Australian Government
Department of Health
Office of the Gene Technology Regulator

April 2018

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 162

Limited and controlled release of bread wheat and durum wheat genetically modified for enhanced rust disease resistance

Applicant: CSIRO

This RARMP is open for consultation until 12 June 2018.

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

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

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

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

PAGE INTENTIONALLY LEFT BLANK

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

for

Licence Application No. DIR 162

Introduction

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

The application

Application number	DIR 162
Applicant	CSIRO
Project Title	Limited and controlled release of bread wheat and durum wheat genetically modified for enhanced rust disease resistance[1]
Parent Organism	Bread wheat (*Triticum aestivum*) Durum wheat (*Triticum turgidum* subsp. *durum*)
Introduced genes and modified traits	• Eight genes involved in stem rust disease resistance • Three genes involved in multi-pathogen (stem rust, leaf rust, stripe rust and powdery mildew) resistance GM lines will contain between one and eight disease resistance genes • Three selectable marker genes will be used across all lines
Proposed location	Ginninderra Experiment Station (ACT) and Boorowa Agricultural Research Station[2], Shire of Boorowa (NSW)
Proposed release size	Up to 40 m^2 per year
Proposed release dates	September 2018 - September 2023
Primary purpose	To evaluate agronomic performance of the GM bread wheat and durum wheat lines under field conditions

[1] The original title for the application was: Limited and controlled release of *Triticum aestivum* and *Triticum turgidum* subsp. *durum* genetically modified for enhanced rust disease resistance

[2] This site was previously named Boorowa Experiment Station.

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.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, exposure of people and other desirable organisms to hybrids between different GMOs, potential for persistence or dispersal of the GMOs, transfer of the introduced genetic material to non-GM bread wheat and durum wheat plants and transfer of the introduced genetic material to plants of related species. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed and that the proposed limits and controls will effectively contain the GMOs and their genetic material and minimise exposure.

Risk management

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

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

Table of contents

Abbreviations

ACT	Australian Capital Territory
Act	*Gene Technology Act 2000*
APR genes	Adult plant resistance genes
APVMA	Australian Pesticides and Veterinary Medicines Authority
Avr	Avirulence
bar	Bialaphos resistance (phosphinothricin N-acetyltransferase) gene
CaMV	Cauliflower mosaic virus
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
Ha	Hectare
hptII	Hygromycin phosphotransferase II gene
HR	Hypersensitive response
LGA	Local Government Area
m	metres
NB-LRR	nucleotide binding site-leucine rich repeat
NLRD	Notifiable Low Risk Dealing
nptII	Neomycin phosphotransferase II
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin N-acetyltransferase
PC2	Physical Containment level 2
Qld	Queensland
R gene	Gene conferring resistance to a specific pathogen
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SA	South Australia
Tas.	Tasmania
USDA	United States Department of Agriculture
Vic.	Victoria

