Australian Government
Department of Health
Office of the Gene Technology Regulator

December 2021

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 186

Limited and controlled release of wheat and barley genetically modified for yield enhancement and improved abiotic stress tolerance

Applicant: The University of Adelaide

This RARMP is open for consultation until 14 January 2022.

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

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601 or

via email to: ogtr@health.gov.au.

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

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

for

Licence Application No. DIR 186

Introduction

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

The application[1]

Project Title	Limited and controlled release of wheat and barley genetically modified for yield enhancement and improved abiotic stress tolerance
Parent organisms	Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)
Genetically modified organisms	
Genetic modifications	• Expression of three genes involved in yield enhancement (expressed both individually and in combination) • Expression of five genes involved in yield and abiotic stress tolerance (water use efficiency) • Expression of three selectable marker genes (expressed both individually and in combination)
Number of lines	Up to 70 lines[2] 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 human food or animal feed is proposed
Proposed locations	The trial is proposed to take place at two sites – one site in South Australia (Light Regional Council), and one site in Western Australia (Shire of Merredin)
Proposed release size	Up to a total of 2 ha per year across both sites
Proposed period of release	From April 2022 to January 2027
Previous releases	Wheat and barley lines containing all or some of the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128 and DIR 152.

[1] The applicant amended their application to remove GM wheat and barley knockout/knockdown lines which had been generated by CRISPR/Cas9 genome editing of endogenous *TaMUTE*, *TaYDA1*, *TaYDA2*, *TaOST1* and *TaSLAC1* genes.

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

Risk assessment

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 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 principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as 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

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

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

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Abbreviations

Act	*The Gene Technology Act 2000*
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
bar	Glufosinate tolerance gene from *Streptomyces hygroscopicus*
CaMV	Cauliflower mosaic virus
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
DPIRD	Department of Primary Industries and Regional Development (Western Australia)
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
g	Gram
GM	genetically modified
GMO	genetically modified organism
ha	Hectare
HGT	Horizontal gene transfer
hptII	Hygromycin phosphotransferase gene
LGA	Local Government Area
m	Metre
µg	Microgram
mg	Milligram
NGNE	New Genes for New Environments
NLRD	Notifiable Low Risk Dealing
nptII	Neomycin phosphotransferase II gene
OGTR	Office of the Gene Technology Regulator
OECD	Organisation for Economic Co-operation and Development
PC2	Physical Containment level 2
pporRFP	Red fluorescent protein gene from *Porites porites*
RAF	Risk Analysis Framework
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
TGA	Therapeutic Goods Administration
USDA	United States Department of Agriculture
WA	Western Australia
WHO	World Health Organisation
WRA	Weed risk assessment

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

6. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. 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.

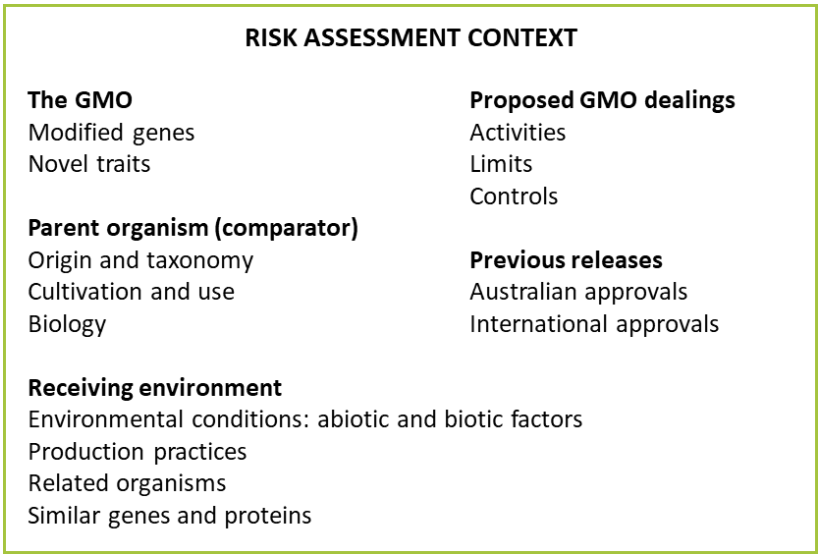

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework.

7. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

Section 2 The proposed dealings

8. The University of Adelaide proposes to release up to 70 genetically modified (GM) wheat and barley lines into the environment under limited and controlled conditions. The GM lines have been genetically modified for yield enhancement and improved abiotic stress tolerance.

9. The purpose of the trial is to evaluate the agronomic performances 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.

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

- conducting experiments with the GMOs
- make, develop, produce or manufacture the GMOs;
- breeding the GMOs
- propagating the GMOs
- growing the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

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

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

11. The release is proposed to take place at up to two sites: one site in South Australia (Light Regional Council), and one site in Western Australia (Shire of Merredin). The release is proposed to take place between April 2022 and January 2027, on a maximum of two sites per year, with a combined total of 2 ha across both sites in any year.

12. 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

13. 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 sites 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 sites with fences 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 sites 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 sites 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.

14. 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). Trial sites would be surrounded by a monitoring zone and an isolation zone.

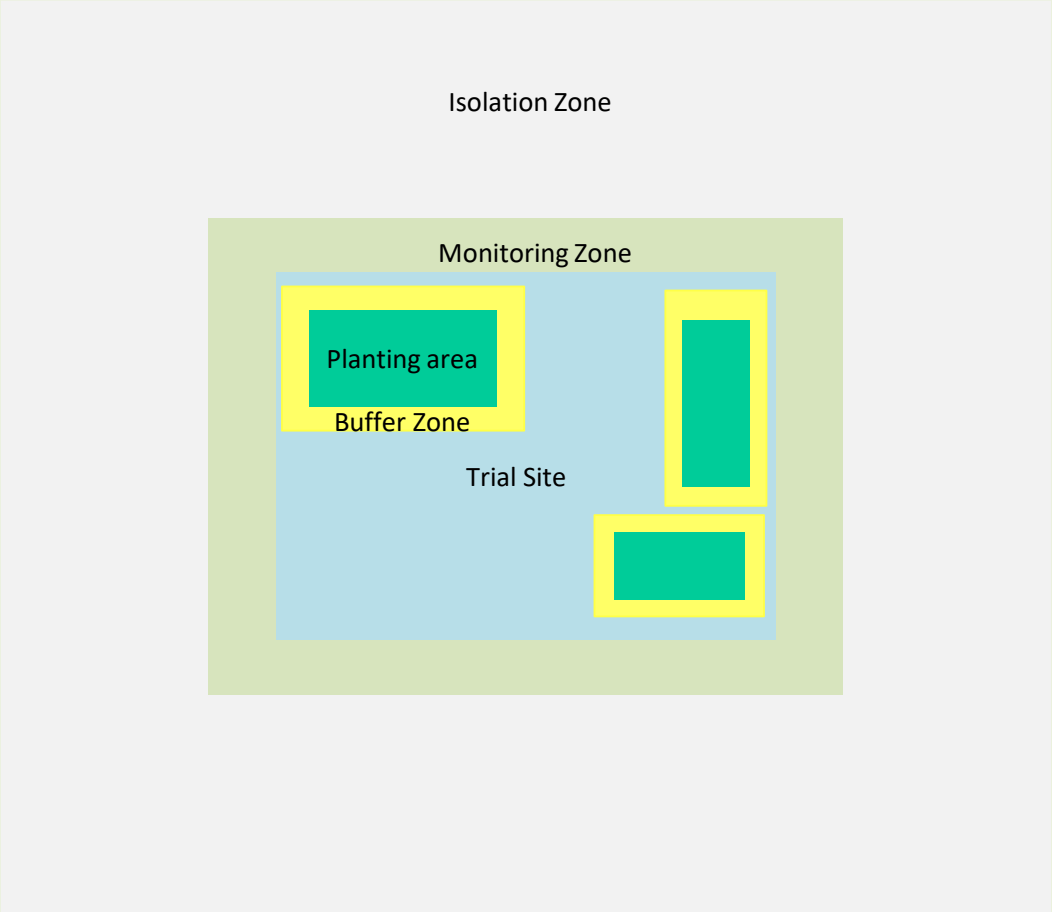

Figure 2 Schematic diagram (not to scale) of trial setup proposed by applicant: Trial site with multiple planting areas.

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

Section 3 The parent organism

16. 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 southeastern Queensland through NSW, Victoria, Tasmania, southern SA and southern WA.

17. 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). Baseline information from these documents will be used and referred to throughout the RARMP. Key points from those discussions are summarised as necessary in this RARMP.

18. There are a number of factors, both biotic and abiotic, 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 limited by a number of pests and diseases.

19. Neither wheat nor barley is regarded as a weed of national significance (National Weeds List) 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). The weed risk assessments included in the biology document for wheat concludes that 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. In the case of barley, it also is a highly domesticated crop. Mutations conferring non-shattering seed heads were selected to enable effective harvesting of barley (Pourkheirandish et al., 2015). Losing the primary seed dispersal system of wild barley reduces the fitness of cultivated barley outside agricultural environments. Many barley cultivars have also been bred to have low seed dormancy. However, as it is more tolerant to drought and salinity, it is somewhat better able to establish in the environment which is confirmed by a medium weed rating in parts of Western Australia and in Victoria. However, it has no weed rating in the other States where it occurs. It also 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

20. The applicant proposes to release up to 70 lines in total of wheat and barley lines genetically modified for yield enhancement and/or improved abiotic stress tolerance (water use efficiency). Details of the introduced genes are listed in Table 1. The applicant has stated that most lines would be GM wheat.

21. The genes AtAVP1, OsNAS2, OsPSTOL1, TaMUTE, TaYDA1, TaYDA2, TaOST1 or TaSLAC1 will be introduced into wheat or barley. Lines containing more than one of these genes will also be examined.

22. Short regulatory sequences that control expression of the genes are also present in the GM wheat and barley lines. 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 1.

23. 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 and barley plants modified for yield enhancement and water use efficiency may also contain the introduced *pporRFP* gene, which encodes a red fluorescent protein used to visually identify GM plant cells. The selectable marker genes and reporter gene are listed in Table 1.

