Australian Government
Department of Health
Office of the Gene Technology Regulator

July 2017

Risk Assessment and Risk Management Plan for

DIR 152

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

Applicant: The University of Adelaide

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 152

Decision

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

The application

Application number	DIR 152
Applicant	The University of Adelaide
Project Title	Limited and controlled release of wheat and barley genetically modified for abiotic stress tolerance and yield improvement.
Parent Organism	Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)
Introduced genes and modified traits	Two groups of introduced genes are proposed: • Group 1: three genes involved in yield enhancement, individually and in combinations • Group 2: seven genes involved in frost tolerance[1] In addition, one selectable marker gene is used across both groups
Proposed location	Maximum of four locations per season across South Australia, Western Australia, and New South Wales.
Proposed release size	Maximum total area of 3.75 ha in Seasons 1 and 2 and 1.5 ha in Season 3.
Proposed release dates	July 2017 – January 2021
Primary purpose	To assess agronomic performance of the GM wheat and barley lines under field conditions.

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings, either in the short or long term, 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 impacts are considered.

[1] The identities and details regarding these genes, some promoters, regulatory sequences and some references have been declared Confidential Commercial Information (CCI).

Credible pathways to potential harm that were considered included exposure of people and desirable animals to the GM plant material; increased potential for spread and persistence of the GMOs in the environment and transfer of introduced genetic material into sexually compatible plants. Potential harms associated with these pathways included increased toxicity or allergenicity to humans or increased toxicity to other desirable organisms and environmental harms due to increased weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed, the imposed limits and controls effectively contain the GMOs and their genetic material and minimise exposure; and the GM wheat and barley have limited ability to establish populations outside cultivation or transfer the introduced genetic material to other plants.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

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

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Abbreviations

ACT	Australian Capital Territory
APVMA	Australian Pesticides and Veterinary Medicines Authority
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
HPH	Hygromycin phosphotransferase protein encoded by *hptII* gene
hptII	Hygromycin phosphotransferase gene
m	Metres
NGNE	New Genes for New Environments (Department of Agriculture and Fisheries Western Australia)
NLRD	Notifiable Low Risk Dealing
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
RARMP	Risk Assessment and Risk Management Plan
Qld	Queensland
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SA	South Australia
the Act	*The Gene Technology Act 2000*
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

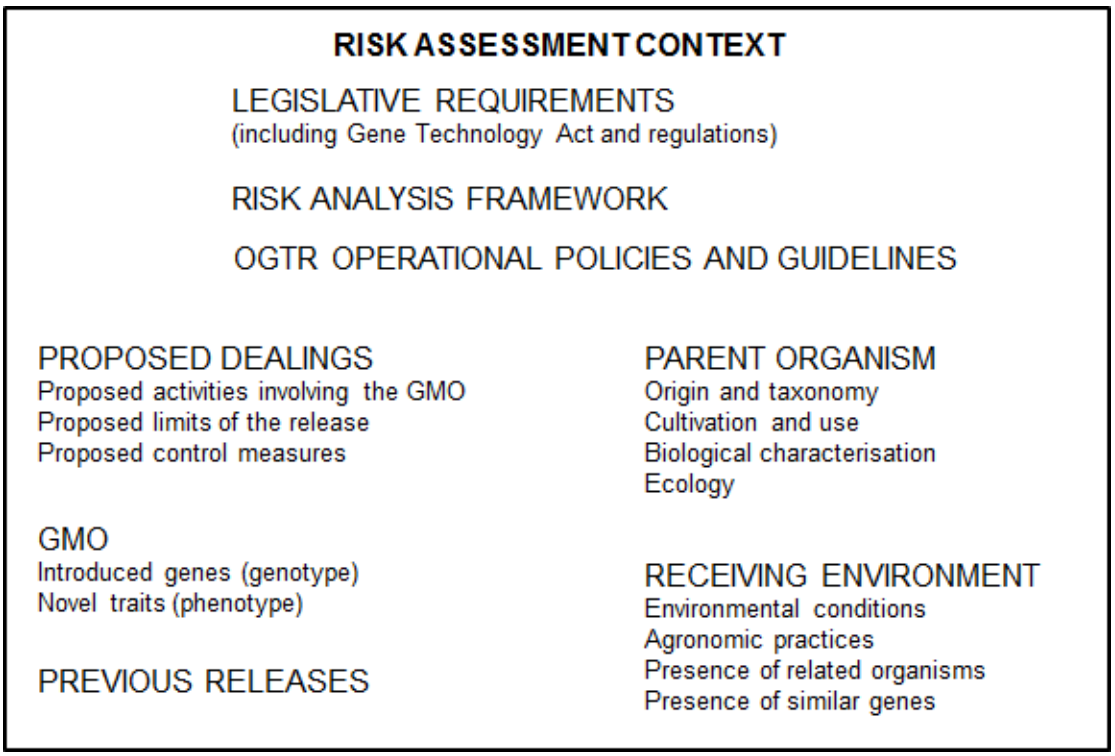

Figure 1 Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public.

7. The Risk Analysis Framework (OGTR 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed dealings

9. The University of Adelaide proposes to release up to 95 genetically modified (GM) wheat lines and up to 18 GM barley lines into the environment under limited and controlled conditions. The wheat lines have been genetically modified for yield enhancement (Group 1, 35 lines) or frost tolerance (Group 2, 60 lines). The barley lines have been genetically modified for frost tolerance (Group 2, 18 lines).

10. Some information including gene identity, accession numbers, associated regulatory elements and relevant references have been declared Confidential Commercial Information (CCI). In this document, CCI gene identities have been replaced with non-CCI identifiers or 'CCI'. All relevant CCI is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

11. 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 under non-stressed and stressed (frost) conditions. The GM wheat and barley lines would not be used for human food or animal feed.

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- growing the GMOs
- transporting the GMOs
- disposing of the GMOs and
- possession, supply or use of the GMOs for any of the purposes above.

These dealings are detailed further below.

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

13. The release is proposed to take place at up to five sites: two in South Australia (SA) at Glenthorne Farm and Loxton; two in Western Australia (WA) at Katanning and Merredin and one in New South Wales (NSW) at Narrabri. The release is proposed to take place over three planting seasons. For each of the first two seasons, planting of the GMOs would occur at up to four sites, with a combined area of up to 3.75 ha per season, with a maximum of 2.5 ha on any single site. In the third season the GMOs would be grown at a single site with an area of up to 1.5 ha.

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

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

15. The applicant has proposed a number of controls to restrict the spread and persistence of the GM 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 the planting area with a 2 m buffer zone, within which plant growth and rodent activity will be controlled
- surrounding the buffer zones with a 10 m monitoring zone in which plant growth will be controlled and a 190 m isolation zone in which no sexually compatible plants will be grown during the cultivation of GM wheat and barley
- only permitting trained and authorised staff to access the site
- restricting human and animal access by surrounding the trial sites with livestock proof fences with lockable gates
- treating non-GM plants used in the trial as if they were GM
- inspecting all equipment for GM plant material, which will be destroyed 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
- not allowing the GM plant materials or products to be used in commercial human food or animal feed

16. Figure 2 shows the layout proposed by the applicant, including some of the proposed controls. The figures show trial sites with either a single planting area (with associated buffer zone) or multiple planting areas (with associated buffer zones). These are surrounded by a monitoring zone and an isolation zone. The proposed limits and controls are taken into account in establishing the risk assessment context (this Chapter) and their suitability for containing the release (Chapter 3).

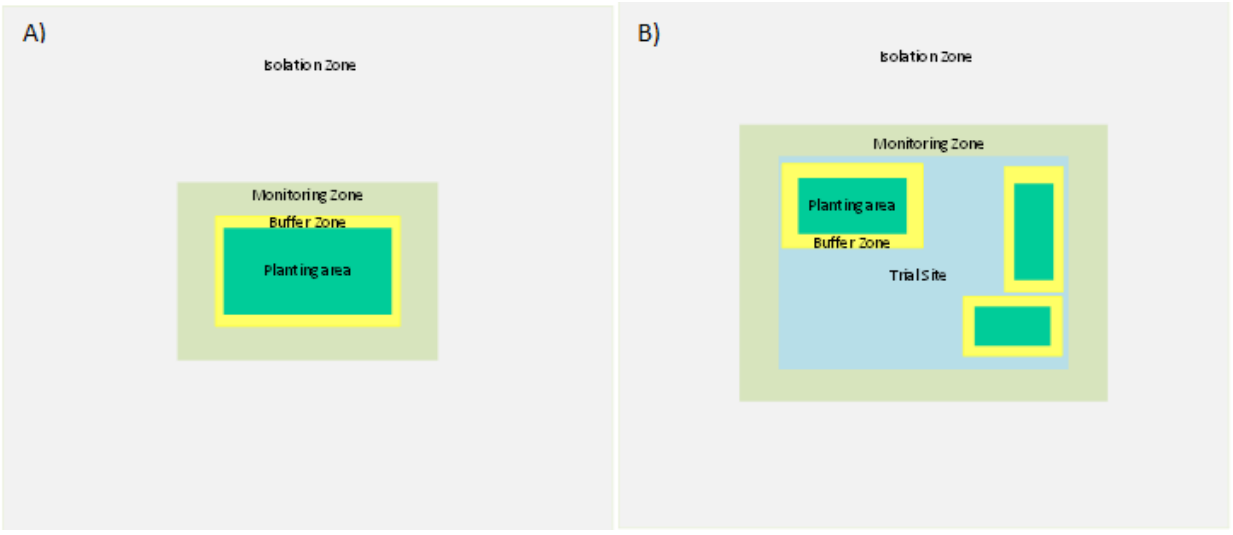

Figure 2 Schematic diagram (not to scale) of trial setup proposed by applicant A) Trial with single planting area; B) Trial with multiple planting areas.