Chapter 1 Risk assessment context

Section 1 Background

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

2. The Act 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).

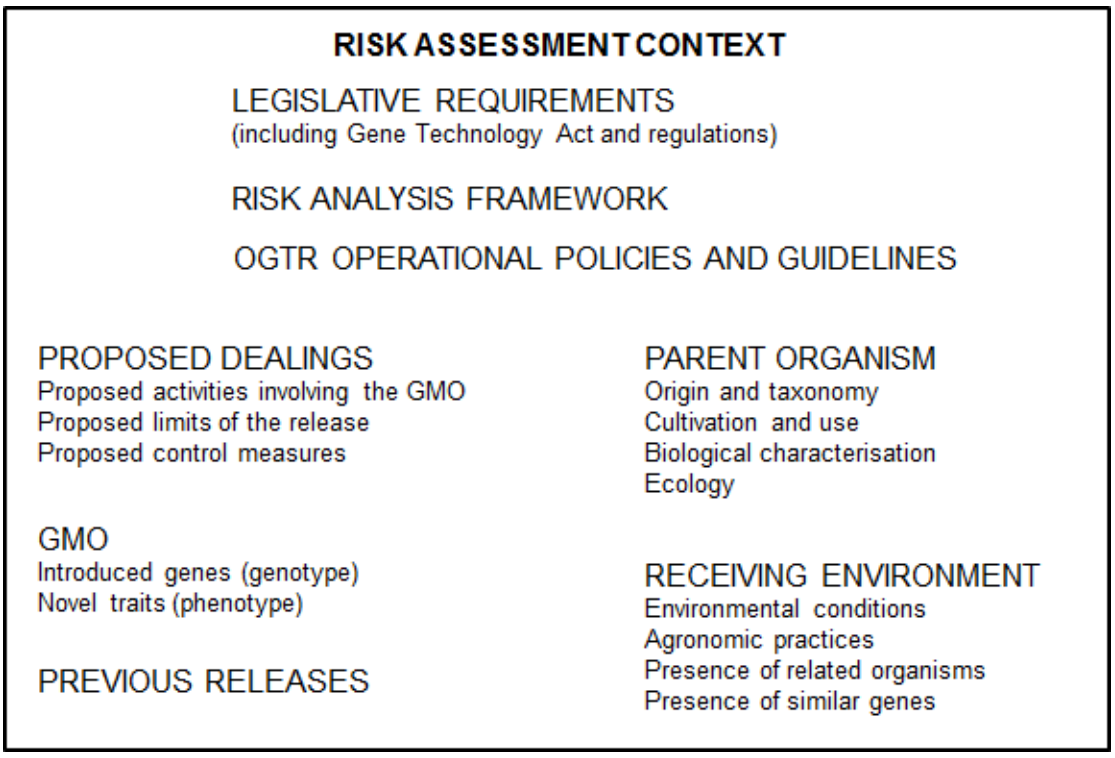

Figure 1 Summary of parameters used to establish the risk assessment context

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 CSIRO proposes to release up to 600 lines of bread wheat and up to 60 lines of durum wheat genetically modified for enhanced rust disease resistance. The purpose of the release is to evaluate the agronomic performance of GM bread wheat and durum wheat lines under field conditions.

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

- conducting experiments with 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.

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

11. The release is proposed to take place for up to five growing seasons, from the issue of the licence until September 2023. GM bread wheat and GM durum wheat[3] would be grown on two trial sites with an area of up to 40 m^2 per season across both sites. The trial sites would be located in Gininderra, ACT and in Boorowa, in the local government area of Hilltops Council in NSW.

12. Only trained and authorised staff would be permitted to deal with the GM bread wheat and durum wheat.

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

13. The applicant has proposed a number of controls to restrict the spread and persistence of the GM bread wheat and durum wheat 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 trial site with a 2 m buffer zone, a 10 m monitoring zone and a 190 m isolation zone in which no sexually compatible plants will be grown
- only permitting trained and authorised staff to access the trial sites

[3] For this document, the term 'wheat' will be used to include both bread wheat and durum wheat where possible. Where reference is made to either species separately they will be identified as bread wheat (*T. aestivum*) or durum wheat (*T. turgidum* subsp. *durum*).

- 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 after use for GM seeds and cleaning as required
- transporting and storing GM plant material in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all plant material from the trial not required for testing or future trials
- post-harvest monitoring of the trial site at least once every 35 days for at least two years and until the site is free of volunteer plants for six months, with any bread wheat or durum wheat volunteers destroyed prior to flowering
- three irrigation events and one tillage to seed depth in the planting area and 2 m buffer zone during the two years of monitoring
- not allowing the GM plant materials or products to be used in commercial human food or animal feed.

Section 4 **The parent organisms**

14. The parent organisms are *Triticum aestivum* L. (bread wheat) and *Triticum turgidum* subsp. *durum* (Desf.) Husn. (durum wheat).

15. Detailed information about bread wheat is contained in the reference document *The Biology of* Triticum aestivum *L. (bread wheat)* (OGTR, 2017), which was produced to inform the risk analysis for licence applications involving GM bread wheat. Baseline information from this document will be used and referred to throughout the RARMP. Information on durum wheat morphology, development and physiology can be found in a review by Bozzini (1988) and a summary of durum wheat information is included in this RARMP document.

16. The great majority of commercially cultivated wheat in Australia is *T. aestivum*. Commercial bread wheat cultivation occurs in the wheat belt from southeastern Queensland (Qld) through New South Wales (NSW), Victoria (Vic.), southern South Australia (SA) and southern Western Australia (WA). The 5-year average production in Australia is 25.8 Mt from 12.4 million ha, with an average yield of 2.1 t/ha. Of this approximately 34% is produced in WA, 30 % in NSW, 18 % in SA, 13% in Vic., 5 % in Qld and less than 1% in Tasmania (Tas.) (ABARES, 2017).