Table 1: Genes and regulatory elements introduced in GM wheat and barley lines

Element	Gene Source	Function
	Yield enhancement	
AtAVP1	*A. thaliana*	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance
OsNAS2	*O. sativa*	Increase in shoot biomass, higher numbers of tillers and grain
OsPSTOL1	*O. sativa*	Enhanced growth vigour and earlier heading, high yield
	Yield enhancement and water use efficiency*	
TaMUTE	*T. aestivum*	Stomatal development, symmetrical division of guard mother cells
TaYDA1	*T. aestivum*	Negatively regulates stomatal development
TaYDA2	*T. aestivum*	Negatively regulates stomatal development

Element	Gene Source	Function
TaOST1	*T. aestivum*	Regulates stomatal aperture
TaSLAC1	*T. aestivum*	Guard cell anion channel
	Promoters	
CaMV35S	Cauliflower mosaic virus	Constitutive
Ubi	*Z. mays*	Constitutive, polyubiquitin
OsAct1	*O. sativa*	Constitutive, rice actin 1
PvUbi+3	*Panicum virgatum*	Constitutive, ubiquitin
	Amplification promoting sequences	
Ubi1 5' UTR	*Z. mays*	Translational modifier
Ubi1 intron	*Z. mays*	Translational modifier
	Selectable Marker Genes	
hptII	*E. coli*	Plant selectable marker – hygromycin resistance gene encoding hygromycin phosphotransferase
nptII	*E. coli K12*	Plant selectable marker – neomycin phosphotransferase gene for resistance against geneticin or kanamycin
bar	*Streptomyces hygroscopicus*	Plant selectable marker – bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate
pporRFP	*Porites porites*	Visual selectable marker gene – red fluorescent protein
	Terminator	
CaMV35S	*Cauliflower mosaic virus*	Viral terminator
nos	*A. tumefaciens*	Terminator of the nopaline synthase gene and polyadenylation signal
OCS	*A. tumefaciens*	Terminator sequence of the octopine synthase gene

*Note: *Triticum aestivum* is a hexaploid plant with three genomes, known as the A, B and D genomes. The genes for yield and water use efficiency from each of the three *T. aestivum* genomes may be used to modify the GM wheat and barley 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.

4.2 Methods of genetic modification

24. The genes for yield enhancement are expressed on their own, or as combinations of genes. Wheat and barley 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.

4.3 The introduced genes, encoded proteins and associated effects

25. The genes and their encoded proteins are summarised in Table 1, with a description of their expected function in the GM wheat and barley lines. Both yield enhancement and water use efficiency are multigenic traits, involving the interaction of genes where the protein products constitute different biochemical pathways.

Gene stacked lines

26. The introduction of each of the genes for yield enhancement or abiotic stress tolerance individually has the potential to improve the yield of wheat and barley. At this stage, there is little information on the phenotypic effect of combined overexpression of the genes. However, as each of the genes is involved in a

different aspect of yield enhancement or water use efficiency, a combination of genes may have the potential to produce wheat and barley plants with increased grain yield under optimal growing conditions. Some of the GM wheat and barley proposed for release contain more than one of the introduced genes. The applicant intends that in future trials, gene stacking between some or all of the GM lines to evaluate if particular gene combinations can enhance yield and water use efficiency to be undertaken (subject of a new application).

4.3.1 Yield enhancement

27. The yield enhancement genes proposed for release are *AtAVP1*, *OsNAS2* and *OsPSTOL1*. Field trials of GM wheat and barley with these genes have been evaluated and licensed previously for DIR 102, DIR 128 and DIR 152, so only a summary and more recent material regarding these genes is presented here.

AtAVP1

28. The *Arabidopsis thaliana* vacuolar H^+-pyrophosphatase (*AtAVP1*) gene encodes an H^+-translocating pyrophosphatase (H^+-PPase) that appears to be localised to the tonoplast and plasma membrane (Gaxiola et al. 1999; Khadilkar et al. 2016). H^+-PPase proteins are proton pumps that use the energy gained from the breakdown of pyrophosphate to pump protons into the vacuoles of plant cells (Khadilkar et al. 2016). However, AtAVP1 can function as both a pyrophosphatase and as PPi-synthase, depending on the electrical gradients across membranes (Pizzio et al., 2017; Scholz-Starke et al., 2019). In addition, other functions have been ascribed to AtAVP1, including a role in auxin distribution within the plant, synthesis of ascorbic acid, acidification of the rhizosphere leading to improved nutrient (e.g. iron, magnesium and potassium) uptake, and sucrose loading of the phloem (Menadue, 2018).

29. 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 H^+-PPases has also been shown to significantly increase 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). Constitutive expression of H^+-PPase proteins in different plant species has produced varying phenotypes. This may indicate that the roles of the protein vary in the different plant species (Menadue, 2018).

OsNAS2

30. Although soils often have a high iron content, plants may not be able to take up the iron as it is not bioavailable (Wang et al., 2019). In crop plants under iron stress, yields suffer as iron is involved in a number of important functions, e.g. chlorophyll synthesis. If iron availability is low, plants respond by expressing various genes that help with iron uptake (Wang et al., 2019). For example, the *OsNAS2* gene encodes a rice nicotianamine synthase (NAS), an enzyme that catalyses the last step in the production of nicotianamine. Nicotianamine is a molecule made by all higher plants that chelates and transports transition metals including iron and zinc (von Wiren 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).

31. Constitutive overexpression of a barley NAS gene, *HvNAS1*, in tobacco led to increased concentrations of iron, zinc, copper, manganese and nickel in shoots and/or seeds, demonstrating enhanced transport of these metals following root uptake (Kim et al., 2005). Constitutive overexpression of rice *OsNAS1*, *OsNAS2* or *OsNAS3* genes in rice led to increased levels of iron and zinc in the grain, but no significant differences in copper, manganese or nickel content compared to non-GM control rice plants (Johnson et al., 2011). Constitutive overexpression of rice *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).

32. Several GM crops overexpressing NAS genes have demonstrated tolerance to low iron availability in alkaline soils, which causes leaf chlorosis and poor yield in control non-GM plants (Nozoye, 2018 and

references cited therein). In addition, GM tobacco and Arabidopsis overexpressing a NAS gene have shown increased tolerance to high levels of heavy metals, particularly nickel, which cause toxicity to non-GM plants (Kim et al., 2005).

OsPSTOL1

33. The Phosphorous Starvation Tolerance 1 (*PSTOL1*) gene occurs within a major quantitative trait locus (QTL) for phosphorus-deficiency tolerance identified in the aus-type rice variety Kasalath. This gene is absent in the genome of phosphorus-starvation-intolerant rice varieties. Overexpression of *PSTOL1* in these varieties enhances 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). A survey of sorghum identified six genes with high sequence similarity to rice *PSTOL1,* two of which were associated with an increased root surface and grain yield under low phosphorus field conditions (Hufnagel et al. 2014).

34. *OsPSTOL1* encodes a functional serine/threonine protein kinase (Gamuyao et al. 2012). Protein kinases are mediators of cellular signalling: they accept input information from receptors that sense environmental conditions, phytohormones and other external factors, and convert it into appropriate outputs such as changes in metabolism, gene expression, and cell growth and division (Hardie 1999). They interact with target proteins and phosphorylate them, resulting in protein activation or deactivation to effect a wide array of processes ranging from disease resistance and developmental regulation to reproduction (Hardie 1999). Os*PSTOL1* shows highest amino acid sequence similarity with serine/threonine receptor-like kinases of the LRK10L-2 family, and may be a receptor-like cytoplasmic kinase (Gamuyao et al. 2012). The molecular mechanism of Os*PSTOL1* that translates into enhanced root growth is not yet fully elucidated.

4.3.2 Yield enhancement and abiotic stress tolerance (water-use efficiency)

35. 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. Stomata are central to plant drought responses because they modulate transpiration and the uptake of carbon dioxide (Lawson and Blatt, 2014). Plants can adjust stomatal aperture to maximise carbon assimilation while also limiting water loss (Raissig et al., 2017).

36. Genetic modification of stomatal development and aperture may protect plants against drought, allowing them to continue to grow in water-limited environments (Franks et al., 2015; Hepworth et al., 2018). The reduction of stomatal density in GM Arabidopsis, maize and barley has been shown to improve water-use efficiency and/or drought tolerance (Buckley et al., 2019). However, modification of stomates may also 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).

37. Genetic modification of stomatal development and aperture may also influence flowering time. Stomatal opening has been shown to be regulated by the FLOWERING LOCUS T (FT), a gene involved in early flowering in *Arabidopsis thaliana* (Kinoshita et al., 2011). FT-like genes have been identified in dicots and monocots, including wheat and barley (Guo et al., 2015; Haiyang et al., 2019). Under short day conditions, GM *Arabidopsis* plants containing a cotton homolog of this gene were found to flower 24 days after sowing when this gene was overexpressed, compared to around 39 days to flowering in wildtype *Arabidopsis* plants (Guo et al., 2015).

TaMUTE gene

38. MUTE is one of three transcription factors that have been shown to positively regulate stomatal development in Arabidopsis (Liu et al., 2019). These three regulators are closely related basic helix-loop-helix (bHLH) domain transcription factors that control stomatal development at the initiation, meristemoid differentiation and guard cell morphogenesis (Pillitteri et al., 2007). In Arabidopsis, MUTE is highly expressed in meristemoids and acts as a molecular switch for meristemoid fate transition (Pillitteri et al., 2007; Liu et al., 2009).

39. 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 genes

40. 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 other two bHLH transcription factors involved in stomatal development (Qi and Torii, 2018; Dunn et al., 2019). In turn, the MAPKKK pathway is regulated by environmental factors, such as light and carbon dioxide, as well as endogenous peptide factors (Dunn et al., 2019).

41. 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). Abrash et al. (2018) found that the YDA gene promoted normal stomatal spacing patterns in both Arabidopsis and Brachypodium (a monocot grass model plant). 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 genes

42. 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). The SLAC1 protein is localised to the plasma membrane of guard cells in Arabidopsis (Negi et al., 2008). Arabidopsis SLAC1 gene mutants exhibit both impaired stomatal closing and slow stomatal opening induced by light, low carbon dioxide and elevated air humidity (Laanemets et al., 2013).

43. In Arabidopsis guard cells, the SLAC1 anion channel is activated by the protein kinase OST1 (Geiger et al., 2009). The OST1 gene encodes a protein kinase (OPEN STOMATA 1) that is expressed in stomatal guard cells and vascular tissue (Mustilli et al., 2002; Acharya et al., 2013). Arabidopsis plants lacking the OST1 gene showed a reduction in their ability to limit transpiration in water-limited environments (Mustilli et al., 2002).

44. The OST1 gene is activated by the plant hormone abscisic acid (ABA), which is synthesised in response to abiotic stress (Mustilli et al., 2002; Geiger et al., 2009).

4.3.3 Marker Genes

45. The GM wheat and barley plants contain selectable marker genes that confer resistance to different classes of antibiotics or to herbicide (Table 1). 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 phosphototransferase II) which encodes an aminoglycoside 3'-phosphotransferase II enzyme that is also known as neomycin phosphototransferase 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).

46. 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*.

47. The GM wheat and barley plants may contain the introduced *pporRFP* gene, which encodes a novel DsRed-type red fluorescent protein (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., 2012a). More information on the use of reporter genes in general may be found in the document *Marker Genes in GM Plants*.

48. The *pporRFP* gene may be used as a real-time visual marker gene in the GM wheat and barley plants. The expression of the *pporRFP* gene can be monitored in the GM plant tissue using *in vivo* fluorescence microscopy with an RFP filter, avoiding the need to destroy the tissue (Mann et al., 2012b). Expression of this reporter gene has been shown to be very effective in rice (*Oryza sativa*) and switchgrass (*Panicum virgatum*) (Mann et al., 2010; Ondzighi-Assoume et al., 2019). The reporter gene has also been used as a phytosensor in GM plants to enable the detection of plant pathogens. In these studies, the *pporRFP* gene was fused to plant pathogen inducible promoters, which were transformed into tobacco and *A. thaliana*. The red fluorescent protein was expressed in the presence of plant pathogens, allowing real-time monitoring and early warning of plant infection (Liu et al., 2011; Fethe et al., 2014).