Section 4 The parent organisms

17. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), which are exotic to Australia. Commercial wheat and barley cultivation occurs in the 'wheat belt' from southeastern Queensland (Qld) through NSW, Victoria, Tasmania, southern SA and southern WA.

18. Some of the GM wheat lines were backcrossed into the varieties Bonnie Rock and IGW-2971. These backcross lines are proposed for use in the field trial. Bonnie Rock is one of the most commonly grown varieties in WA.

19. The GM barley lines were backcrossed into elite varieties Hindmarsh and Compass. These backcross lines are proposed for use in the field trial. Hindmarsh is an early maturing feed or food barley variety, listed as having exceptional yield potential (Agriculture Victoria 2016). Compass is also an early yielding variety listed as high yielding, currently used as a feed barley and being assessed as a malting barley (South Australian Research and Development Institute (SARDI) 2016).

20. Detailed information about the parent organisms is contained in the reference documents produced to inform the risk assessment process for licence applications involving GM crops: *The Biology of* Triticum aestivum *L. (Bread Wheat)* (OGTR 2017b) and *The Biology of* Hordeum vulgare *L. (barley)* (OGTR 2017a). Baseline information from these documents will be used and referred to throughout the RARMP. Of particular interest are the characteristics of the parent plant that relate to spread and persistence and therefore to potential weediness. Key points from those discussions are summarised in the individual risk scenarios for this RARMP.

21. 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.

22. 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 documents conclude that both species possess few attributes which would make them weedy and this is supported by the observation that there are very few weedy populations of wheat or barley in the Australian environment.

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

5.1 Introduction to the GMOs

23. The applicant proposes the release of up to 95 GM wheat lines and 18 GM barley lines into the environment under limited and controlled conditions. The GMOs are classified into two groups (Table 1), designated Group 1 (Yield enhancement) and Group 2 (Frost tolerance). Further details are provided in Table 2.

Table 1: The GM wheat and barley lines proposed for release

Group	GMO	Modified trait	Genes	Number of lines
Group 1	Wheat	Yield enhancement	3	35
Group 2	Wheat	Frost tolerance	7	60
Group 2	Barley	Frost tolerance	2	18

24. The applicant proposes to release up to 35 lines of GM wheat plants containing up to three yield enhancement genes. The genes are expressed singly or as combinations of two or three genes (Table 2). One gene is derived from thale cress (*A. thaliana*) and two from rice (*O. sativa*). Wheat plants with single genes were transformed either with biolistic transformation (*AtAVP1*, *OsNAS2*) or *Agrobacterium*-mediated transformation (*OsPSTOL1*). Information about these methods can be found in the document *Methods of plant genetic modification*, available from the OGTR Risk Assessment References page. Plants containing two or three genes were generated using controlled crossing of the GM plants containing single genes.

25. The applicant proposes to release up to 60 wheat lines and 18 barley lines each containing one of seven individual frost tolerance genes (Table 2). Gene 1 and Gene 4 in the frost tolerance group are derived from wheat. Source information for the other genes in this group is CCI. Wheat lines containing these genes were transformed using biolistic methods and barley lines were generated by *A. tumefaciens*-mediated transformation.

26. Short regulatory sequences that control expression of the genes are also present in the GM wheat and barley lines. The promoters used to drive expression of the introduced genes are inducible promoters, with the exception of *CaMV35S* and *Ubi* promoters. Details of regulatory elements are shown in Table 2.

27. The GM wheat and barley plants also contain the *hptII* selectable marker gene. This gene is derived from the bacteria *Escherichia coli* and it encodes the hygromycin phosphotransferase (HPT) enzyme conferring antibiotic resistance. This selectable marker was used in the laboratory to select transformed GM plants during early stages of development.

Table 2: Genes and regulatory elements[a] introduced to 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
OsNAS2	*O. sativa*	Increase in shoot biomass, higher numbers of tillers and grain
OsPSTOL1	*O. sativa*	Enhanced growth vigour and earlier heading, high yield
AtAVP1+ OsNAS2	*A. thaliana; O. sativa*	Combinations of traits listed for single genes as above
AtAVP1+ OsPSTOL1	*A. thaliana; O. sativa*	Combinations of traits listed for single genes as above
OsNAS2+ OsPSTOL1	*O. sativa*	Combinations of traits listed for single genes as above
AtAVP1+ OsNAS2+ OsPSTOL1	*A. thaliana; O. sativa*	Combinations of traits listed for single genes as above
	Frost Tolerance	
Gene 1	*T. aestivum*	Increases vegetative drought and frost tolerance, regulator of LEA (drought and frost inducible) genes
Gene 2	*[CCI]*	[CCI]
Gene 3	*[CCI]*	[CCI]
Gene 4	*T. aestivum*	Improved frost tolerance
Gene 5	*[CCI]*	[CCI]
Gene 6	*[CCI]*	[CCI]
Gene 7	*[CCI]*	[CCI]
	Promoters	
CaMV35S	Cauliflower mosaic virus	Constitutive
Ubi	*Z. mays*	Constitutive, polyubiquitin
Promoter 3	*Z. mays*	Inducible (drought, salt) very strong, some basal activity
Promoter 4	*T. durum*	Inducible (cold, drought) relatively strong
Promoter 5	*T. durum*	Inducible (drought, cold salt, wounding) relatively strong
Promoter 6	*T. durum*	Inducible (drought, cold, salt, ABA) moderate, prolonged
Promoter 7	*T. durum*	Inducible (stress) moderate
Promoter 8	*O. sativa*	Inducible (cold, drought) moderate
Promoter 9	*T. durum*	Inducible (drought, cold, ABA) moderate
	Amplification promoting sequences	
Ubi1 5' UTR	*Z. mays*	Translational modifier
Promoting sequences 2	*[CCI]*	[CCI]
Ubi1 intron	*Z. mays*	Translational modifier
Promoting sequences 4	*[CCI]*	[CCI]

Element	Gene Source	Function
	Selectable Marker Genes	
hptII	*E. coli*	Plant selectable marker – hygromycin
	Terminator	
nos	*A. tumefaciens*	Terminator of the nopaline synthase gene and polyadenylation signal
Sb-GKAF ter[b]	*Sorghum bicolor*	Terminator of the *S. bicolor* gamma-kafirin gene

[a] The identities and details of some genes, promoters and regulatory sequences have been declared CCI under section 185 of the Act.
[b] Inclusion of this terminator was requested by the applicant following the release of the consultation RARMP

5.2 The introduced genes, encoded proteins and associated effects

28. The genes and their encoded proteins are summarised in Table 2, with a description of their potential function in the GM wheat and barley lines. Both yield enhancement and frost tolerance are multigenic traits, involving the interaction of genes where the protein products constitute different biochemical pathways. Frost tolerance can be grouped with other abiotic stresses, such as drought, temperature, salt or nutrient stresses and mineral toxicities. More detailed discussion of plant responses to abiotic stresses can be found in the RARMPs for DIR 102 and DIR 128.

5.2.1 Group 1: Yield enhancement

29. The *AtAVP1* and *OsNAS2* genes for yield enhancement have been discussed previously in RARMPs for DIR 102 and DIR 128, so only a summary and more recent material regarding these genes is presented here. The *OsPSTOL1* gene will be discussed in more detail.

AtAVP1

30. The *Arabidopsis thaliana* vacuolar H^+-pyrophosphatase (*AtAVP1*) gene encodes an H^+-translocating pyrophosphatase (H^+-PPase) that appears to be localised to the tonoplast and the 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).

31. 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).

32. It has been suggested that overexpression of *AtAVP1* increases biomass by enhancing phloem loading (Gaxiola et al. 2012; Pizzio et al. 2015; Khadilkar et al. 2016). Efficient phloem loading and long-distance carbon partitioning may improve plant productivity by decreasing feedback inhibition of photosynthesis in leaves and mobilising more resources for the growth of sink organs like roots. An increase in root growth may explain improved tolerance to nutrient deficiency.

OsNAS2

33. The *OsNAS2* gene encodes a rice nicotianamine synthase (NAS), an enzyme that catalyses the last step in the production of nicotianamine, which is a chelator and long distance transporter of transition metals such as iron (Inoue et al. 2003). In grasses, nicotianamine is also used by other enzymes to synthesize phytosiderophores, which are molecules involved in the acquisition of iron from the soil (Inoue et al. 2003). Overexpression of *NAS* genes in plants has been shown to increase the levels of both nicotianamine and transition metals in cells (Kim et al. 2005; Ishimaru et al. 2007; Wirth et al. 2009;

Johnson et al. 2011). More information about the role of NAS genes in iron and other transition metals homeostasis can be found in DIR 128.

OsPSTOL1

34. 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 recent 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).

35. *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.

Gene stacked lines

36. The overexpression of each of the *AtAVP1*, *OsNAS2* or *OsPSTOL1* genes individually, has the potential to improve the yield of wheat. 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, the combination of these genes may have the potential to produce wheat plants with increased grain yield under optimal growing conditions.

