varieties. *International Journal of Poultry Science* 2, 62-70.
- Yoneyama, K., and Brewer, P.B. (2021). Strigolactones, how are they synthesized to regulate plant growth and development? *Current Opinion in Plant Biology* 63, 102072.
- Yoshida, A., Suzaki, T., Tanaka, W., and Hirano, H.-Y. (2009). The homeotic gene long sterile lemma (G1) specifies sterile lemma identity in the rice spikelet. *Proceedings of the National Academy of Sciences* 106, 20103-20108.

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities³ 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	Notes that the licence will prohibit use of the GM plant material in human food or animal feed. No further comments on the licence application at this stage.	Noted.
2	Does not have any advice or comments on the RARMP.	Noted.
3	Agrees that all plausible risk scenarios have been identified and did not identify additional relevant information that should be considered. Agrees that the proposed limits and controls for the GM wheat and barley are appropriate. Agrees with the overall conclusions of the RARMP.	Noted.
4	Notes that the RARMP concludes that the proposed release poses negligible risks to human health and safety and the environment, and that these negligible risks do not require specific risk treatment measures. Notes that if a licence is issued, conditions would be imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.	Noted.
	Notes some uncertainty around expression of introduced genes, potential increased toxicity or allergenicity to people and potential for increased weediness in the GM wheat and barley. However, given that these issues were not detected in previous releases, agrees that the limited and controlled release of these GM wheat and barley lines pose negligible risks to the health and safety of people or the environment.	Noted. Uncertainty for DIR-201 is discussed in Chapter 2, Section 3 of the RARMP. 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.

³ Prescribed experts, agencies and authorities include GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
5	Agrees that the proposed work poses minimal risks to humans, plants and the environment, and that the level of proposed risk minimisation activities is acceptable.	Noted.
6	No concerns regarding the DIR 201 application.	Noted.

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

The Regulator received three 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	Against the GM wheat and barley field trial and the genetic modification of these grains.	Noted.
	Wheat and barley are widely used in foods and there is no easy way to track GM grains once they are produced.	DIR-201 is a limited and controlled release to assess the performance of the GM wheat and barley under field conditions. Strict licence conditions are imposed to ensure the GM plant material stays within the field trial sites. The GM wheat and barley grown in this field trial would not be used in human food or animal feed.
	The introduction of GM wheat and barley may benefit short-term sales at the expense of the long-term health of people, which may not be known for generations.	DIR-201 is a short-term field trial of the GM wheat and barley. Risks to the health and safety of people as a result of this trial were considered to be negligible.
	Australia would be a stronger export nation by growing and moving to organic wheat and barley.	Matters related to marketing or trade issues, consumer preferences and coexistence regimes are outside the Regulator's legislative responsibility. These issues are the responsibility of the State and Territory governments and industry.
2	Stop messing up lives by allowing genetic modification.	The functions of the Gene Technology Regulator (the Regulator) are defined by the <i>Gene Technology Act 2000</i> (the Act) which is legislation passed by the Parliament of Australia. The Regulator must consider each application for a licence for dealings with GMOs based on criteria listed in the Act.
3	Against the proposed DIR-201 field trial of GM wheat and barley.	Noted.
	Concerned about the potential risk to human health. Long-term effects are uncertain. Unintended consequences may trigger allergic reactions and unforeseen health issues among consumers. The lack of conclusive evidence on the safety of GMOs for human consumption warrants caution and thorough investigation before allowing such trials.	Any use of GM crops for production of human food would need to be approved by Food Standards Australia New Zealand (FSANZ), which has responsibility for food safety and labelling. DIR-201 is a limited and controlled release to assess the performance of the GM wheat and barley under field conditions. The GM wheat and barley grown in this field trial is not permitted for use in human food or animal feed.

Submission	Summary of issues raised	Comment
		<p>Under the <i>Gene Technology Act 2000</i> (the Act), the Gene Technology Regulator (the Regulator) considers both the short- and long-term risks to human health and safety and the environment. This information is detailed in the Risk Assessment and Risk Management Plan (RARMP) prepared for this application (Chapter 2). Harms resulting from exposure of people to the GM wheat and barley were assessed in Risk scenario 1 (Chapter 2). The risk to the health and safety of people from this exposure was considered negligible.</p> <p>As field trials of GMOs are designed to gather data, there are generally data gaps for field trial applications and this uncertainty is considered when assessing the risks and when considering risk management. 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. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk (Chapter 3 of the RARMP).</p> <p>Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs. Information needed for future release is discussed in Chapter 3, Section 4.</p>
	Concerned about the environmental impact of GM crops, particularly the potential for cross-contamination with wild or traditional crop varieties. This raises serious concerns about biodiversity and ecosystem stability. While effort has been made to limit the spread of the GMOs, there are environmental factors outside of the study controls that could result in GMO contamination of the area.	<p>The environmental impact of the proposed release of GM wheat and barley was assessed in Risk scenarios 3 and 4 (Chapter 2). The risk to the environment from this limited and controlled release was considered negligible. As noted in the RARMP, the likelihood of outcrossing of the GM wheat and barley with other non-GM wheat and barley is highly unlikely as wheat and barley are mainly self-pollinating with low levels of outcrossing (Chapter 2).</p> <p>Licence conditions have been imposed to limit and control the scope of the dealings and to manage risk to people and the environment. For example, the site is surrounded by an isolation zone in which no wheat, barley or related species may be grown.</p>
	The trial poses a threat to organic farming in the vicinity.	Matters related to marketing or trade issues, consumer preferences and coexistence regimes are outside the Regulator's legislative responsibility. These issues are the responsibility of the State and Territory governments and industry.

Submission	Summary of issues raised	Comment
	The use of GM crops may lead to increased dependence on specific agrochemicals, posing a threat to beneficial insects, soil health, and water resources.	<p>While some of the GM wheat lines contain a gene which confers tolerance to glufosinate herbicides, this gene is only used for GM plant selection during development of the GM lines in the laboratory. Therefore, glufosinate herbicides will not be sprayed on the GM wheat plants in the field.</p> <p>It should be noted that issues relating to agricultural chemical use are outside the scope of the Regulator's responsibilities. The Australian Pesticides and Veterinary Medicines Authority (APVMA) has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. The APVMA considers risks to human health, animals and the environment in assessing agricultural chemicals for registration and in setting maximum application rates. See the APVMA website for further information.</p>
	Numerous scientific studies have highlighted the uncertainties surrounding the long-term environmental and health effects of GMOs. Imprudent to proceed with trials until we have comprehensive and independently verified data demonstrating the safety of GM wheat and barley.	<p>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. Both the short- and long-term risks are considered.</p> <p>The risk assessment concluded that risks to the health and safety of people or the environment from the proposed dealings are negligible.</p> <p>The principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as the small trial size and not using GM plant material in 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.</p>
	Reconsider the decision to conduct the trial and prioritise practices that promote the well-being of our environment and safeguard the health of our community. It is essential to prioritise the precautionary principle and exercise due diligence in evaluating the potential risks before exposing our community to the uncertainties associated with genetically modified crops.	<p>The functions of the Gene Technology Regulator (the Regulator) are defined by the <i>Gene Technology Act 2000</i> (the Act) which is legislation passed by the Parliament of Australia. The Regulator must consider each application for a licence for dealings with GMOs based on criteria listed in the Act.</p> <p>In the case of licence application DIR 201, the RARMP concludes that the proposed field trial poses negligible risks to human health and safety or the environment. Licence conditions regarding limits and controls have been imposed to maintain the context in which the risks have been assessed.</p>