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

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

50. The applicant has not yet performed any toxicity or allergenicity studies on the GM wheat and barley lines proposed for release. Apart from *pporRFP*, all of the genes introduced into the GM plants were isolated from common sources, thus people and other organisms have a long history of exposure to them.

51. A comprehensive search of the scientific literature yielded no information to suggest that the 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, no toxicity/allergenicity tests have been performed on any of the introduced proteins.

52. 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 been studied by other research groups with the aim of increasing levels of iron in plant tissues and biofortification. Iron content in whole wheat plants is approximately 30 µg/g plant material, with a biofortification target of 52 µg/g (Bouis et al. 2011). People must obtain iron from their diet as it is involved in several essential processes in the body. However, excessive iron in the diet can result in toxicity, i.e. more than 20 mg/kg of body weight (Balmadrid & Bono 2009). Even in research aimed at producing biofortified wheat lines, the targeted concentrations of iron are such that these levels are unlikely to occur as a result of typical consumption. Certain conditions such as thalassemia (Tanno et al. 2007; Nemeth 2010) and hereditary haemochromatosis (Barlow-Stewart et al. 2007) may be further complicated by iron overload.

53. For dogs, no clinical signs of iron toxicity are expected after oral ingestion of less than 20 mg iron/kg of body weight. In all companion animals, oral doses between 100 and 200 mg iron/kg of body weight are potentially lethal (Albretsen, 2006). This suggests that susceptibility to excess iron toxicity is similar in people and other mammals.

54. *OsNAS2* introduction or overexpression could lead to accumulation of metals other than iron. Several heavy metals are toxic to humans and animals, e.g. arsenic, lead, mercury and cadmium (Flora et al., 2008; Jaishankar et al., 2014; Clemens and Ma, 2016). Of these, cadmium is of interest because: (a) more than 80% of human cadmium exposure is from consumption of cereals and vegetables; (b) many populations around the world already have cadmium intake above recommended levels, so moderate increases in cadmium exposure could have toxic effects; and (c) in terms of chemical characteristics, cadmium mimics iron and zinc, so may be taken up by biological pathways that are used for biofortification (Khan et al., 2014; Clemens and Ma, 2016). It is recommended to monitor levels of cadmium in agricultural produce in general in Australia, as it is naturally present in Australian soils from less than 0.1 to 0.5 mg/kg in the top 10 cm of soil. It can be present at higher levels in the vicinity of, e.g. smelters (Horticulture Australia, 2003).

55. 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).

56. There have been no adverse effects reported from similar GM lines planted under DIR 102, DIR 128 or DIR 152. It should be noted that neither licence permitted use of the GM lines in human food or animal feed.

57. 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 20 September 2021).

58. The *bar* gene and the protein it encodes (phosphinothricin N-acetyl transferase or PAT) has been extensively assessed in other RARMPs most recently in DIR 178, and in scientific literature. The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the bar gene was derived from the common soil bacterium *S. hygroscopicus*, which is not considered a pathogen of humans or other animals
- no sequence homology has been found between PAT and any known toxic or allergenic proteins
- the PAT protein does not possess any of the characteristics associated with food allergens
- the PAT protein is inactivated by heat, e.g. through cooking, and by low pH, e.g. in the human stomach
- purified PAT protein was not toxic to mice and rats when administered at high doses in acute toxicity studies.

59. 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).

60. The *pporRFP* gene and its encoded protein have not been previously assessed by the OGTR. 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, 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 desirable organisms.

4.5 Characterisation of the GMOs

61. Although these lines are at an early stage of development, the applicant has provided some preliminary information on expected phenotypes for some of the genes.

62. Some GM wheat lines constitutively overexpressing *OsNAS2* have increased iron concentration in grains (Beasley et al., 2017). 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.

63. The applicant also claims that overexpression of *OsPSTOL1* in GM wheat resulted in enhanced plant vigour and earlier heading. In GM rice, *OsPSTOL1* conferred enhanced root growth, thus increasing uptake of phosphorous as well as nitrogen and potassium (data not supplied). Recently, 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). The applicant also stated that data from DIR 152 shows field grown GM wheat expressing *OsPSTOL1* has enhanced grain yield.

64. The genetic 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 and barley proposed for release, however there is no information on phenotype changes in the GM wheat and barley proposed for release.

65. Genetic modification of the OST1 and SLAC1 genes in the GM wheat and barley may also affect 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).

66. The applicant has stated that one of the unintended changes from the proposed genetic modification may be reduced time to flowering. Some of the GM lines approved for release under DIR 152, have been shown to flower 5-10 days earlier than non-GM plants within the same cultivar in the glasshouse and under field conditions.

67. The GM wheat lines for yield enhancement (*AtAVP1*, *OsNAS2* or *OsPSTOL1,* individually and in combination) were grown under DIR 152. 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. Only yield was successfully increased by up to 30% under these field conditions.

Section 5 The receiving environment

68. 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).

69. 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

70. 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

71. It is proposed that the GMOs will be grown at either one or two sites: one is a field trial facility at Rosedale in SA, the other is in Merredin in WA. The applicant intends to plant the GMOs in more than one planting area per site, which allows for the analysis of seasonal and environmental stress variation. The total planting area across both sites would be up to 2 ha per year. GM plants approved under other DIR licences, including DIR 152 (in SA) and future DIR licences, if approved, would also be grown at the sites, although in different planting areas.

72. The site in Rosedale is located in Light Regional Council, a local government area 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 South Australia, 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 South Australia based on Bureau of Meteorology climate data, which shows a concentration of rainfall during the winter months and drier summer months.

73. The Merredin location is a New Genes for New Environments (NGNE) facility that is owned and operated by the WA Department of Primary Industries and Regional Development (DPIRD). This facility was

set up for conducting GM field trials under differing environmental conditions, representing abiotic stresses which occur in WA agricultural environments. The Merredin site represents the low rainfall environment used for growing wheat and barley in WA (see the DPIRD NGNE facilities website). The soil of Merredin is a mixture of yellow sands, gravels, loamy earth and loamy duplex soils, but with also calcareous subsoils.

74. 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 with 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

75. 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.

76. Seeds would be harvested either by hand or with a machine (e.g. plot harvester) which 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.

77. 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

78. Both proposed locations are within cereal-producing regions.

79. The Rosedale site has previously been used for sheep grazing for over 10 years. No wheat or barley has been sown in surrounding fields. The NGNE facility in Merredin has been used for University of Adelaide GM field trials, most recently for DIR 128 and DIR 152, with sites either signed off or in post-harvest monitoring. The DIR 128 licence has now been surrendered, so no further planting can occur under this licence. However, planting of GM wheat and barley can occur at both sites until (and including) the 2022/2023 growing season under the DIR 152 licence, so planting could occur under DIR 152 concurrently with that proposed under DIR 186.

80. One limited and controlled GM wheat trial is approved for planting in the Shire of Merredin (DIR 165); however, there are no current plantings of GM wheat in Merredin under this licence.

81. Cultivated wheat and barley are not known to hybridise with one another naturally, but each can hybridise with other species. Details are given in the biology documents for these species and briefly summarised below.

5.4.1 Wheat

82. Bread wheat (*Triticum aestivum* L.) is sexually compatible with other bread wheat or durum plants. Bread wheat is cultivated in the LGAs where proposed field trial sites may be located.

83. *Triticum aestivum* can spontaneously hybridise with a number of closely related species from the *Triticum-Aegilops* genera complex (Zaharieva and Monneveux, 2006). The only other *Triticum* species present in Australia is *T. turgidum* (durum wheat), which is cultivated for pasta production (Atlas of Living Australia, accessed 22 September 2021). No *Aegilops* species (goatgrasses) are cultivated or naturalised in Australia (Weeds Australia, accessed 22 September 2021).

84. There have been occasional reports of natural hybridisation of wheat with rye (*Secale cereal*) or triticale (*xTriticosecale*), which are minor crops in Australia. However, these hybridisation events are rare and progeny are usually sterile (Hegde and Waines, 2004; Kavanagh et al., 2010).

85. A European study of gene flow from wheat to *Hordeum marinum* found no first-generation hybrids, however one *H. marinum* plant contained a low level of introgressed genetic material from wheat (Guadagnuolo et al., 2001). It is unclear whether this gene flow occurred directly from wheat to *H. marinum* or via one or more bridge species.

5.4.2 Barley

86. Barley has a primary gene pool consisting of *H. vulgare* and *H. vulgare ssp. spontaneum*, which produce completely fertile offspring following crossing. The secondary gene pool consists of *H. bulbosum* L. where mating can occur but often hybrids are sterile, and a tertiary gene pool containing all other *Hordeum* species (Pickering & Johnston 2005). 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.

87. Although there have been interspecific crosses within the *Hordeum* genus and intergeneric crosses across a number of genera, all have been under experimental conditions and successful hybrids have not been observed under natural conditions. Details of experimental crosses are provided in the barley biology document.

5.5 Presence of similar genes and their products in the environment

88. The introduced genes listed in Table 1 were originally isolated from naturally occurring organisms most of which are already widespread and prevalent in the environment. Thus, humans and animals have been exposed to these genes and their encoded proteins either through consumption of the parent organisms or through other exposures in the environment. In addition, homologues of the genes and encoded proteins occur naturally in animals, plants, yeast and bacteria.

89. 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.

90. The *bar* gene was isolated from the common bacterium *S. hygroscopicus*, which is a saprophytic, soil-borne 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 (Hérouet et al., 2005).

91. 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.

92. All promoters used to drive expression of the introduced genes 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*) and *A. tumefaciens*, a common bacterium that can cause galls in various plants.

93. While some of the source organisms can cause allergies (e.g. wheat), the introduced proteins are not known to cause harm.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

94. 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) and DIR 152 (*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.

95. Information on previous DIR licences for GM wheat and barley is available from the OGTR GMO Record. The Regulator has previously approved 22 field trial releases of GM wheat, of which ten are licences for both wheat and barley. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.

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

6.2 International approvals

97. 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 September 2021).