5.2.2 Group 2: Frost tolerance

37. The frost tolerance genes used in the proposed release improve plant protection and thus plant survival under strong or prolonged stresses such as cold, drought and salinity. The genes are all transcription factors, however the classification of each gene to a specific family of transcription factors is CCI.

38. A transcription factor (TF) is any protein required for recognition, by RNA polymerases, of specific sequences in genes (Lewin 1994). Transcription factors are involved in regulating expression of downstream genes and signalling pathways. GM plants overexpressing transcription factors have shown increased drought and often cold and salinity tolerance (Yamaguchi-Shinozaki & Shinozaki 2006; Cabello et al. 2007; Lu et al. 2009).

39. In glasshouse experiments it was observed that constitutive overexpression of genes in one of the TF families can slow barley plant growth and reduce grain yield. Some lines containing constitutively overexpressed transcription factors showed delayed flowering (up to 10 days) in the glasshouse.

5.2.3 Marker Genes

40. The vectors used to transform plant tissue contain a selectable marker gene *hygromycin phosphotransferase* (*hptII*). This gene is derived from *Escherichia coli*, a common gut bacterium. The hygromycin phosphotransferase (HPH) protein confers resistance to hygromycin antibiotic. More information on marker genes in general and on this gene in particular, may be found in the document *Marker Genes in GM Plants*.

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

41. 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. Non-GM wheat and barley contain a number of anti-nutritional factors and allergens that, in extreme cases, may have a toxic effect (OGTR 2017a; OGTR 2017b). The proteins encoded by the introduced genes are not expected to have any toxic or allergenic effects.

42. A comprehensive search of the scientific literature yielded no information to suggest that the genes themselves, their protein products, or any associated products (except iron, see below) were toxic or allergenic to people, or toxic to other organisms. This includes homologues isolated from other species. However, no toxicity/allergenicity tests have been performed on any of the proteins.

43. In the current application, the introduction of the *OsNAS2* gene is being examined for its role in yield enhancement. This gene has been studied by other research groups with the aim of increasing levels of iron in plant tissues (biofortification). Iron content in wheat (whole plant) is approximately 30µg/g, with a biofortification target of 52µg/g (Bouis et al. 2011). Iron must be obtained from the diet and is involved in a number of essential processes in the body. However, excessive iron (over 20 mg/kg for any toxic effects) in the diet can result in toxicity (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.

44. No adverse health effects were reported by the staff who handled the GMOs during screening trials in the glasshouse. There have been no adverse effects reported from similar GM lines planted under DIR 077/2007, DIR 102 or DIR 128.

45. There is no evidence that the HPH protein is toxic or allergenic (OGTR Risk Assessment documents and references therein). GM foods containing the HPH protein have been assessed and approved for sale in Australia (FSANZ 2004).

5.4 Characterisation of the GMOs

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

47. Some GM wheat lines constitutively overexpressing *OsNAS2* have increased iron concentration in grains. 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 (unpublished data).

48. 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 (unpublished data).

49. Some GM lines in which transcription factors were constitutively overexpressed showed delayed flowering in glasshouse trials. Other lines grown in the glasshouse did not show any unexpected phenotype.

50. In the glasshouse, constitutive overexpression of some frost tolerance genes has slowed plant growth and had negative effects on yields.

Section 6 The receiving environment

51. The receiving environment forms part of the context in which the risks associated with dealings with 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).

52. Information relevant to the commercial cultivation and distribution of wheat in Australia is discussed in the wheat biology document. Information relevant to the commercial cultivation and distribution of barley in Australia is available in the barley biology document.

6.1 Relevant biotic factors

53. A number of biotic factors are important in the cultivation of both wheat and barley and these are discussed in detail in the biology documents for these plants. There are a number of 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.

6.2 Relevant abiotic factors

54. It is proposed that the GMOs will be grown at five potential locations. Three locations are proposed for dealings with Group 1 (yield enhancement) GM plants. One is Glenthorne Farm in SA, the other two are in WA, in Katanning and Merredin. One location will be chosen each season for planting GM plants expressing the Group 2 (frost tolerance) genes, selected from four locations including the Merredin and Katanning locations used for Group 1 GM plants, as well as one at Loxton (SA) and one at Narrabri (NSW). A different location may be chosen in each season.

55. Glenthorne Farm is a University of Adelaide property located close to urban Adelaide. Information provided for DIR 128 indicates that this site has a climate typical of rain-fed wheat production areas for South Australia .

56. The Merredin and Katanning locations are New Genes for New Environments (NGNE) facilities that are owned and operated by the WA Department of Agriculture and Food (DAFWA). These two facilities were set up for conducting GM field trials under differing environmental conditions, representing abiotic stresses which occur in WA agricultural environments. The Merredin site has lower rainfall and higher temperatures, while the Katanning site has frost and higher rainfall with winter waterlogging.

57. As mentioned previously both wheat and barley are affected by a number of abiotic stresses and information can be found in the biology documents. Nutrient stress, particularly nitrogen, potassium and phosphorus, affects both species. Both are affected by drought, although barley is generally regarded as more tolerant to drought than wheat with better water use efficiency than wheat. However, barley is susceptible to waterlogging. Heat stress impacts on wheat and barley production, with barley 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. Barley is also sensitive to acidic soils and to aluminium and boron toxicity.

6.3 Relevant agricultural practices

58. The limits and controls of the proposed release are outlined in Section 3.1 and Section 3.2 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.

59. Seeds would be harvested either by hand or with a plot harvester dedicated for use on GM plants. Threshing will occur within the planting area or heads transported to approved facilities for threshing, analysis or other processing.

60. Waste material derived from 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.

6.4 Presence of related plants in the receiving environment

61. Glenthorne Farm is surrounded by urban areas of Adelaide and is not in a cereal-producing region. The other four proposed locations are within cereal-producing regions.

62. Glenthorne Farm and the NGNE facilities in Merredin and Katanning have been used for University of Adelaide GM field trials, most recently for DIR 102 and DIR 128, with sites either signed off or in postharvest monitoring. However, planting of GM wheat and barley can occur at these locations until (and including) December 2019 under the DIR 128 licence, so planting could occur under DIR 128 concurrently with that proposed under DIR 152.

63. Some wheat and barley production occurs in both Narrabri and Loxton, however no GM wheat or barley trials have been conducted in these areas recently. Two limited and controlled GM wheat trials were approved for planting at properties in Narrabri, but both licences have been surrendered so no further planting could occur.

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

6.4.1 Wheat

65. Gene flow can occur between cultivated varieties of wheat, although pollen flow is limited, generally occurring at low frequency and/or over short distances (Gatford et al. 2006). Wheat is considered a low-risk crop for both intraspecific and interspecific gene flow (Eastham & Sweet 2002).

66. Wheat is sexually compatible with a number of species within the tribe Triticeae that occur in Australia, including other cereal crops. It hybridises naturally with *T. turgidum* (durum wheat), which is cultivated in areas that overlap with bread wheat production (OGTR 2017b). Hybridisation with rye (*Secale cereale*) is rare despite the use of this cross to generate Triticale (X *Triticosecale*) (Ammar et al. 2004) and generally requires intervention to produce fertile hybrids. Crossing between Triticale and wheat has been performed under laboratory conditions but rates of natural outcrossing are unknown (Kavanagh et al. 2010). In wheat x Triticale crosses using hand pollination and embryo rescue, hybrids were almost completely self-sterile, with severe hybrid necrosis also observed (Bizimungu et al. 1997).

67. There are four Australasian Triticae genera, of which *Australopyrum* and *Anthosachne* (*Elymus*) have Australian species, while *Stenostachys* and *Connorochloa* occur only in New Zealand and/or New Guinea (Barkworth & Jacobs 2011). A number of introduced Triticeae species are also present in Australia including *Elytrigia repens* (couch grass) and at least four *Thinopyrum* species (Bell et al. 2010), some of which are classified as weeds in particular regions (Barrett-Lennard 2003; NYNRMB 2011). A review of pollen-mediated gene flow from GM wheat to wild relatives in Europe concluded that there was a minimal possibility of gene flow from wheat to *Elytrigia* spp. (Eastham & Sweet 2002). There has been no concerted investigation of natural hybridisation of these native and introduced Triticeae species with wheat. Factors such as genome incompatibilities, the necessity for the parent plants to be in close proximity, concurrent flowering, and the ability of the hybrid progeny to set viable seed, combine to make it extremely unlikely that any of these Triticeae would ever naturally cross with wheat.

68. There has been one report of natural hybridisation between wheat and *Hordeum marinum* in a European study, however, it is likely to be a rare event (Guadagnuolo et al. 2001). *H. marinum* is found in wheat growing areas of Australia, however, there are no reports of natural hybridisation between the two under Australian conditions. Wheat also readily hybridises with *Aegilops* species (goatgrasses), but no *Aegilops* species are considered to be naturalised in Australia. Any specimens of *Aegilops* that have been collected in Australia presumably originate from seed accidently introduced amongst wheat seed, or straying from that brought in for breeding programs (Weeds in Australia).

6.4.2 Barley

69. Barley has a primary gene pool consisting of *H. vulgare* and *H. vulgare subsp. 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 subsp. spontaneum,* with which it may outcross, is not known to be present in Australia.

70. Although there have been a number of 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 summarised in the barley biology document.

6.5 Presence of similar genes and encoded proteins in the environment

71. The genes in this application are all derived from organisms that are widespread 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.

72. The *hptII* gene is 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 gene and its encoded protein through contact with plants or food.

73. All promoters used to drive expression of the introduced genes are derived from plant species (durum wheat, maize and rice), with the exception of the *CaMV35S* promoter from a plant virus. Humans and animals have been safely consuming these plants for centuries. Other regulatory sequences are from common organisms including maize (*Z. mays*) and *A. tumefaciens.*

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

74. Wheat and barley lines containing the genes proposed for release under the current application (except *OsPSTOL1* and Gene 4), have been approved by the Regulator for limited and controlled release under licences including DIR 102 or DIR 128. There have been no reports of adverse effects on human health and safety or the environment resulting from those releases.

75. Information on previous DIR licences for GM wheat and barley is available from the OGTR GMO Record. The Regulator has previously approved 18 field trial releases of GM wheat, of which nine are licences for wheat and barley. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.

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

7.2 International approvals

77. Field trials of 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 GM Register; accessed 14 February 2017).

78. None of the lines in the current application have been approved for release in any other country.

Chapter 2 Risk assessment

Section 1 Introduction

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

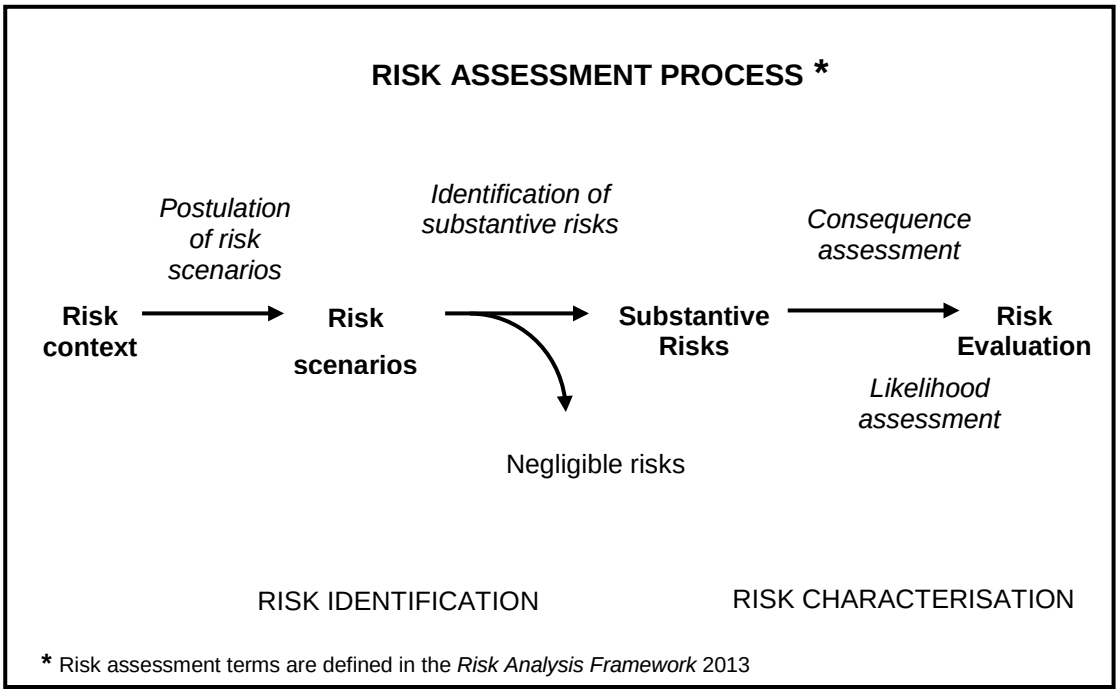

Figure 3 The risk assessment process

80. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

81. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

82. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al. 2014). In addition, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

83. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

Section 2 Risk Identification

84. Postulated risk scenarios are comprised of three components:

i. The source of potential harm (risk source).

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

iii. Potential harm to an object of value (people or the environment).

85. In addition, the following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMOs
- the characteristics of the parent organism(s).

2.1 Risk source

86. The source 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.

2.1.1 The introduced genes

87. As discussed in Chapter 1 (Table 1 and Table 2), the GM wheat lines have been modified by the introduction of one to three genes for yield enhancement or one of seven genes for frost tolerance. Each of the GM barley lines has been modified by the introduction of one of two genes for frost tolerance (a subset of the genes introduced into the GM wheat lines). The introduced genes will be considered further as potential sources of risk.

2.1.2 The introduced marker gene

88. The GM wheat and barley lines contain the *hptII* gene, which confers antibiotic resistance and was used as selectable marker gene. The *hptII* gene and its products have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas. Further information about *hptII* is available in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website.

89. As the marker gene has not been found to pose a substantive risk to either people or the environment, its potential effects will **not** be further considered for this application.

2.1.3 The introduced regulatory sequences

90. The introduced genes are controlled by introduced regulatory sequences. These are derived from a number of common sources including plants, a bacterium and a plant virus (CaMV) (see Chapter 1, Table 2). Information regarding some of the regulatory elements has been declared CCI.

91. Regulatory sequences are naturally present in plants and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, risks from these regulatory sequences will not be further assessed for this application.

2.1.4 Unintended effects

92. The genetic modifications have the potential to cause unintended effects in several ways. These include altered expression of endogenous genes by random insertion of introduced DNA in the genome,

increased metabolic burden due to expression of the proteins encoded by the introduced genes, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, the range of unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. Unintended effects also occur spontaneously and in plants generated by conventional breeding (Bradford et al. 2005; Ladics et al. 2015; Schnell et al. 2015). In general, the crossing of plants, each of which will possess a range of innate traits, does not lead to the generation of progeny that have health or environmental effects significantly different from the parents (Weber et al. 2012; Steiner et al. 2013). Therefore, unintended effects resulting from the process of genetic modification will **not** be considered further in this application.

2.2 Causal pathway

93. 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 effects of the introduced gene(s) and gene product(s) on the properties of the organism
- 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 GM plants (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer
- unauthorised activities.

94. Although all of these factors are taken into account, some may have been considered in previous RARMPs or are not expected to give rise to substantive risks.

2.2.1 Horizontal gene transfer

95. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese 2008) and has been assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108. Due to the rarity of these events and because the gene sequences (or sequences that are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms, horizontal gene transfer will **not** be assessed further.

2.2.2 Unauthorised activities

96. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome and no substantive risk was identified. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore unauthorised activities will **not** be considered further.

2.3 Potential harm

97. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity

- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment of desirable plants, including having an advantage in comparison to related plants
- reduced yield of desirable vegetation
- 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).

98. These harms are based on those used to assess risk from weeds (Standards Australia Ltd et al. 2006). Judgements of what is considered harm depend on the management objectives of the land into which the GM plant is expected to spread and persist. 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

99. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 3 and examined in detail in Sections 2.4.1 – 2.4.4. Postulation of risk scenarios considers impacts of the GM wheat and GM barley or their products on people undertaking the dealings, as well as impacts on people, other desirable organisms and the environment if the GM plants or genetic material were to spread and/or persist.

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

Table 3: 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 for yield enhancement and frost tolerance	Growing GM wheat and barley at the field trial sites ⬇ Expression of the introduced genes in GM plants ⬇ Exposure of humans and other desirable organisms by ingestion of, or contact with, the plant material	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms	No	• The source organisms for the introduced genes are routinely used for food or feed or are commonly found in the environment. • Encoded proteins and similar proteins occur naturally in the environment and are not known to be toxic or allergenic to people or other desirable organisms • GM plant material would not be used in food or feed • The limited scale, short duration and other proposed limits minimise exposure of people and other desirable organisms to the GM plant material

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes for yield enhancement and frost tolerance	Cultivation of GM wheat and barley ⬇ Expression of the introduced genes in GM plants ⬇ Hybridisation with other GM wheat or barley ⬇ Expression of the introduced genes from both parental GM lines ⬇ Exposure of humans and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms	No	• The source organisms for the introduced genes are routinely used for food and feed or are commonly found in the environment. • Encoded proteins and similar proteins occur naturally in the environment and are not known to be toxic or allergenic to people or other desirable organisms • No reason to expected that novel proteins would be expressed in hybrids nor that the expressed proteins would behave differently in a hybrid background. • The limited time, small scale and other proposed limits minimise exposure of people and other desirable organisms to the GM plant material.
3	Introduced genes for yield enhancement and frost tolerance	Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Increased exposure of humans and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material OR Establishment of GM wheat or barley plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans 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 seed distribution outside the trial site • There is no expectation the introduced gene constructs confer other characteristics to enhance the spread and persistence of the GM wheat lines. • Wheat and barley grains have limited dispersal by animals. • Wheat and barley have limited ability to survive outside agricultural settings. • The GM wheat and barley lines used in this trial are susceptible to standard weed control measures. • Risk Scenarios 1 and 2 did not identify any increased risk of toxicity or allergenicity in the GM plants
4	Introduced genes for yield enhancement and frost tolerance	Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or GM barley plants ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇ Increased exposure of humans and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material OR Establishment of GM wheat or barley plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans 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 yield enhancement and frost tolerance genes
Causal Pathway	⬇ Growing GM wheat and barley plants at the field trial sites ⬇ Expression of the introduced genes in GM plants ⬇ Exposure of humans and other desirable organisms at the trial sites by ingestion of, or contact with the GM plant material ⬇
Potential Harm	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms

Risk source

101. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement in GM wheat or frost tolerance in GM wheat or GM barley lines.