98. On a commercial scale, drought tolerant HB4 GM wheat has been approved in Argentina.

99. 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

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

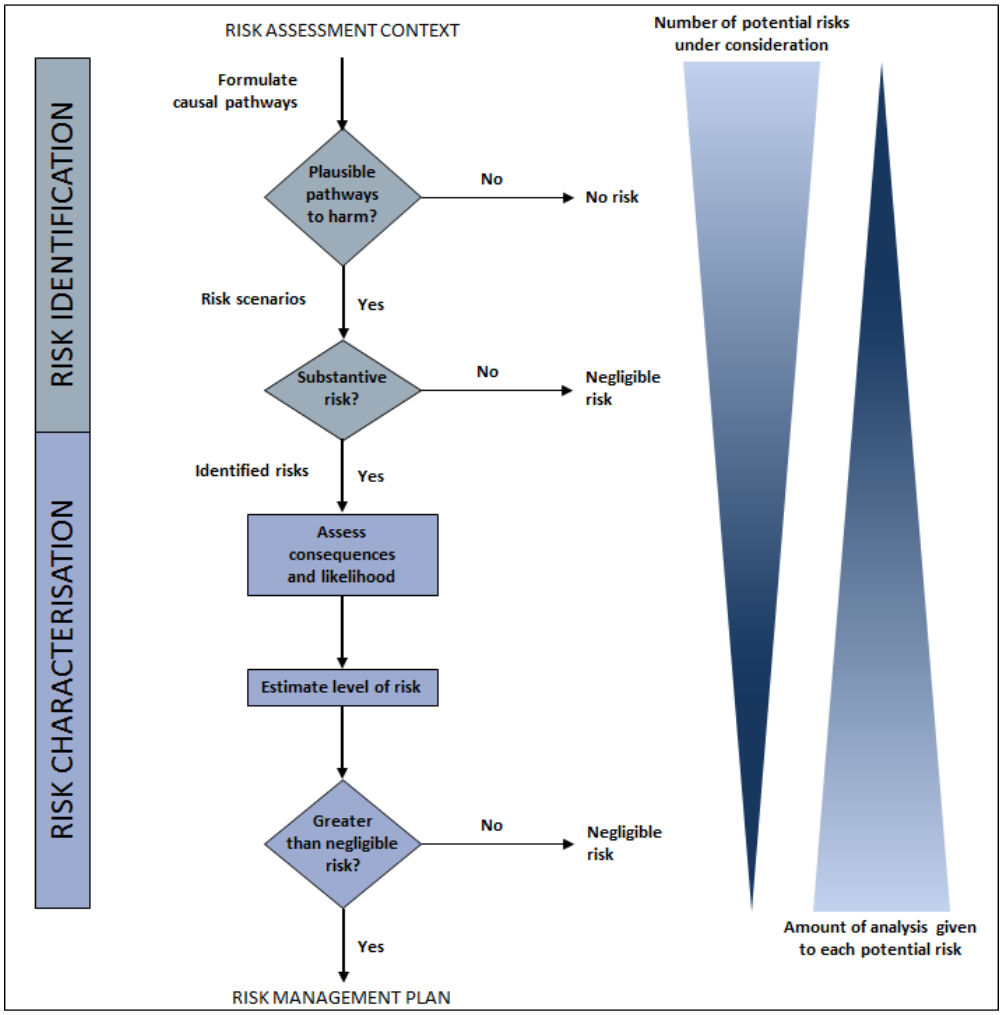

Figure 3 The risk assessment process

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

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

those that could not plausibly occur, do not advance in the risk assessment process (Figure 3) i.e. the risk is considered to be no greater than negligible.

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

Section 2 Risk Identification

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

i. the source of potential harm (risk source)

ii. a plausible causal linkage to potential harm (causal pathway)

iii. potential harm to people or the environment.

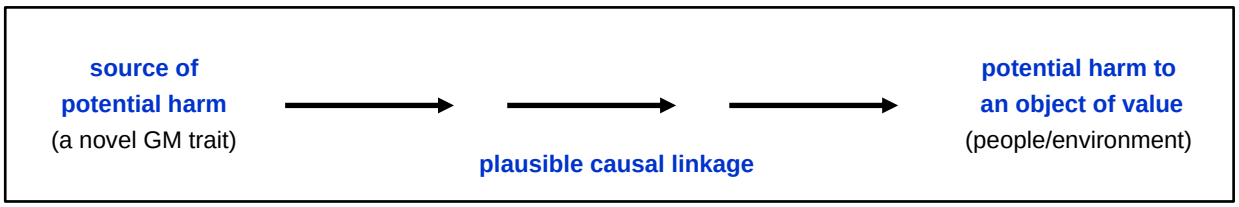

Figure 4 Risk scenario

105. 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

106. 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.

107. As discussed in Chapter 1, the GM wheat and barley lines have been modified by overexpression of genes. The intended effect of insertion of the genes is yield enhancement or yield enhancement and water use efficiency. Also, a red colour marker gene, *pporRFP*, has been introduced into some of the GM wheat and barley lines, the effect of which has not been risk assessed in previous DIR RARMPs. These introduced genes will be considered further as a potential source of risk.

108. The GM wheat and barley also contains other marker genes, *nptII* and *hptII* from *E. coli* that confer antibiotic resistance and were used as selectable marker genes. Similarly, the GM wheat and barley contain the *bar* gene conferring herbicide tolerance for GM plant selection. These 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. Further information about the antibiotic resistance genes can be found in the document *Marker genes in GM plants* on the OGTR website. As the 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, other than the presence of the herbicide tolerance trait potentially impacting the spread and persistence of the GM plants.

109. The introduced genes are controlled by introduced regulatory sequences. These were originally 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.

110. The genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

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 risk scenarios because they have been considered in previous RARMPs.

113. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for DIR 108. Although the DIR 108 RARMP is for GM canola, the HGT considerations are the same for the current RARMP: HGT events rarely occur and the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

114. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR 117. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have

regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

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. 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 2 and examined in detail in Sections 2.4.1– 2.4.4.

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

Table 2: Summary of risk scenarios from the proposed dealings with the GM wheat and barley

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	GM wheat and barley grows at the field trial sites ⬇ Expression of the introduced genes results in the GM wheat and barley composition being different from non-GM wheat and barley ⬇ Exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms	No	• GM plant material would not be used as human food or animal feed. • Other proposed limits and controls would further minimise the exposure of people to GM plant material. • The source organisms for most introduced genes are routinely used for food or feed or are commonly found in the environment.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	GM wheat and barley grows at the field sites ⬇ Hybridisation with other GM wheat or barley grown under a different licence at the trial site ⬇ Expression in the GM hybrids of the introduced genes from both parental GM lines ⬇ Exposure of people and other desirable organisms at the trial sites by ingestion of, or contact with, the GM hybrid wheat or barley	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms	No	• The limited time, small scale and other proposed limits and controls minimise exposure of people and other desirable organisms to the GM hybrid seeds and other plant material, including buffer zones, site monitoring and post-harvest monitoring. • Wheat and barley are mostly self-pollinating, and outcrossing occurs at low levels. • Most introduced genes were sourced from common food plants and the encoded proteins are not known to be toxic or allergenic.
3	Introduced genes conferring yield enhancement, improved abiotic stress tolerance, herbicide tolerance and a visual marker	Presence of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment OR Increased ability to provide a reservoir for pathogens or shelter for pests	No	• The proposed limits and controls minimise the likelihood of seed dispersal or persistence outside the trial limits. • There is no expectation the introduced genes confer characteristics in the GM wheat and barley that may lead to adverse environmental effects. • Dispersal by natural means, and ability to establish outside agriculture is limited in wheat and barley. • The GM wheat and barley is susceptible to most standard weed control measures.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
4	Introduced genes conferring yield enhancement, improved abiotic stress tolerance, herbicide tolerance and a visual marker	Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or barley ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment	No	• The proposed limits and controls minimise the likelihood of pollen flow from the trial site to sexually compatible plants. • Wheat and barley have limited ability to outcross. • 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

Risk Source	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
Causal Pathway	⬇ GM wheat and barley grows at the field trial sites ⬇ Expression of the introduced genes results in the GM wheat and barley composition being different from non-GM wheat and barley ⬇ Exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley ⬇
Potential Harm	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms

Risk source

119. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat and barley plants.

Causal pathway

120. The GM wheat and barley would be planted at the trial sites. The aim of the introduced genes is to enhance yield and improve abiotic stress tolerance in the GM wheat and barley plants. The purpose of the visual marker is to identify GM wheat and barley plants in the laboratory. The encoded proteins could potentially be produced in all plant tissues throughout plant development, but this has not yet been determined. This is an area of uncertainty for this risk assessment.

121. The GM wheat and barley would not be used as human food, so people would not be exposed to plant material from the trial through ingestion. People may be exposed to GM plant material through inhalation of pollen when the GMOs flower, or through direct skin contact with GM plant material. This contact is most likely at the trial sites. 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.

122. The applicant proposes that only authorised persons would be permitted to deal with the GM wheat and barley, or to access the trial sites. These authorised staff could have direct skin contact with GM plant material or could inhale GM pollen.

123. 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 & 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 a 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 small size and limited duration of the proposed trial, and the fact that both proposed trial sites are 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.

124. The GM wheat and barley would not be used as animal feed. However, animals, including mammals, birds and invertebrates, and other desirable organisms, may have direct contact with the GM wheat and barley at the trial sites. A range of animals consume cereals (Hill et al. 1988; AGRI-FACTS 2002; OGTR 2021a; OGTR 2021b) and may be attracted to the GM plant material. The applicant proposes to surround each trial site with a fence and locked gates that would restrict access to large animals. The NGNE facility at Merredin is a purpose-built facility with secure fencing and locked gates to restrict access. Desirable animals such as small native mammals or birds could enter the trial sites 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 desirable organisms that would be exposed to the GM plant material.

Potential harm

125. 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).

126. Potentially, people exposed to the proteins expressed by the introduced genes may show increased toxic reactions or increased allergenic reactions. From consideration of the causal pathway, exposure would be mostly limited to staff involved in handling and harvesting the GM wheat and barley plants during the field trial. Similarly, exposure to the proteins expressed by the introduced genes may lead to increased toxicity to other desirable organisms.

127. No toxicity or allergenicity studies have been performed on the GM plant material and this is an area of uncertainty for this risk assessment. Most of the introduced genes were isolated from naturally occurring organisms that are widespread and prevalent in the environment, including common food sources such as rice and wheat. The fluorescent protein marker gene, *pporRFP*, was sourced from a coral, *Porites porites*, which is distributed in a variety of coral reefs environments and used for aquarium and home decoration (Chapter 1, Section 5.5). Thus, people and other organisms are exposed to the same or similar proteins through their diet and/or in the environment. There is no information in the literature to suggest that the introduced genes or their products are toxic or allergenic to people or toxic to other desirable organisms.

128. GM wheat or barley containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128 and DIR 152. 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 . As noted in Chapter 1, section 4.3 and in the RARMPs for DIR 128 and DIR 152, the *OsNAS2* gene is associated with increased iron uptake in plant tissues and excessively high dietary iron can have toxic effects. Preliminary data suggest that the iron levels in these plants will not be in a range of concern for iron toxicity.

129. A further uncertainty is whether the *OsNAS2* modified plants increase uptake of cadmium or other heavy metals, which are toxic in people and other desirable animals. The level of cadmium accumulation in the GM wheat and barley will depend on soil cadmium concentration. It is noted that some plants naturally accumulate higher levels of cadmium than wheat (Brennan and Bolland, 2004). In people, absorption of cadmium through the skin is negligible (Agency for Toxic Substances and Disease Registry, accessed 9 November 2021). Absorption of cadmium through inhalation of plant material is known to occur, as smokers on average accumulate twice the cadmium burden of non-smokers, due to high cadmium levels in tobacco leaves. However, even heavy smokers receive only about 10% of the FAO/WHO Provisional Tolerable Weekly Intake for cadmium from smoking (EFSA, 2009). In addition, there is uncertainty regarding potential harm because sensitivity to cadmium toxicity varies between animal species and between different developmental stages in the same species (Furness, 1996; EFSA, 2004).

130. Five genes that have not been assessed in previous RARMPs are derived from wheat, and people and other desirable organisms have been exposed to these genes and their products through the consumption of, or other exposure to, wheat products. However, since the GM wheat and barley lines overexpressing these genes have not been studied in detail, this is an area of uncertainty for this assessment.

131. The visual marker gene *pporRFP* was isolated from the coral *P. porites* and encodes a DsRed-like red fluorescent protein pporRFP (Alieva et al., 2008). DsRed and its derivatives have been used in the genetic modification of a variety of organisms, including plants (Jach et al., 2001; Nishizawa et al., 2006; Sun et al., 2018). The original DsRed protein sourced from *Discosoma* sp. has demonstrated cytotoxic effects (Strack et al., 2008; Shemiakina et al., 2012). Low or non-cytotoxic versions of DsRed have been generated and are currently in use (Bevis and Glick, 2002; Clontech Laboratories, 2003; Shemiakina et al., 2012). If the protein encoded by *pporRFP* were also cytotoxic, then the cells of the GM wheat and barley plants could be affected by this introduced protein. However, even if the pporRFP protein were cytotoxic, this does not

necessarily mean that it would cause toxicity in people or other desirable organisms after ingestion as most proteins are digested before the resulting protein pieces or amino acids can be taken up by interstitial cells. Only proteins with (some) resistance to digestion may cause toxicity upon ingestion. The DsRed-like fluorescent protein encoded by the visual reporter gene, *pporRFP*, has not been characterised with regard to its digestibility, or toxicity or allergenicity in general, and this is an uncertainty for this assessment.

132. 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, 2021a, b). There is no reasonable expectation that any of the genes introduce into the GM wheat or barley proposed for this trial would be allergenic or influence the pathways producing known allergens in wheat or barley.

Conclusion

133. Risk scenario 1 is not identified as a substantive risk due to the proposed limits and controls, including not permitting the GM plant material in food or feed, and the fact that most of the introduced genes are already present in wheat or rice and lack toxicity or allergenicity in people as well as toxicity in other organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk Source	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
Causal Pathway	⬇ GM wheat and barley grows at the field sites ⬇ Hybridisation between GM lines or with other GM wheat or barley grown under a different licence at the trial site ⬇ Expression in the GM hybrids of the introduced genes from both parental GM lines ⬇ Exposure of people and other desirable organisms at the trial sites by ingestion of, or contact with, the GM hybrid wheat or barley ⬇
Potential Harm	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms

Risk source

134. The source of potential harm for this postulated risk scenario are the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat and barley plants.

Causal pathway

135. GM wheat and barley would be planted at the trial sites. 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 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 sites.

136. During this field trial, it is possible that different lines grown under DIR 186 would be planted in close proximity to one another. In addition, the GM wheat and barley proposed for release may be grown in close proximity to other GM wheat or barley planted under licence DIR 152. Given that the different GM lines are sexually compatible and that they may have similar flowering times, pollen flow between plants

with different introduced genes may occur. This may result in hybrid GM wheat and barley seeds with additional – 'stacked' – introduced genes for yield enhancement and/or abiotic stress tolerance. People and other organisms may be exposed to this hybrid GM wheat or barley.

137. 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.

138. Both the current DIR 186 proposal and the licence for DIR 152 include several lines in which either one, two or all three of the genes for yield enhancement, i.e. *AtAVP1*, *OsNAS2* and *OsPSTL1*, are present. The proposed DIR 186 trial also includes another five genes for abiotic stress tolerance. In addition, lines containing one of seven individual genes for frost tolerance is also approved under DIR 152.

139. 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, 2021a, b).

140. A Canadian study of gene flow in wheat detected trace rates (≤ 0.01%) up to 300 m (for a 16 ha pollinator block) or 2.75 km (for a 30 ha pollinator block) away from the pollen source (Matus-Cádiz et al., 2004; Matus-Cádiz et al., 2007). Another highly relevant study for the current application was conducted in south-eastern Australia and has shown much lower rates of intraspecific gene flow than that observed overseas (Gatford et al., 2006). Using three blocks of 1.2 m x 2.5 m planted to GM wheat surrounded by 27 blocks of the same size planted to non-GM wheat, the authors measured gene flow rates far lower than those observed in similar conditions overseas, i.e. up to 0.055% at 8 m from the pollen source (Gatford et al., 2006).

141. In a study using male sterile barley at a distance of 1 m as the recipient, viable pollen flow resulted in an average of less than half a seed to one seed per head, and seed set diminished with distance (Ritala et al. 2002). In normal fertile barley, the cross-pollination frequency was between 0 and 7% at a distance of 1 m. This study used open flowered barley as the recipient, so outcrossing would be expected to be lower in most cultivated barley varieties as many of these are closed-flowering for part or all of their flowering period (Ritala et al. 2002). In observations of pollen migration between commercial barley fields, outcrossing rates were 0.05% and 0.01% for distances of 1 m and 10 m, respectively. No pollen migrants were observed in these studies at distances of 20 m or 50 m (Allard unpublished, cited in Wagner & Allard 1991; Ritala et al. 2002). However, in other studies, cross fertilisation with very low frequencies has been observed at distances of up to 50 m (Ritala et al. 2002) and 60 m (Wagner & Allard 1991), although cross pollination at such distances is rare.

142. Outcrossing rates in both wheat and barley are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity (OGTR, 2021a, b). There is currently no information that would indicate an effect of the introduced genes on pollen characteristics leading to an increase in the likelihood of outcrossing.

143. 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 152 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, buffer zones, site monitoring and post-harvest monitoring requirements have been imposed under licence DIR 152. In addition, seeds, including any possible hybrid seeds, obtained from the trial authorised under DIR 152 must not be used for breeding or propagation to produce cultivars for future commercial release. Taken together, exposure of people or other desirable organisms to any hybrid GM wheat and barley would be highly unlikely.

Potential Harm

144. If pollen flow occurred between the GM wheat or GM barley lines grown under DIR 186 or between lines from DIR 152 and DIR 186, it is possible that some GM hybrid seed may occur. For example, these plants could contain additional yield and water use efficiency genes, or a yield enhancement gene and a yield and water use efficiency gene. If this occurs, hybrid seeds and any resulting plants may express a

combination of introduced genes. These proteins may be toxic or allergenic to people or toxic to other organisms.

145. Risk Scenario 1 (above) and the RARMP for DIR 152, did not identify toxicity or allergenicity of any of the individual genes as a substantive risk. Likewise, there is no expectation that combinations of 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. The genes are sourced from organisms already present in the environment, most of the genes from dominant food crops, suggesting that people and other organisms have a long history of exposure to them.

Conclusion

146. Risk scenario 2 is not identified as a substantive risk due to the proposed limits and controls, wheat and barley being mainly self-pollinating with low levels of outcrossing, and the fact that most introduced genes were sourced from common food plants and the encoded proteins are not known to be toxic or allergenic. 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 genes conferring yield enhancement, improved abiotic stress tolerance, herbicide tolerance and a visual marker
Causal Pathway	⬇ Presence of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment OR Increased ability to provide a reservoir for pathogens or shelter for pests

Risk source

147. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat or barley.

Causal pathway

148. GM wheat and barley would be grown at the trial sites, and viable seeds would be available for dispersal when the trial is sown and as the GM wheat and barley reaches maturity. If viable GM wheat and barley seeds remained at the trial sites after completion of the trial, or if GM seed dispersed outside the trial sites, 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 animals to the GM wheat and barley, and the introduced genes and their products.

149. Similarly, if GM hybrids were formed between the GM wheat and barley in this application and those authorised for release at the same sites under DIR 152, then GM hybrid seed with combined traits could also be available for this scenario.

150. The features of both non-GM wheat and barley relevant to their spread and persistence in the environment are summarised in the biology documents.

Persistence of GM wheat and barley on the trial sites

151. For GM wheat and barley seeds to be available to persist at the proposed trial sites, seeds from any GM wheat or barley would need to drop to the ground either near maturity or during sowing 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. The introduced genes have not been linked to alterations in this trait in GM wheat and barley authorised proposed for release under the current application or under DIR 152.

152. While in a commercial setting loss of seeds during harvest is common (e.g. up to 10% in the case of non-GM barley; (OGTR, 2021a), the applicant proposes hand harvesting of seeds or use of a plot harvester. Both proposed methods of harvest would reduce the likelihood of seeds ending up on, or in the ground, when compared to commercial equipment.

153. GM wheat or barley at trial sites 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.

154. The applicant proposes to remove or destroy all GM wheat and barley plants at the trial sites 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 trial sites

155. Dispersal of GMOs outside the trial sites could occur through the activity of people or through natural means, such as animals, wind and water. There is no reasonable expectation that the introduced genes would affect any of the seed characteristics important for dispersal. In addition, the applicant has not reported any such changes in the GM wheat and barley already planted under DIR 152 and proposed for further release under the current application.

156. The main means of dispersal in non-GM wheat and barley is deliberate and inadvertent dispersal by people (OGTR 2021a; OGTR 2021b). 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; 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 sites by people.

157. Animals, including birds, could spread viable mature seeds:

- through seeds adhering to fur, feathers or feet, e.g. wheat seeds in sheep wool (Ryves 1988) or on muddy feet or legs of birds (Cummings et al. 2008), or barley seeds in fur and feathers (Von Bothmer 1992; Von Bothmer et al. 1995)
- by removing and hoarding seed, e.g. by ants (up to a few metres; (Gómez and Espadaler, 1998)), rodents (up to 50 metres; (Andersson and deVicente, 2010) or birds (Chambers and MacMahon, 1994)

- by consuming and excreting whole seeds, e.g. wheat seeds by birds, including emus (Calvino-Cancela et al. 2006).

158. While seed dispersed using the first two mechanisms mentioned above would retain viability, ingestion and excretion can affect seed viability and reduce the likelihood of germination:

- physically intact seed may make up to 30% (wheat) or 15% (barley) of dry matter in the faeces of cattle fed grain (Beauchemin et al. 1994), noting that germination rates were not measured. Viable wheat seeds have not been found in rabbit dung (Malo & Suárez 1995), and viable barley seeds have not been found in sheep and goat manure (Oveisi et al., 2021).
- after consumption by birds, a small proportion of intact wheat seed can be excreted by corellas (0.25%) and galahs (0.1%), with varying germination rates (Woodgate et al. 2011). Wheat seed may be dispersed by emus, however germination rates were very low (Rogers et al. 1993; McGrath & Bass 1999). Viable barley seed is not excreted by a range of birds (Cummings et al. 2008).

159. The applicant proposes controls that would reduce the likelihood of seed dispersal by animals, e.g. fencing the sites 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.

160. 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, 2021a, b). 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 the trial sites.

161. Seeds could also be transported off a site by water during heavy runoff or flooding. Proposed controls, including locating the trial sites at least 50 m from any natural waterway in areas not prone to flooding, would minimise the potential for seed dispersal through flooding.

Establishment of GM volunteer populations in the environment

162. If GM wheat and barley seed were dispersed beyond the trial, they require an environment conducive to germination and establishment. Non-GM wheat and barley are domesticated plants that have limited ability to survive outside cultivation. This is also evident from the weed risk ratings for wheat and barley, respectively (OGTR, 2021a, b).