Causal pathway

102. The overexpression of some genes in GM plants are driven by constitutive promoters, so those genes are potentially expressed in all tissues at all developmental stages. Therefore, the encoded proteins are potentially produced in all tissues at all developmental stages.

103. Thus people may be exposed to GM plant material and the expressed proteins, either by direct contact with the plant material or through inhalation of pollen. This is most likely to occur at the trial site but may also occur during transport and handling of GM plant material. Other organisms such as rodents, birds or invertebrates may be exposed at the trial site through contact with, or ingestion of GM plant material. A range of animals (including stock and wildlife) and birds may consume cereals (Hill et al. 1988; AGRI-FACTS 2002; Chapter 1, Section 4; OGTR 2017a and references therein; OGTR 2017b), thus they may have direct contact with or ingest the GM plant material.

104. However, there are a number of limits and controls proposed for this trial that will limit the exposure of people or animals to the GM plants and their products, including access to planting areas, duration and size of the trial. In addition, no material from this trial will be used for human food or animal feed. Only trained and authorised people would be permitted to enter the trial sites and to handle the GM plants proposed for this trial. The three sites proposed for planting with the yield enhancement GM wheat lines are on properties which have fences and locked gates to limit access to the properties. The University of Adelaide owns and operates Glenthorne farm and the farm manager must be notified of any intention to enter the site prior to access. The NGNE facilities at Merredin and Katanning are purpose built facilities with secure fencing and locked gates to restrict access. The applicant has proposed similar conditions for other sites should they be used.

105. The trial is proposed for two growing seasons for yield enhancement and for three growing seasons for frost tolerance. The potential for exposure is limited to a short period during these growing seasons. In addition, the areas proposed are small, thus further limiting exposure. The maximum planting area in each of the first two seasons is 3.75 ha across all sites and all GM lines, with a maximum of 2.5 ha at any single site. In the third season, a maximum of 1.5 ha at a single site is proposed.

Potential harm

106. 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).

107. 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).

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

109. Although no toxicity or allergenicity studies have been performed on the GM plant material, the introduced genes were isolated from naturally occurring organisms that are already widespread and prevalent in the environment, including common food sources such as rice and wheat (Chapter 1, Section 5.1). Thus, people and other organisms are exposed to the same or similar proteins through their diet and in the environment. There is no information to suggest that the introduced genes or their products are toxic or allergenic to people or toxic to other desirable organisms.

110. All but two of the genes in this application have been assessed for previous applications (DIR 102 and DIR 128) and no substantive risks for toxicity or allergenicity of the proteins were identified. Nor have there been any reports of adverse reactions from either of those earlier releases. As noted in Chapter 1, section 5.3 and in DIR 128 RARMP, the *OsNAS2* gene is associated with increased iron uptake in plant tissues and high dietary iron can have toxic effects. However, it is unlikely that the iron levels in these plants will be in a range of concern for iron toxicity and plant material from this trial may not be used for food or feed.

111. Of the two genes which have not been assessed in previous RARMPs, the yield enhancement gene *OsPSTOL1* is derived from rice and has been well characterised (Chapter 1, section 5.2.1 and references therein) and is one of a broad class of serine/threonine protein kinases. In plants these kinases are involved in tolerance of phosphorous deficiency and have roles in a range of processes in the plant. Gene 4 (frost tolerance) belongs to a well-characterised class of genes, some of which have been assessed in previous RARMPs as posing negligible risk. In addition, this gene is derived from wheat, so it is likely that humans and animals have been exposed to the gene and its products.

112. Non-GM wheat and barley are not regarded as toxic to humans or other desirable organisms. However, both 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 2017a; OGTR 2017b). There is no reasonable expectation that any of the genes proposed for this trial would influence the pathways producing known allergens in wheat or barley. Also, as mentioned, plant material from this trial may not be used for food or feed.

Conclusion

113. Risk scenario 1 is not identified as a substantive risk due to limited exposure and the lack of toxicity or allergenicity of the introduced genes and their encoded proteins to humans and lack of toxicity to 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 yield enhancement and frost tolerance genes
Causal Pathway	⬇ Growing GM wheat and barley plants at the field trial sites ⬇ Expression of the gene constructs in GM plants ⬇ Pollen flow from GM plants to other GM wheat or barley plants growing at the sites ⬇ Hybridisation of different GM wheat or barley lines producing lines with additional introduced genes ⬇ Exposure of humans and other desirable organisms at the trial sites by ingestion of, or contact with the hybrid GM plant material ⬇
Potential Harm	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms

Risk source

114. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement in GM wheat or frost tolerance in GM wheat or GM barley lines.

Causal pathway

115. Due to the small size of the planting areas proposed for this field trial, it is likely that different lines grown under DIR 152 would be planted in close proximity to one another. In addition, the GM wheat grown at the Glenthorne Farm or the GM wheat and GM barley grown at NGNE Merredin or NGNE Katanning sites may be grown in close proximity to GM wheat or barley lines modified for abiotic stress tolerance planted under licence DIR 128. Given that different GM lines are sexually compatible and that they may have similar flowering times, pollen flow between plants with different introduced genes is likely. Thus, there is potential for the production of hybrid GM wheat plants containing additional – 'stacked' - introduced genes for yield enhancement, frost tolerance and/or other abiotic stress tolerances; or hybrid GM barley plants containing stacked genes for frost tolerance and/or other abiotic stress tolerances. People and other desirable organisms may be exposed to hybrid GM wheat or barley plants containing proteins encoded by the stacked genes.

116. A number of the genes and lines proposed for DIR 152 are the same as those included under DIR 128. The remaining lines contain genes from common sources, including edible plants or plants to which humans and other desirable organisms have long been exposed. The genes introduced to wheat and barley under DIR 128 are involved in abiotic stress tolerance and micronutrient uptake. Two of those genes (*OsNAS2* and *AtAVP1*) are being examined for yield enhancement in the current application. As discussed in the RARMPs for DIR 102 and DIR 128, abiotic stress tolerances are generally multigenic traits, involving genes for which the expressed proteins are involved in different biochemical pathways and tolerance to one abiotic stress may also confer tolerance to other abiotic or indeed biotic stresses.

Potential harm

117. If pollen flow occurs between GM plants grown under DIR 152 or between lines from DIR 128 and DIR 152, it is likely that some hybrid plants may occur. These plants could contain additional genes from the same group (e.g. two frost tolerance genes in wheat), or genes from different groups (e.g. a yield enhancement gene and a frost tolerance gene in wheat, or a frost tolerance gene and an aluminium tolerance gene in barley). If this occurs, lines may contain one or more proteins produced as a result of expression of the introduced genes. These proteins may be toxic or allergenic to humans or toxic to other desirable organisms.

118. However, Risk Scenario 1 (above) and the RARMPs for DIR 102 and DIR 128, 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 there is minimal likelihood of novel allergens or toxins. The genes are sourced from common organisms widely present in the environment suggesting that humans and other desirable organisms have a long history of exposure to them.

119. It is also unlikely that hybrid progeny would persist, due to post-harvest control measures to ensure removal of GM volunteers. Thus, exposure of people or other desirable organisms to hybrids would be minimal.

120. Additionally, for reasons outlined in Risk scenario 1, the proposed limits and controls would minimise exposure of people and other organisms to the GM plant material.

Conclusion

121. Risk scenario 2 is not identified as a substantive risk due to limited exposure and to the lack of toxicity or allergenicity of the introduced genes and their encoded proteins or hybrid plants containing combinations of these proteins to humans or lack of toxicity to other organisms. 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 yield enhancement and frost tolerance genes
Causal Pathway	Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Establishment of GM wheat or barley plants in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment

Risk source

122. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement in GM wheat or frost tolerance in GM wheat or GM barley lines.

Causal pathway

123. If GM wheat or barley seed were dispersed outside the trial site, or persisted at the trial sites after completion of the trial, this seed could germinate and give rise to plants expressing the introduced genes. These plants could spread and persist in the environment and establish populations of GM wheat or GM barley expressing genes for yield enhancement or frost tolerance. This could increase the likelihood of exposure of people or other desirable organisms to the proteins expressed in GM plants.

124. Similarly, pollen from GM wheat could fertilise other GM wheat lines from this trial or from lines licenced under DIR 128, as could GM barley lines from both trials, resulting in hybrid wheat and barley progeny with stacked traits for yield enhancement, frost, drought, aluminium or salt tolerance, or nitrogen use efficiency. It is unlikely that such progeny would survive to produce seed, due to the requirements to remove volunteer plants from the trial site prior to flowering, as discussed in Risk Scenario 2. However, there is a small possibility that hybrid GM wheat or GM barley seed with enhanced yield, multiple abiotic stress tolerances, and/or nitrogen use efficiency could also be dispersed from the trial site.

125. There are a number of routes for dispersal of GM seed from the trial site. The main methods of seed dispersal are through human or animal activity, or spread through extreme weather.

126. There are some features of both wheat and barley which generally limit the likelihood of spread and persistence in the environment, as summarised in Chapter 1, Section 4 and in the biology documents for

wheat and barley. Both wheat and barley have been selected during domestication for reduced shattering of seed heads - a mechanism for seed dispersal in ancestral wheat and barley plants. The presence of GM wheat or barley at trial sites could persist through dormancy of seeds in the seed bank. This could potentially increase the number of volunteers persisting at the site after the trial and provide seeds for spread to other areas. Although a range of factors in the environment can influence dormancy in both wheat and barley, neither species shows a high degree of dormancy or a persistent seed bank under Australian conditions (OGTR 2017a; OGTR 2017b).

127. Dispersal of GMOs outside the limits of the trial sites could occur through the activity of people or animals and through extreme weather events.

Dispersal through human activity

128. Although human activity is generally one of the main mechanisms for seed dispersal from wheat and barley crops (OGTR 2017a; OGTR 2017b) the applicant has proposed limits and controls to prevent the spread of GM wheat or barley seed from the trial site. Access to the site is restricted to authorised, trained staff. The applicant has proposed harvesting by hand or using dedicated small plot harvesters. All equipment used at the trial site will be cleaned in a designated clean-down area before leaving the site or being used for any other purpose. All GM plant material will be transported in accordance with the Regulator's Transport, Storage and Disposal of GMOs guidelines, which would minimise the opportunity for dispersal of GM material or of contact with any GM plant material during transport from the trial site to other facilities for analysis.

Dispersal by animals

129. Animals can potentially spread seed by consumption and excretion of whole seeds, movement of seeds in hair, fur, feathers or on muddy feet, or by removing and hoarding seed. Wheat seeds can be dispersed in sheep wool (Ryves 1988) and barley seeds adhere well to the fur of large animals, feathers and clothing, all which can facilitate seed dispersal (Von Bothmer 1992; Von Bothmer et al. 1995). Dispersal on animal hooves is probable but not well reported.

130. 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), however the germination rates of this seed were not measured. Kangaroos, mice, rats and rabbits are known pests of wheat (Hill et al. 1988; AGRI-FACTS 2002) and could potentially distribute viable seeds, although viable seeds have not been found in rabbit dung (Malo & Suárez 1995). Rodents hoard cereal seeds including wheat and barley, and may distribute seed from crops or volunteers in this manner.

131. Studies examining the dispersal of viable seed after consumption by birds indicate that viable barley seed is not excreted by a range of birds (Cummings et al. 2008), while a small proportion of intact wheat seed can be excreted by corellas and galahs, with varying germination rates (Woodgate et al. 2011). Wheat seed may be dispersed by emus (Calvino-Cancela et al. 2006), however germination rates were very low (Rogers et al. 1993; McGrath & Bass 1999), or in some cases not provided (Davies 1978). Intact wheat and barley seed can be distributed on the muddy feet and legs of some birds (Cummings et al. 2008).

132. Although dispersal by most insects is unlikely, ants may move wheat seeds short distances, but often bury such seeds at depths at which germination is highly unlikely and therefore have a limited role in dispersal of wheat seeds (OGTR 2017b).

133. The proposed trial sites are small and the period during which viable seed is available for animal consumption or for spread of viable seeds via animal fur, feathers or muddy feet is short (during sowing and immediately prior to harvest) thus limiting the opportunity for consumption or spread of viable seed. The applicant also proposes rodent control by means such as trapping and baiting in the planting areas and management of vegetation in the buffer zone and monitoring zones. The applicant proposes fencing the trial sites to minimise access by large animals, however the likelihood of spread via farm animals is minimal. The weed risk assessments for wheat and barley completed as part of the biology documents consider a range of factors with respect to the spread of wheat or barley seeds. The likelihood of dispersal of viable

plant parts by land-based animals is rated as 'unlikely to occasional' for wheat and 'occasional' for barley in those weed risk assessments. The limited size and duration of the current trial further limits the availability of viable seed for spread. There are also a number of factors which limit the survival of wheat or barley plants outside cultivation if seeds were spread from the trial site (OGTR 2017a; OGTR 2017b).

Dispersal in extreme weather

134. Extreme weather events have the potential to spread plant material outside a trial, with the most likely means of spread through wind or water. It is possible that plant material such as leaves, stalks or indeed whole plants may be moved by extreme winds, but it is not clear that this could move plant material outside the trial site. It is unlikely that wheat or barley seed would be spread by wind as both have non-shattering seed heads, seeds are heavy and lack specific structures associated with wind transport. Dispersal by water is possible, but is unlikely as wheat and barley ears and seeds are heavy and not adapted for water dispersal. In addition, trial sites will be at least 50 m from any natural watercourse and in areas that are not prone to flooding.

Potential Harm

135. If GM plants were able to establish outside the trial site they could potentially cause increased toxicity to humans or desirable animals or increased allergenicity for humans through increased exposure. However, as discussed in Chapter 1 (section 5.3) and in Risk Scenarios 1 and 2, there is no reasonable expectation that the GM wheat and barley and their products, alone or in combination through hybridisation, would be any more toxic or allergenic than non-GM wheat or barley.

136. Establishment of GM wheat or barley outside the trial site could potentially reduce the establishment and/or yield of desirable plants by a number of means. This could occur through reduced establishment or yield of desirable agricultural crops; reduced establishment of desirable native vegetation; reduced utility of roadsides, drains, channels and other intensive use areas; or by providing a reservoir for pathogens or pests.

137. Although both wheat and barley have a long history of cultivation in Australia, neither is listed as a weed of national significance (National Weeds List), nor as a significant weed in Australian ecosystems (Groves et al. 2003). Large weedy populations of wheat and barley are not observed in the agricultural or natural environment. There is no reasonable expectation that any of the introduced genes will alter characteristics such as seed shattering, other seed dispersal characteristics or seed dormancy which would alter the GMOs' ability to disperse and establish outside an agricultural setting.

138. The introduced genes involved in yield enhancement have been observed as increasing shoot biomass, root biomass, plant vigour and root growth, number of grains, photosynthetic ability, or increasing tillering (related to increased shoot biomass), improving nitrogen use efficiency, or promoting early heading (Chapter 1, Section 5.4). Thus, there is potential for increased vigour, increased biomass and/or increased seed production in the GM wheat plants and it might be expected that their competitive ability may be increased. However, in order to increase weediness these characteristics would need to be coupled with other mechanisms that increase invasiveness through increased spread and persistence in the environment, through changes in dispersal, establishment and survival. These characteristics would not reasonably be expected to change as a result of the introduced genes.

139. The introduced genes for yield enhancement and for frost tolerance are likely to be pleiotropic (that is, they have effects on a number of 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 competitiveness of the plants in agricultural and natural settings. The field performance of the GM plants is to be assessed in the proposed release, to determine their tolerance to abiotic stress conditions. However, tolerance to abiotic stress(es) or enhanced yield in an agricultural setting will not in isolation increase the invasiveness and persistence of the plants, due to the complexity of environmental conditions.

140. None of the introduced traits are likely to change the susceptibility of the GM wheat and barley lines to conventional weed controls. Thus, the GM wheat and barley plants in this trial can be controlled by standard weed control measures, such as cultivation or the use of herbicides, if required.

141. Risk Scenarios 1 and 2 (above) did not identify toxicity or allergenicity of any of the individual genes as a substantive risk. In addition the limits and controls outlined in Risk Scenario 1 further limit the likelihood of exposure to GM plants. The limits and controls reduce the potential amount of seed available for dispersal outside the trial site, as well as the opportunities for spreading seeds.

Conclusion

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

2.4.4 Risk scenario 4

Risk Source	Introduced yield enhancement and frost tolerance genes
Causal Pathway	Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or barley plants ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment

Risk source

143. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement in GM wheat or frost tolerance in GM wheat or GM barley lines.

Causal pathway

144. Pollen from GM wheat and GM barley lines could be transferred outside the trial sites and fertilise sexually compatible plants, either non-GM wheat or barley, or plants from another sexually compatible species. Hybrid plants carrying the genes of interest could form the basis for spread and dispersal of these genes in other varieties of wheat or barley, or other sexually compatible plant species.

145. People and other desirable organisms could then be exposed to the proteins expressed by the introduced genes through ingestion, contact with plant material or inhalation of pollen from hybrid plants.

146. 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. Baseline information on vertical gene transfer associated with non GM wheat and barley plants can be found in the wheat and barley biology documents. This information is also summarised in Chapter 1, Sections 6.4.1 and 6.4.2.

147. Wheat is mainly self-pollinating and where pollen dispersal does occur, the main method is wind, with the role of insects considered minimal. Wheat pollen is heavy and short-lived, with most pollen falling within the first few metres. Field trials conducted in Australian Capital Territory (ACT) and SA investigating gene flow from GM lines to non-GM crops have shown a cross-pollination frequency of 0.012% to 0.055%, over a distance of less than 12 m (Gatford et al. 2006). Cross-pollination rates are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity. The

introduced genes for yield enhancement or frost tolerance are unlikely to increase the likelihood of wheat outcrossing.

148. Wheat is sexually compatible with a number of species within the tribe Triticeae that occur in Australia, including other cereal crops, however, such crosses are highly unlikely under field conditions. Hybrids with potentially compatible weedy species are rare, indeed hybrids with *H. marinum* have not been reported in Australia and *Aegilops* species (goatgrasses) are not considered to be naturalised in Australia.

149. There has been no concerted investigation of natural hybridisation of the native and introduced Triticeae species with wheat. However, factors such as genome incompatibilities, the necessity for the parent plants to be in close proximity, concurrent flowering, and the ability of the hybrid progeny to set viable seed, combine to make it extremely unlikely that any of these Triticeae would ever naturally cross with wheat.