163. The introduced genes for yield enhancement and improved abiotic stress tolerance are likely to be pleiotropic (that is, they have effects on several traits) thus potentially enhancing their ability to thrive in sub-optimal conditions. A gene involved in abiotic stress tolerance may impart tolerance to a number of abiotic stresses or to biotic stresses (Howles & Smith 2013). This may increase the ability to establish the GM wheat and barley in agricultural, natural and intensive use areas, and may provide the GM wheat and barley with an advantage over non-GM wheat and barley. However, no studies have been conducted and this is an area of uncertainty for this risk assessment.

164. The fluorescent visual marker gene and its product are not expected to alter characteristics that may affect the establishment of the GM wheat and barley as they are not involved in a relevant biochemical pathway.

165. The GM wheat and barley has tolerance to glufosinate-based herbicides, and therefore may offer a selective advantage in areas where these herbicides are used, such as in agricultural settings. However, GM wheat and barley volunteers could be controlled by application of alternative herbicides and other weed management measures.

Potential Harm

166. If GM plants were able to establish outside the trial sites, they could cause increased toxicity to people or desirable organisms, 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.

167. Establishment of the GM wheat or barley outside the trial limits could also reduce the establishment or yield of desirable plants in agricultural or natural land uses; reduce the utility of intensive use areas, such as roadsides, drains or channels; or increase its ability to provide a reservoir for pathogens or shelter for pests. However, none of the introduced genes has 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 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 introduced genes or their products are involved in relevant pathways, there is no reasonable expectation this may occur.

Conclusion

168. 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 wheat or barley to spread and persist outside the trial site and their susceptibility to a number of 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

Risk Source	Introduced or modified genes conferring yield enhancement, improved abiotic stress tolerance, herbicide tolerance and a visual marker
Causal Pathway	⬇ Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or barley ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity to people or toxicity to other organisms OR Other environmental harms (see risk scenario 3)

Risk source

169. The source of potential harm for this postulated risk scenario is the introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker in the GM wheat and barley.

Causal pathway

170. GM wheat and barley would be planted at the trial sites. When these plants flower, their pollen could be carried by wind to sexually compatible crops growing in the vicinity of the trial sites. If these related crops are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM seed. Hybrid GM plants carrying the introduced genes could form the basis for establishment, spread and dispersal of the introduced genes in other varieties of wheat or barley, or other sexually compatible plant species. Exposure of people and other organisms could increase to the proteins expressed by the introduced genes through ingestion, contact with plant material or inhalation of pollen.

171. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the wheat and barley biology documents (see Chapter 1, Section 5.4), and relevant details have also been provided in the pathway section for risk scenario 2.

172. 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).

173. 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 destruction of GM wheat and barley before flowering during post-harvest monitoring.

174. Any hybrid seed resulting from vertical gene flow would need a suitable environment for germination, plant establishment and persistence. Volunteers can be managed with alternative herbicides and other weed management measures.

Potential harm

175. If GM hybrid plants spread and persisted in the environment, this may lead to increased toxicity to people or other desirable organisms, or allergenicity to people. Hybrids expressing the introduced genes could also reduce the establishment and yield of desired plants and cause other environmental harms as per risk scenario 3.

176. The introduced genes could combine, via vertical gene transfer, with those of other non-GM wheat, barley or other sexually compatible species. The properties that the introduced 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 introduced genes in any subsequent hybrids would confer the same properties as the GM parent.

177. As discussed in risk scenarios 1-3, the introduced genes are unlikely to change the GM wheat or barley characteristics such that they would cause more harm than the non-GM parents.

Conclusion

178. Risk scenario 4 is not identified as a substantive risk due to the proposed limits and controls, the limited ability of long distance pollen flow in wheat and barley, and the limited ability of wheat and barley to survive outside of cultivation. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

180. 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.

181. 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.

182. For DIR 186, uncertainty is noted particularly in relation to:

- expression patterns of the introduced genes in the GM plants

- potential for increase in toxicity or allergenicity, including potential for uptake of heavy metals by the GM plants
- potential for the introduced genes to increase spread and persistence of the GM plants.

183. 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.

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

Section 4 Risk evaluation

185. 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.

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

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

187. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- no GM plant material would enter human food or animal feed
- limits on the size and duration of the proposed release
- controls proposed by the applicant to restrict the spread and persistence of the GM wheat and barley plants and their genetic material (see Chapter 3 for their suitability)
- 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.

188. 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

189. 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.

190. 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 can be managed in a way that protects the health and safety of people and the environment.

191. 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.

192. 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

193. 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

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

3.1 Draft licence conditions to limit and control the release

3.1.1 Consideration of limits proposed by The University of Adelaide

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

196. The applicant proposes that the release would take place at up to two sites per year. These sites are in Rosedale (SA) and at the NGNE facility in Merredin (WA). The field trial would run between April 2022 and January 2027, inclusive. A combined total of 2 hectares across both sites in any year would be used for planting of the GM plants. The applicant has stated that for each site, more than one planting area may be used. The small size and short duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk Scenario 1), and limit the opportunity for presence of the GM outside or after the trial (remaining risk scenarios).

197. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions included in the draft licence state that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions. These measures would limit the exposure of people to potential harm from the GM wheat and barley (Risk Scenario 1).

198. GM wheat and GM barley have previously been planted at Merredin under the licence for DIR 152. The Rosedale site is also approved for planting of GM wheat and barley under licence DIR 152. The licence for DIR 152 permits planting until the end of the 2022/23 growing season (inclusive), so GMOs from both DIR 152 and DIR 186 (if approved) could be grown concurrently at the same sites. Pollen transfer between individual GM lines proposed to be grown under DIR 186 has been considered, as has the risk of gene flow between GM lines from DIR 152 and DIR 186 (Risk Scenario 2). Like the DIR 152 licence, the DIR 186 draft licence include requirements to treat any collected seeds as GMOs, i.e. the seeds cannot be released into the environment unless an appropriate authorisation has been obtained. Thus, even if outcrossing between GM plants from both trials were to occur, exposure of humans or other desirable organisms to GM hybrid plants (Risk Scenario 2) or the spread of any GM hybrid plants outside the trial site (Risk Scenario 3) would be highly unlikely.

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

199. The applicant proposes not allowing the GMOs or GM products to be used for human food or animal feed. A draft licence condition states that GM plant material must not be used as food for humans or feed for animals. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenario 1).

200. The applicant has indicated that all properties 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 toxic or allergenic to people 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

201. The applicant proposes surrounding each GM wheat and barley planting area 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 152). These isolation distances are expected to minimise pollen flow from the GMOs to non-GM plants outside the trial sites, so are included in the licence. The 2 m buffer zone and pollen trap are not required to minimise pollen flow in addition to these isolation distances, so they are not proposed under the conditions of the licence.

202. 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 draft 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.

203. The applicant has stated that, under field conditions, the GM lines for yield enhancement flower 5-10 days earlier than non-GM plants within the same cultivar. The introduced genes for yield enhancement may also influence tillering in the GM wheat lines (Chapter 1, Section 4.5). Additionally, genetic modification of stomatal development and aperture may also reduce time to flowering in the GM wheat and barley plants (Chapter 1, Section 4.3.2). 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 to be destroyed or prevented from flowering.

204. The applicant proposes that more than one planting area could be established at each trial site. Under the conditions proposed 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

205. 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. Draft licence conditions require that each 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, draft licence conditions require that GMOs must be harvested or destroyed within eight months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been cleaned.

206. 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, with requirements for monitoring of burial sites, have been included in the draft licence. To ensure the effectiveness of destruction by seed burial, a draft 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.

207. 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 draft 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.

208. 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 after harvest in the same manner as GM wheat and barley, to manage persistence of the GMOs, and this measure is included in the draft licence.

209. At both trial sites, where GMOs from DIR 152 could be planted in close proximity, the GM lines from each licence could hybridise with one another, or with future trials approved at the sites, resulting in hybrid lines containing additional introduced genes and/or traits. Therefore, if seed from DIR 186 trials was used to develop future GM wheat or barley lines, there is a possibility that other genes could be unintentionally present. Therefore, as in the licence for DIR 152, a licence condition for DIR 186 has been proposed to prevent seed from trials where such gene flow could have occurred being used for development of cultivars for potential future commercial release (Risk Scenarios 2 and 4). On sites where no other GM trials have been planted, the seed could be used for future variety development, subject to appropriate approvals from the Regulator.

210. 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 draft licence condition requires that any area used to clean equipment used 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.

211. After harvest, the applicant proposes to inspect the planting areas and monitoring zone at least once every 35 days for two years, and until the site is free of volunteer plants for at least 6 months. 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-days 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 and barley lines (Chapter 1, Section 4.3.2), monitoring the trial sites 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 sites, so is included in a draft licence condition.

212. 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 & 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 (Ogg & 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 & Parker 2000).

213. 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 draft licence. The licence contains draft conditions requiring that after harvest, the trial sites 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 would minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4), if a licence were issued.

214. 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 draft 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).

215. 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 is not proposed under the conditions of the draft licence, however draft 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 proposed to manage the potential risks 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

216. 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. Both the Rosedale and NGNE Merredin sites have dedicated washdown facilities at their 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).

217. 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 draft 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 GMOs 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).

218. The applicant has proposed to fence the trial sites. 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 draft 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.

219. 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 & 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 & 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 propose measures to control access of birds to the planting areas (Risk Scenario 3).

220. In addition, there is no evidence that the GM wheat and GM barley lines 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.

221. 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 trials 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 proposed requirement that livestock not be allowed to access viable grain further limits the likelihood of spread of with wheat or barley seed via these routes (Risk Scenario 3).

222. 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 sites, 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).

223. 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 draft conditions of the licence.

224. Both of the draft licence conditions (keeping vegetation short and rodent controls) would apply while the GMOs are being grown and until the planting area is cleaned. Cleaning of a planting area, as defined in the draft 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.

225. The applicant has proposed that all trial sites 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 draft 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 draft licence conditions to be implemented to limit and control the release

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

- limit the duration of the release to the period from April 2022 to January 2027
- limit the release to a maximum of two site; one in SA (Rosedale) and the other in Western Australia (NGNE Merredin)

- limit the release to a combined total of 2 ha across both sites in any year
- locate trial sites 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 allow the GM plant material to be used for human food or animal feed.

3.2 Other risk management considerations

227. 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

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

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

229. 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.

230. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2.2 Contingency plan

231. 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.

232. 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

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

3.2.4 Reporting requirements

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

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

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

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

3.2.5 Monitoring for compliance

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

237. 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.

238. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

239. Additional information has been identified that may be required to assess an application for a commercial release of these GM wheat and barley lines, 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.

Section 5 Conclusions of the consultation RARMP

240. The RARMP 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, and that these negligible risks do not require specific risk treatment measures.

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

Chapter 4 Proposed licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

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

    b. words denoting a gender include any other gender;

    c. words in the singular include the plural and words in the plural include the singular;

    d. words denoting persons include a partnership and a body whether corporate or otherwise;

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

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

    g. specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

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

'Barley' means plants of the species *Hordeum vulgare* L.