150. Barley has a primary gene pool containing only one *H. vulgare* subspecies – which is not known to be present in Australia - and there are strict isolation barriers to gene flow between *Hordeum* species. Interspecific crosses within the *Hordeum* genus and intergeneric crosses have been made under experimental conditions and successful hybrids have not been observed under natural conditions.

151. The proposed limits and controls for this trial would also minimise the likelihood of pollen flow from the trial to related species. No wheat or barley crops may be planted within at least 200 m of a planting area while GM wheat or GM barley are being cultivated and any related species must be controlled within at least 60 m of a planting area during flowering. This would greatly reduce the potential for pollen flow from the trial to related species including cultivated wheat and barley. In addition to this, the applicant proposes postharvest monitoring of the site for any volunteer GM wheat or barley to prevent production of plants that could hybridise with related species through pollen flow.

Potential Harm

152. If pollen from GM wheat and barley lines was dispersed, any resulting hybrid plants could spread and persist in the environment, leading to increased exposure and potentially toxicity to more people and/or other desirable organisms, or allergenicity to more people. Hybrids expressing the introduced genes could also reduce the establishment and yield of desired plants and subsequently reduce biodiversity.

153. The traits that have been introduced into the GM plants of this application could combine, via vertical gene transfer, with traits of other non-GM commercially cultivated wheat or barley plants, or with sexually compatible species. Durum wheat is the only related species present in Australia with which wheat can readily hybridise, while barley has no related species present. However, there is no reason to believe that the resulting plants would possess a level of toxicity or allergenicity greater than that of either parent. Nor is it likely that such hybrids would possess a level of weediness greater than that of either parent.

154. As discussed in Risk scenario 1 and Risk scenario 2, the introduced gene products are not expected to be toxic to humans or other organisms. Properties of these genes are not expected to differ in a hybrid background. Therefore, in the rare 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 have the same properties as the GM parent.

155. As discussed in Risk scenario 3, the introduced genes are unlikely to make the GM wheat or barley plant more weedy. As mentioned, the properties of the introduced genes are not expected to change in a hybrid background resulting from cross-pollination.

156. As discussed in the previous scenarios, the limits on access, total area and timeframe for this trial further restrict the likelihood of increased exposure of humans or other desirable organisms to the GM plants and their products or spread of the genes through seed dispersal or pollen flow.

Conclusion

157. Risk scenario 4 is not identified as a substantive risk due to the limited occurrence of long distance pollen flow for wheat and barley and to the strict reproductive barriers for barley. In addition, Risk

scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs or their hybrids as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

158. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis[2].

159. There are several types of uncertainty in risk analysis (Clark & Brinkley 2001; Hayes 2004; Bammer & Smithson 2008). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

160. 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.

161. 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.

162. For DIR 152, uncertainty is noted particularly in relation to:

- phenotype of GM wheat and barley plants, with respect to the desired traits of enhanced yield and frost tolerance
- potential increases in toxicity or allergenicity as a result of the genetic modification
- potential for increased spread and persistence of the GMOs, including land uses outside agriculture

163. 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.

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

Section 4 Risk evaluation

165. 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.

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

[2] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

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

167. 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 3 and include:

- the limits and controls of the trial are such that exposure of humans and other desirable organisms to the GM plants is minimal
- the risk of seed dispersal or pollen flow is minimised
- none of the GM plant material is to be used for human food or animal feed
- the introduced genes and their expressed proteins are unlikely to be toxic or allergenic
- the expressed genes would not reasonably be expected to behave differently in stacked lines or in a hybrid background, thus there would be no increased risk if hybrids were to occur
- the introduced genes are not involved in regulation of characteristics that facilitate the spread of seeds, nor in any changes to seed dormancy
- the expressed genes are unlikely to alter the establishment and persistence of GM plants outside cultivation, nor to change their susceptibility to conventional weed control measures

168. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM wheat plants into the environment are considered to be negligible. The Risk Analysis Framework, 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 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.[3]

[3] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities, and the public.

Chapter 3 Risk management plan

Section 1 Background

169. 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.

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

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

172. 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. 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

173. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM wheat and barley. These risk scenarios were considered in the context of the scale of the proposed release, the proposed containment measures, and the receiving environment, 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

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

3.1 Licence conditions to limit and control the release

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

175. Sections 3.1 and 3.2 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 controls is considered further in the following sections.

176. The applicant proposes up to five sites for the release of GM wheat lines and of GM barley lines. These are in SA (Glenthorne Farm and Loxton), WA (Merredin and Katanning) and in NSW (Narrabri). For

each site, more than one planting area may be used. The field trial would run for three and a half years, which includes three planting seasons. The maximum total area planted would be 3.75 ha per season for 2018/19 and 2019/20, with a maximum of 2.5 ha on any single site, and up to 1.5 ha planted on a single site in 2020/21. GM wheat expressing genes for yield enhancement would be planted at three sites in the 2018/19 and 2019/20 seasons. GM wheat and barley expressing genes for frost tolerance would be planted at a one site per season for 2018/19, 2019/20 and 2020/21.

177. These conditions will limit the potential exposure of humans and other desirable organisms to GM wheat and GM barley (Risk scenarios 1-2) and will limit the opportunity for dispersal of seed and establishment of GM lines outside the trial site (Risk Scenarios 3 – 4).

178. GM wheat and GM barley have previously been planted at Glenthorne Farm, Katanning and Merredin currently under the licence for DIR 128. The licence for DIR 128 permits planting until December 2019 (inclusive), so potentially GMOs from both DIR 128 and DIR 152 (if approved) could be grown concurrently at the same sites. Pollen transfer between GMOs grown under the licence for DIR 128, or between those grown under DIR 152 has been considered, as has the risk of gene flow between lines from the DIR 128 and DIR 152 with one another (Risk Scenarios 1 and 2). There is a requirement in the licence for DIR 128 that buffer zones and monitoring zones must be inspected for the presence of wheat or barley volunteers during cultivation of the GMOs and that any such plants must be destroyed or prevented from flowering. Thus even if hybrids were to occur between plants from the two licences, they would be destroyed prior to setting any seed, thus reducing the likelihood of exposure of humans or other desirable organisms to hybrid plants (Risk Scenario 2) or the spread of any hybrid plants outside the trial site (Risk Scenario 3).

179. The applicant has indicated that all properties will have lockable gates on perimeter fences. The applicant also proposed that only authorised personnel would be permitted to deal with the GMOs. A standard licence condition requires all people dealing with the GMOs to be informed of relevant licence conditions. 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 to people than the non-GM parental wheat or barley lines (Risk Scenarios 1 and 2).

180. The applicant has proposed to fence the trial sites. Whilst animals will consume wheat or barley plant material, there is minimal risk of seed spread via livestock and there is no evidence that the GM wheat and barley would be more toxic to livestock than non-GM wheat or barley. A standard licence condition has been included in the licence which prohibits the use of plant material in this trial for food or feed, thus livestock cannot 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 is not a requirement under the licence.

181. 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), but 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 impose measures to control access of birds to the planting areas (Risk Scenario 3).

182. 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.

183. 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 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).

184. 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 clear of vegetation. 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 serves a number of purposes:

- reduces rodent activity (see discussion above and Risk Scenario 3), and
- facilitates detection of GM plant material that has been dispersed during sowing or harvesting (Risk Scenario 3). This is discussed later in Chapter 3.

185. 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 is not required as a condition of the licence. Rodent control measures such as traps and/or baits in the planting area are a requirement under the conditions of the licence.

186. The applicant has stated that in some GM lines, particularly those where transcription factors were constitutively overexpressed, flowering was delayed by up to ten days in glasshouse trials. Additionally some of the introduced genes for yield enhancement may influence tillering in the GM wheat lines, which could potentially alter or spread the flowering period for different 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). The licence contains a condition that requires inspection of the monitoring and inspection zones for volunteers and related species during the period from two weeks before the expected start of flowering of the GMOs until four weeks after flowering has finished in all GMOs. Any volunteers or related species must be destroyed or prevented from flowering, thus minimising the risk of gene flow from the GMOs (Risk Scenario 4).

187. The applicant has also proposed an isolation zone of 190 m surrounding the monitoring zone in which no sexually compatible species may be grown. This area must be inspected during flowering of the GMOs for the presence of volunteers and related species. They have requested that for trial sites where there has been no cultivation of wheat or barley or no detection of volunteers in the isolation zone in the previous two years, that the inspection area within the isolation zone be reduced to the 50 m closest to the monitoring zone.

188. The potential for outcrossing in wheat and barley has been discussed in detail in the biology documents for wheat and barley and in a number of RARMPs. The most recent detailed discussion is in DIR 112 and DIR 102, with summaries in DIR 128 and DIR 151 (wheat) and in Chapter 1, Section 4. There are a number of environmental factors which influence the rates of gene flow for wheat and barley. Both species are largely self-pollinated (94 - 99 %), but may otherwise be wind-pollinated. Based on the evidence presented, including scientific literature on gene flow, international containment measures for GM wheat and barley trials and for producing basic and certified seed, an isolation distance of 200 m is considered adequate to minimise gene flow from the GM wheat and barley plants to another wheat or barley crop outside the planting areas. Therefore, the combination of a 10 m monitoring zone, the 50 m inspection

zone and a 140 m isolation zone would manage any risk of gene flow to wheat and barley crops (Risk Scenario 4).