'Clean' means, as the case requires:

    a. in relation to Equipment or a facility, remove and/or Destroy the GMOs; or

    b. in relation to an area of land specified in this licence as requiring Cleaning:

        i. Destroy wheat and barley plants, if present, to the reasonable satisfaction of the Regulator, and

        ii. remove wheat and barley seeds from the soil surface to the reasonable satisfaction of the Regulator.

Note: The intent of removing seeds from the soil surface is to minimise seed dispersal. One method of removing seeds from the soil surface is Tillage, which moves seeds to under the soil. Tillage must be in accordance with condition 39.

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

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

    b. recover and/or Destroy the GMOs to the reasonable satisfaction of the Regulator; and

    c. inspect for and Destroy any Volunteers that may exist as a result of the event to the reasonable satisfaction of the Regulator.

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

    a. uprooting;

    b. root cutting and shredding/mulching;

c. tillage, but only in accordance with condition 39;

d. treatment with herbicide;

e. burning/incineration;

f. autoclaving;

g. milling/hammer milling;

h. crushing or grinding of seed;

i. burial, but only in accordance with condition 40;

j. a method approved in writing by the Regulator.

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

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

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

'Flowering' is taken to begin when anthers emerge from any plant of the class of plants referred to in a particular condition, and is taken to end when anthers have dried up or dropped off all plants in the class of plants.

'GM' means genetically modified.

'GMOs' means the genetically modified organisms that are the subject of the dealings authorised by this licence. GMOs include live plants and viable seed.

'Inspection Zone' means an area of land extending outwards at least 50 metres from the outer edge of a Monitoring Zone, as shown in Figure 1.

'Isolation Zone' means an area of land extending outwards at least 140 metres from the outer edge of an Inspection Zone, as shown in Figure 1.

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

'Monitoring Zone' means an area of land extending outwards at least 10 metres from the outer edge of a Planting Area, as shown in Figure 1. If multiple Planting Areas are present in a Site, the Monitoring Zone also includes the areas of land, of any size, between Planting Areas, as shown in Figure 1.

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

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

a. whether the information or opinion is true or not; and

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

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

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

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

'Regulator' means the Gene Technology Regulator.

'Related Species' means durum wheat, rye or triticale plants.

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

'Site' means an area of land containing one or more Planting Areas and their joint Monitoring Zone, as shown in Figure 1.

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

Note: Tillage must be in accordance with condition 39.

'Volunteers' means GM or non-GM wheat and barley plants, which have not been intentionally grown.

'Wheat' means plants of the species *Triticum aestivum* L. em Thell.

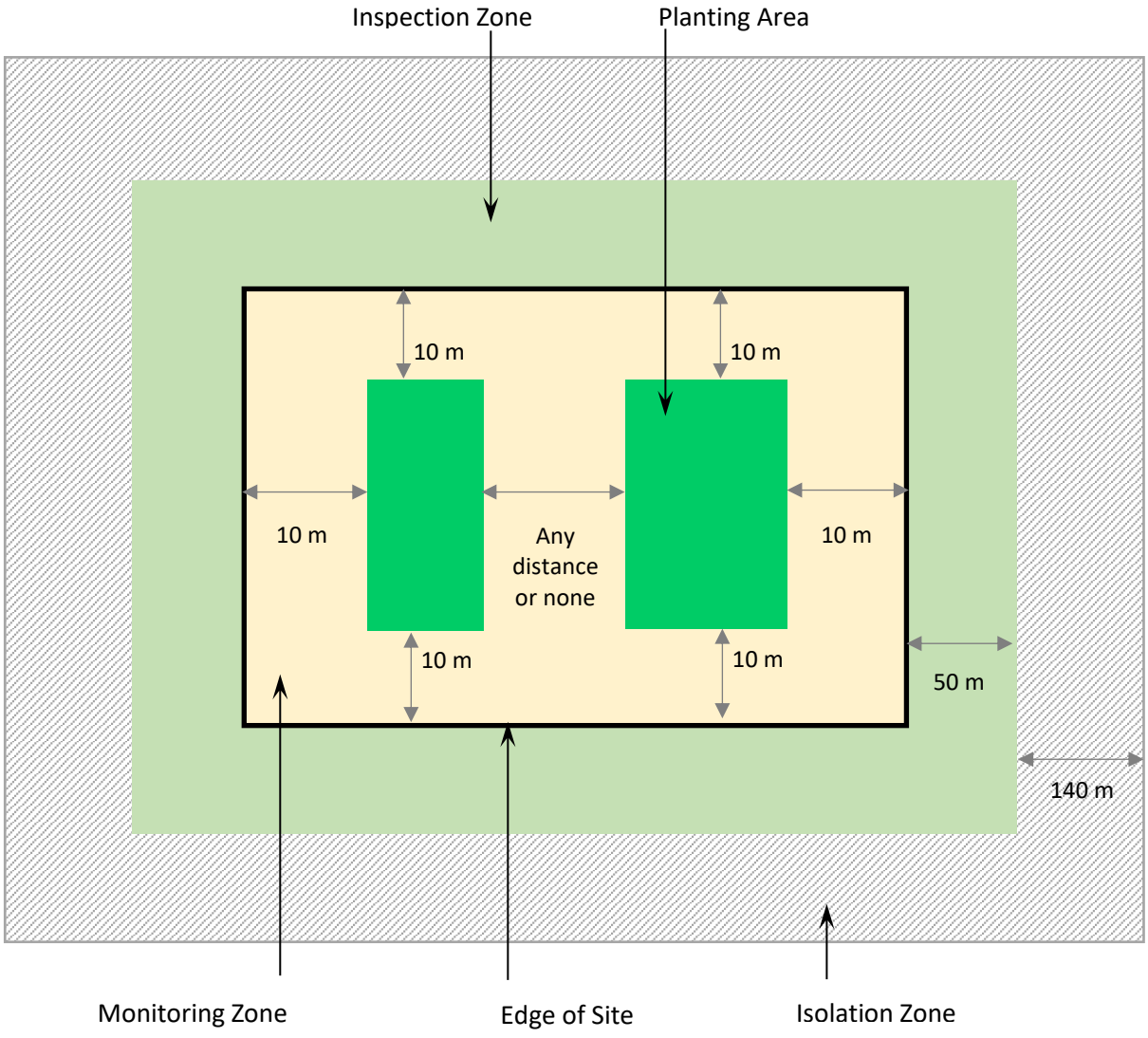

Figure 1. Diagram (not to scale) showing the relationship between Planting Area, Monitoring Zone, Site, Inspection Zone and Isolation Zone.

Section 2 General conditions and obligations

3. This licence does not authorise dealings with the GMOs that are otherwise prohibited as a result of the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

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

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

5. The licence holder is The University of Adelaide.

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

7. The GMOs with which dealings are authorised by this licence are those listed at **Attachment A**.

8. The dealings authorised by the licence are to:

    a. conduct experiments with the GMOs;

    b. make the GMOs;

    c. breed the GMOs;

    d. propagate the GMOs;

    e. grow or culture the GMOs;

    f. import the GMOs;

    g. transport the GMOs;

    h. dispose of the GMOs;

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

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

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

General obligations of the licence holder

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

11. The licence holder must be able to access and control all Planting Areas, Monitoring Zones, Inspection Zones, Isolation Zones and approved facilities to the extent necessary to comply with this licence.

Note: Arrangements to access and control these areas must be notified to the Regulator as part of each planting notification (Condition 48(a)).

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

    a. the particular condition, including any variations of it;

    b. the cancellation or suspension of the licence;

    c. the surrender of the licence.

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

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

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

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

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

14. The licence holder must inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator.

General Obligations of persons covered by the licence

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

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

Section 3 Limits and Control Measures

3.1 Limits on the release

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

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

Area and duration

Period	Maximum number of Sites per year	Maximum combined area of Planting Areas at both Sites
April 2022 – January 2027	2	2 ha

Possible locations of Sites

Location	Local government area	State
Rosedale	Light Regional Council	SA
NGNE Merredin	Shire of Merredin	WA

3.2 Control measures

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

GMOs must not enter food or feed

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

Conditions to restrict pollen flow

20. A Planting Area must be surrounded by a Monitoring Zone (as shown in Figure 1). Multiple Planting Areas may be contained within a single Monitoring Zone. No Planting Area may be less than 10 metres from the outer edge of the Monitoring Zone.

21. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and Destruction of Volunteers and Related Species while the GMOs are growing in the Planting Areas and until the Planting Areas are Cleaned.

Note: Acceptable measures to achieve this include keeping land free of vegetation or keeping vegetation mown to a height of less than 10 centimetres. Condition 491.d) requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

22. The Monitoring Zone must be surrounded by an Inspection Zone (as shown in Figure 1).

23. The Inspection Zone must be surrounded by an Isolation Zone (as shown in Figure 1).

24. The GMOs must not be grown in a Planting Area if any crop of wheat and barley or a Related Species is present in the Monitoring Zone, Inspection Zone or Isolation Zone.

25. While the GMOs are growing in a Planting Area, associated areas must be inspected by people trained to recognise wheat and barley and Related Species, and actions must be taken as follows:

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

**Condition 48(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.*

Note: Details of any inspection activity must be must be recorded in a Logbook (Condition 49) and reported to the Regulator (Condition 48).

Conditions to restrict seed dispersal

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

27. Planting Areas must be at least 50 metres away from any permanent natural watercourses or man-made drainage features that flow into natural watercourses.

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

28. Planting Areas must not be located in flood prone areas.

29. Measures must be implemented to control rodents within each Planting Area while GMOs are being grown and until the Planting Area is Cleaned.

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

30. The Monitoring Zone must be maintained in a manner that does not attract or harbour rodents while the GMOs are being grown at a Planting Area and until the Planting Area is Cleaned.

Note: Acceptable measures to achieve this include keeping land free of vegetation or keeping vegetation mown to a height of less than 10 centimetres.

Conditions relating to harvesting

31. GMOs must be harvested or Destroyed within eight months after planting.

32. If all GMOs in a Planting Area have been Destroyed, then for the purposes of this licence:

    a. the GMOs are taken to have been harvested; and

    b. the Planting Area is taken to have been Cleaned.

Note: Cleaning activities must be reported to the Regulator (Condition 48). Areas of land that have been Cleaned are subject to inspections (Condition 37).

33. GMOs must be harvested in a manner that minimises dispersal of GMOs outside the Planting Area.

34. The GMOs must be harvested and threshed separately from any other crop.

35. Harvested GM seed not required for experimentation or future planting must be Destroyed as soon as practicable.

Conditions to restrict persistence of GMOs on trial sites

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

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

Note: Cleaning activities must be reported to the Regulator (Condition 48). Areas of land that have been Cleaned are subject to inspections (Condition 37).

37. After Cleaning, areas of land must be inspected by people trained to recognise wheat and barley. Inspections must cover the entirety of the areas to be inspected. Actions must be taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area, Monitoring Zone and other areas of land that were Cleaned in accordance with Condition 36	From the day of Cleaning, until: i. the area is planted as a new Planting Area in accordance with condition 16; or ii. the Regulator has issued a Sign off for the area.	At least once every 35 days	Volunteers	Destroy before Flowering

Note: Details of any inspection activity must be must be recorded in a Logbook (Condition 49) and reported to the Regulator (Condition 48).

38. While post-Cleaning inspection requirements apply to an area:

    a. the area must be maintained in a manner appropriate to allow identification of Volunteers; and

    b. no plants may intentionally be grown in the area unless:

        i. the area is planted as a new Planting Area in accordance with condition 16; or

ii. the plants are agreed to in writing by the Regulator; and

c. the area must not be used for grazing livestock; and

d. prior to an application for Sign off, the area must receive at least three watering events as described in **Attachment B**, at intervals of at least 28 days, with the final required watering event occurring within the six months prior to submission of the Sign off application; and

e. within the six months prior to submission of the Sign off application, and before the final required watering event, the area must be Tilled.

Tillage

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

Destruction by burial

40. If Destruction of GMOs occurs by burial:

a. the GMOs must be buried in a pit and covered by a layer of soil at least one metre in depth, the top of which is no higher than the surrounding soil surface; and

b. seeds must be wet when buried to encourage decomposition; and

c. the licence holder must take measures to ensure that the burial site is not disturbed for a period of at least 12 months from the date of burial.

Note: If GMOs are dispersed on the soil surface during the process of burial, the burial site becomes an area of land that requires Cleaning under Condition 36 and is subject to post-Cleaning requirements.

Note: The date and location of burial, and measures used to ensure that the burial site is not disturbed, must be reported to the Regulator (Condition 48(f)).

Processing or experimentation with the GMOs

41. Treatment, threshing or processing of GM seed, or experimentation or analysis with the GMOs may only be undertaken within:

a. a Planting Area before Cleaning; or

b. a Monitoring Zone before Cleaning; or

c. a facility approved in writing by the Regulator.

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

42. Within a facility approved in writing by the Regulator in accordance with Condition 41, any area that is used for treatment, threshing, processing, experimentation or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

Transport or storage of the GMOs

43. Transport or storage of the GMOs must:

a. only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation under the Act, or to the extent necessary to enable export of the GMOs; and

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

c. comply with all other conditions of this licence.

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

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

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

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

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 45).

Contingency plan

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

Section 4 Sign off

46. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and other areas requiring Cleaning if:
    a. post-Cleaning inspection activities have been conducted for at least 24 months on the area; and
    b. conditions have been conducive for germination and detection of Volunteers; and
    c. no Volunteers have been detected in the area during the six months prior to the Sign off request.

Note: An area requires Tillage and three watering events prior to a Sign off application (Condition 38).

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

Section 5 Reporting and documentation

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

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

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

Notice	Content of notice	Timeframe
a. Changes to contact details	Changes to any of the contact details of the project supervisor that were notified in the licence application or subsequently	As soon as practicable
b. Ongoing suitability to hold a licence	i. any relevant conviction of the licence holder; or ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and	As soon as practicable after any of these events occur
	iv. any information related to the licence holder's ongoing suitability to hold a licence, that is requested by the Regulator	Within the timeframe stipulated by the Regulator
c. People covered by the licence	i. names of all organisations and persons, or functions or positions of the persons, who will	At least 14 days prior to conducting any dealings with the GMOs (to be updated

	be covered by the licence, with a description of their responsibilities; and *Note: Examples of functions or positions are 'project supervisor', 'site manager', 'farm labourer' etc*. ii. detail of how the persons covered by the licence will be informed of licence conditions	within 14 days if the notified details change)
d. Testing methodology	A written methodology to reliably detect the genetic modifications described in this licence. The detection method/s must be capable of identifying each GM wheat and barley line planted under this licence	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
e. Contingency plan	A Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
f. Training records	Copies of the signed and dated statements referred to in condition 13 if requested by the Regulator	Within the timeframe stipulated by the Regulator
g. Additional information required by the Act	i. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or ii. any contraventions of the licence by a person covered by the licence; or iii. any unintended effects of the dealings authorised by the licence *Note: The Act requires, for the purposes of the condition 47(g), that:* • *the licence holder will be taken to have become aware of additional information of a kind mentioned in Condition 47(g) if he or she was reckless as to whether such information existed; and* • *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in Condition 47(g), if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed* *Note: Contraventions of the licence may occur through the action or inaction of a person.*	Without delay after becoming aware of any new information *Note: An example of notification without delay is contact made within a day of a contravention of the licence via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location, if required*
h. Further details regarding additional information	Any further details requested by the Regulator in relation to information provided under condition 47(g)	Within the timeframe stipulated by the Regulator

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

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

Notice	Content of notice	Timeframe
a. Intention to plant	i. Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the Site (e.g. Google Maps) and any other descriptions ii. Detail of how the licence holder will access and control the Planting Area and the associated *Monitoring Zone, Inspection Zone and Isolation Zone*, in accordance with condition 11 *Note: this should include a description of any contracts, agreements, or other enforceable arrangements.* iii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details) iv. Date on which the GMOs will be planted v. Period when the GMOs are expected to Flower vi. Period when harvesting is expected to commence vii. How all areas requiring post-Cleaning inspections are intended to be used until Sign off, including proposed post-harvest crops (if any) viii. Details of how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers ix. History of how the Site has been used for the previous two years	At least 7 days prior to each planting (to be updated as soon as practicable if the notified details change)
b. Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition	Within 7 days of any planting
c. Extreme Weather	Any Extreme Weather event that is expected to affect or has already affected an area where the GMOs are or may be present. *Note: The Contingency Plan must be implemented if the GMOs are detected outside areas requiring Cleaning (Condition 45).*	As soon as practicable
d. Harvest	Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting
e. Cleaning	i. Date(s) on which required Cleaning was performed on any areas of land ii. Method(s) of Cleaning	Within 7 days of completion of Cleaning
f. Destruction by burial	Date of burial, location of burial including GPS co-ordinates, and details of measures used to ensure that the burial site will not be disturbed for the period required by Condition 40	Within 7 days of burial of any GMOs
g. Inspection activities	Information recorded in a Logbook as per the inspection requirements (Conditions 25, 37 and 49).	Within 35 days of inspection

Note: Additional records must be provided to the Regulator, if requested, in accordance with condition 44.

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

    a. date of the inspections; and

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

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

d. details of areas inspected including current land use (including any post-harvest crops) and recent management practices applied; and

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

f. details of the developmental stage of the GMOs while they are being grown; and

g. details of any post-Cleaning rainfall events including measurements at or near the area, or any irrigation events; and

h. details of any Volunteers and/or Related Species observed during inspections or during land-management activities, including number, developmental stage and approximate position of the Volunteers and/or Related Species within each area inspected†; and

i. date(s) and method(s) of Destruction of or preventing Flowering of any Volunteers and/or Related Species, including destruction of Volunteers and/or Related Species during land-management activities; and

j. details of rodent control methods used and any evidence of rodent activity, while rodent control methods are required.

† Examples of acceptable ways to record the positional information for Volunteers and/or Related Species in the Logbook include:
- descriptive text
- marking on a diagram
- indicating grid references on a corresponding map/sketch.

Note: Details of inspection activities must be provided to the Regulator (Condition 48).The Regulator has developed a standardised proforma for recording inspection activities. This can be made available on request.

ATTACHMENT A

DIR No: 186

Full Title: Limited and controlled release of wheat and barley genetically modified for yield enhancement and improved abiotic stress tolerance

Organisation Details

Postal address: The University of Adelaide
SA 5005

Phone No: (08) 8313 5208

IBC Details

IBC Name: The University of Adelaide Institutional Biosafety Committee

GMO Description

GMOs covered by this licence

Wheat and Barley plants genetically modified by introduction of only the genes and genetic elements listed below.

Parent Organisms

Common Name: Wheat and Barley

Scientific Name: *Triticum aestivum* L. and *Hordeum vulgare* L.

Modified traits

Category: Enhanced yield
Abiotic stress tolerance
Selectable markers – antibiotic resistance, herbicide tolerance, visual marker

Description: Wheat and barley plants have been genetically modified for yield enhancement and/or improved abiotic stress tolerance (water-use efficiency). The plants have been modified by the introduction of either one, two or three of the genes for yield enhancement, or by the introduction of one of five genes for yield enhancement and abiotic stress tolerance. The introduced genes and associated regulatory sequences for the genes are listed in Table 1.

Purpose of the dealings with the GMO

The purpose of the release is to evaluate the agronomic performance of the GM wheat and barley under Australian field conditions. The GM Wheat and barley is not permitted to be used for human food or animal feed.

Subject to any conditions imposed by DIR 152, this licence does not prohibit the planting of GMOs authorised under this licence on Planting Areas previously used for DIR 152 or adjacent to Planting Areas concurrently planted under DIR 152.

Table 1. Introduced genes in the GM Wheat and Barley

Element	Gene Source	Function
		Yield enhancement
AtAVP1	*A. thaliana*	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance

Element	Gene Source	Function
OsNAS2	*O. sativa*	Increase in shoot biomass, higher numbers of tillers and grain
OsPSTOL1	*O. sativa*	Enhanced growth vigour and earlier heading, high yield
	Yield enhancement and water use efficiency*	
TaMUTE	*T. aestivum*	Stomatal development, symmetrical division of guard mother cells
TaYDA1	*T. aestivum*	Negatively regulates stomatal development
TaYDA2	*T. aestivum*	Negatively regulates stomatal development
TaOST1	*T. aestivum*	Regulates stomatal aperture
TaSLAC1	*T. aestivum*	Guard cell anion channel
	Promoters	
CaMV35S	Cauliflower mosaic virus	Constitutive
Ubi	*Z. mays*	Constitutive, polyubiquitin
OsAct1	*O. sativa*	Constitutive, rice actin 1
PvUbi+3	*Panicum virgatum*	Constitutive, ubiquitin
	Amplification promoting sequences	
Ubi1 5' UTR	*Z. mays*	Translational modifier
Ubi1 intron	*Z. mays*	Translational modifier
	Selectable Marker Genes	
hptII	*E. coli*	Plant selectable marker – hygromycin resistance gene encoding hygromycin phosphotransferase
nptII	*E. coli K12*	Plant selectable marker – neomycin phosphotransferase gene for resistance against geneticin or kanamycin
bar	*Streptomyces hygroscopicus*	Plant selectable marker – bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate
pporRFP	*Porites porites*	Visual plant selectable marker – red fluorescent protein
	Terminator	
CaMV35S	*Cauliflower mosaic virus*	Viral terminator
nos	*A. tumefaciens*	Terminator of the nopaline synthase gene and polyadenylation signal
OCS	*A. tumefaciens*	Terminator sequence of the octopine synthase gene

ATTACHMENT B

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

Examples of acceptable watering events are:

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

Rainfall measurements must be taken on the site or within 3 km of the site. An irrigation or natural rainfall that matches one of the examples listed above, and occurs during the time period specified for a watering event in Condition 38 of the licence, is considered a valid watering event. The licence holder should keep records of the date/s and amount of water applied during the watering event, and provide this information when requesting Sign off of the relevant site.

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

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

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

References

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