189. Apart from the potential for gene flow between the planting area and commercial wheat or barley crops, pollen mediated gene flow may occur between GMOs at the planting area and flowering volunteers or related species in the isolation zone itself. A standard licence condition in recent wheat and barley licences requires inspection of the 190 m isolation zone for flowering volunteers or related species. However, as noted above, the applicant has requested that, for trial sites where there has been no cultivation of wheat or barley or no detection of volunteers in the isolation zone in the previous two years, the inspection area within the isolation zone be reduced to 50 m.

190. In considering the distance across which pollen mediated gene flow may occur, it should be noted that many seed certification schemes require only short distances between crops for seed production. The Canadian Seed Growers Association requires 3 m between wheat or barley crops grown to produce certified seed and other varieties of wheat or barley or related species, as well as restrictions regarding the recent cropping history of the land used (Canadian Seed Growers' Association 2005). The California Crop Improvement Association requires a definite boundary between crops grown for certified seed and other cereal crops or a barren strip of 10 feet, as well as restrictions on the recent cropping history of the land used to grow crops for certified seed (California Crop Improvement Association 2003). Basic and certified barley seed through Seed Services Australia in South Australia must be separated from other cereal crops by at least a 2 m strip or a physical barrier (Smith & Baxter 2002). The OECD seed scheme requires a distance of 25 m from the female parent to any variety of the same species (except the male parent variety) for production of certified hybrid cereal seed, although in some cases a distance of 100 m is required (OECD 2016).

191. Based on this information and on the evidence of gene flow from small scale trials, it is considered that a distance of 60 m (the minimum distance (including the monitoring zone) between a planting area and the outer edge of a 50 m inspection zone) is sufficient to prevent gene flow from GMOs in the planting area to any volunteers within the isolation zone. For wheat, observations indicate that the majority of pollen falls within 3 m of the parent plant (Hegde & Waines 2004). For barley although some pollen has been detected 60 m from parent plants under experimental conditions (Wagner & Allard 1991), even at shorter distances rates of outcrossing were very low (Allard unpublished, cited in Wagner & Allard 1991; Ritala et al. 2002). The outcrossing rates in field trials have been shown to be very low – with a cross-pollination frequency of 0.012% to 0.055%, over a distance of less than 12 m (Gatford et al. 2006). The possibility of gene flow from a small scale trial crop to isolated volunteers is likely to be less than that assessed from a small scale trial plot to another crop. The monitoring zone and inspection zone must be inspected for any volunteers or related species during flowering of the GMOs. Any volunteers or related species found must be destroyed or prevented from flowering. The condition in the licence requiring an inspection zone of 50 m surrounding the monitoring zone of at least 10 m is considered sufficient to manage the risk of gene flow from the GMOs to any volunteer wheat or barley or to sexually related plants (Risk Scenario 4).

192. Additionally, information from recent GM wheat or GM wheat and barley licences indicates that very few volunteers have been detected in the isolation zones of the trial sites during the inspections conducted to satisfy licence conditions.

193. In light of the discussion above, a combination of a 10m monitoring zone and 50 m inspection zone should be adequate to manage gene flow to volunteers or sexually related species outside the planting area. Thus a licence condition is imposed requiring a 50 m inspection to be maintained, surrounding the outer edge of the monitoring zone, which may not be planted with wheat, barley or related species and must be inspected for volunteers and related species during flowering of the GMOs in the planting areas. The licence imposes a condition that the inspection zone must be surrounded by an isolation zone of 140 m in which no wheat, barley or related species may be planted, but does not require inspection. This also maintains the requirement for an isolation distance of 200 m between other wheat or barley crops and any planting area.

194. The applicant has proposed the use of multiple planting areas at the trial sites. Under the conditions imposed in the licence, where more than one planting area is established at a field trial site, the monitoring zone must extend at least 10 m from the outer edge of the outermost (in each direction) planting areas within the trial site (See Figure 1, Chapter 4). Where multiple planting areas are established, any land between planting areas is included in the monitoring zone and must be maintained as such.

195. At Glenthorne Farm, Merredin and Katanning, where GMOs from DIR 128 and 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 152 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 128, a licence condition for DIR 152 has been imposed 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 1 and 2). On sites where no other GM trials have been planted, the seed can be used for future variety development, subject to appropriate approvals from the Regulator.

196. 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 licence requiring immediate notification of any extreme weather event affecting the properties during the release to allow assessment and management of any risks.

197. The applicant has proposed a number of measures to minimise the persistence of GM wheat and barley plants and seeds in the seedbank at the field trials after harvest of the GM plants. These measures include tillage to the depth of seeding within the planting areas, three irrigations during the two years following harvest to encourage germination of any remaining seed and inspection of the planting areas and monitoring zone at least once every 35 days for two years after harvest.

198. 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). 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).

199. The Regulator considers that under Australian conditions, a post-harvest monitoring period of at least two years, with monthly inspections, and with no volunteers detected for a minimum of 6 months prior to the end of the time period, would effectively manage survival and persistence of viable wheat and barley seeds in the soil. Therefore, these measures are included in the licence. The licence contains conditions requiring that after harvest, the trial 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 will minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4).

200. The applicant proposes that rainfall events of greater than 10 mm in a 24 h period would be deemed to be equivalent to an irrigation event. A licence condition states that a period of natural rainfall may be taken as irrigation only with the agreement of the Regulator. Evidence (such as rainfall measurements, photos etc.) that the rainfall has been sufficient to promote germination needs 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 will 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).

201. 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 imposed under the conditions of the licence, however licence conditions do require any other areas where GM material has

been dispersed, including during planting, harvest or threshing, must be inspected and volunteers and related species must be destroyed or prevented from flowering. The licence also requires harvest of GM wheat and barley to be conducted separately from other crops. These conditions are imposed to manage the potential risks for spread and persistence of the GMOs due to mechanical dispersal of grain during sowing and harvesting (Risk Scenario 3).

202. The applicant proposes to conduct harvest by hand or using 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 on site. The NGNE Katanning and Merredin properties both have dedicated field equipment for use at either of the sites. These properties each have a dedicated washdown facility for cleaning equipment after use. At the other properties, a clean down area will be marked out near the exit point for the trial site and used for cleaning prior to exit or removal from the area. These measures would minimise human-mediated dispersal of GM plant material (Risk Scenario 3).

203. 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. 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. Any seed heads or grain for analysis would be bagged in the planting area 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 will 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).

204. The GM wheat and barley lines have not been assessed by FSANZ, however the applicant does not propose to use GM plant material from the field trial for animal feed or human food. Licence conditions have been imposed such that GM plant material may not be used as food for humans or feed for animals (Risk Scenario 1 and Risk Scenario 2).

205. 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, milling, incineration or burial. Autoclaving, crushing and milling are considered effective for destruction, as they render seed non-viable, therefore minimising the risk 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 licence. Conditions have been included in the licence requiring the cleaning of planting areas and other areas in which GMOs have been detected, inspection for volunteers and destruction of waste materials. These conditions are imposed to manage the risk of spread of GMOs from the trial site (Risk Scenario 3).

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

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

- limit the duration of the release to the period from July 2017 to the end of January 2021
- limit the release to a maximum of five locations, two in SA (Glenthorne Farm and Loxton), two in Western Australia (NGNE Katanning and NGNE Merredin) and one in New South Wales (Narrabri)
- limit the release to a maximum total area of 3.75 ha per season in 2018/19 and 2019/20 and 1.5 ha in the 2020/21. Maximum total area on a single site is 2.5 ha per season
- 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 month 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

207. 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

208. 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.

209. 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.

210. 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

211. 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 outside permitted areas.

212. The University of Adelaide would also be required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. This methodology would be required before planting the GMOs.

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

213. 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

214. 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.

215. 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

216. 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.

217. 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.

218. 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

219. Additional information has been identified that may be required to assess an application for a commercial release of these GM wheat 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

220. The RARMP concludes that the proposed limited and controlled release of GM wheat and GM 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.

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

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Appendix A

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

Abbreviations: CCI: Commercial confidential information; **DIR**: Dealing involving Intentional Release; **GM**: genetically modified, **RARMP**: Risk Assessment and Risk Management Plan

Sub. No.	Summary of issues raised	Comment
1	Agrees with the overall conclusions of the consultation RARMP, while noting the following: There should be further separation of the discussion of the two species (wheat and barley) with regard to • factors affecting the dispersal of barley seed by birds • the different abilities for GM wheat and barley to outcross to weedy relatives	 The spread of wheat and barley seeds by animals is discussed in Chapter 2, Risk Scenario 3 and Chapter 3. The text has been amended to provide greater clarity. The RARMP discusses the outcrossing potential of wheat and barley separately and in some detail in Chapter 2, Risk Scenario 4, with additional discussion in Chapter 3.
2	Negligible risk to environment and human health. The proposed control measures seem adequate. The application is outside area of core expertise. Cannot see any faults in the risk assessment and support the trials proceeding.	Noted
3	Agree with the overall conclusion of the RARMP	Noted
4	Notes that the licence prohibits the use of GM plant material in human food or animal feed. No further comments on the application.	Noted
5	Overall support for the conclusion that DIR 152 poses negligible risk of harm to human health and safety and the environment. Notes that having more information on the genes (declared Commercial Confidential Information - CCI) would help the decision making process.	Noted Information declared CCI under the Act may be supplied to prescribed agencies upon request, however in this case it was not requested.