17. *Triticum turgidum* subsp. *durum* (durum wheat) is a closely related species and is the second *Triticum* species cultivated commercially in Australia. It generally accounts for less than 5 % of commercially-cultivated wheat (Kneipp, 2008; ABS, 2013). It is produced as a high-quality product for production of pasta and sold as a premium product in export markets, which account for around 50% of production (Kneipp, 2008). The flour produced from durum wheat has high gluten making it suitable for pasta and semolina products (Feldman, 1976, 2001).

18. Durum wheat is cultivated in smaller areas within the wheat belt, mainly southern Queensland, northern New South Wales, western Victoria and south-eastern South Australia. Australian production is generally approximately 500,000 t annually (Kneipp, 2008; Ranieri, 2015). There are currently 15 cultivars listed on the Wheat Variety Master List for 2017/18, with three new varieties currently being tested (NVT Online New varieties). More detailed information on durum wheat varieties and cultivation practices can be found in industry publications (Hare, 2006; Kneipp, 2008; GRDC, 2016a; WQA, 2017).

19. Worldwide, durum wheat makes up approximately 5 % of the world wheat production (Ranieri, 2015; Taylor and Koo, 2015), with world production of 38 MT in 2013/14 (Ranieri, 2015). In the US, durum wheat prices are higher than those for all classes of bread wheat (Taylor and Koo, 2015). Durum wheat is the most widely cultivated tetraploid wheat (Matsuoka, 2011) and is generally grown under relatively dry conditions (Feldman, 1976, 2001; Matsuoka, 2011). It is a free-threshing wheat with soft glumes and non-hulled seeds (Matsuoka, 2011) with large hard grains (Feldman, 1976, 2001).

20. There are a number of factors, both biotic and abiotic, that limit the growth and survival of bread wheat and durum wheat, with both species grown in similar areas and conditions. These include abiotic

stresses - water stress (drought or waterlogging), heat and cold stress, nutrient deficiencies, salinity - and biotic stresses including pests and diseases. For bread wheat these are detailed in the biology document (OGTR, 2017). Although durum wheat is a separate species, it is closely related to bread wheat and many of the cultivation requirements and interactions with abiotic and biotic factors in the growing environment are similar to those for bread wheat. Whilst details of these may vary slightly, the overall requirements for, and limitations to, durum production are similar to those of bread wheat. More details of these can be found in a number of industry publications (Hare, 2006; Kneipp, 2008; QDAF, 2012a, b; GRDC, 2016a, b, c; SARDI, 2017).

21. Neither bread wheat nor durum wheat is regarded as a weed of national significance (National Weeds List). Bread wheat is regarded as naturalised non-native species present in all Australian states and territories with the exception of the Northern Territory, while durum wheat is not listed in any naturalised or weedy category (Groves et al. 2003). The weed risk assessment included in the biology document concludes that bread wheat possesses few attributes that would make it weedy and this is supported by the observation that there are very few weedy populations of bread wheat or durum wheat in the Australian environment.

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

5.1 Introduction to the GMOs

22. The applicant proposes to release up to 600 lines of GM bread wheat and up to 60 lines of GM durum wheat. The GMOs can be assigned to four groups, based on the genetic modifications (Table 1).

Table 1: The GM bread wheat and durum wheat lines proposed for release

Group	Modified trait	Genes	Modified Species	# Lines
A	Stem rust and multi-pathogen resistance[a], single plasmid	9[b]	Bread wheat	340
B	Stem rust and multi-pathogen resistance[a], two plasmids	8	Bread wheat	100
C	Stem rust resistance, single plasmid	7	Bread wheat	140
D	Multi-pathogen resistance, single plasmid	3	Bread wheat, durum wheat	80

[a] Lines in this group may contain combinations of genes for rust resistance or combinations of genes for rust resistance with a gene for multi-pathogen resistance.
[b] Genes in Group A are also used in other Groups.

23. The GM wheat lines were produced using *Agrobacterium*-mediated transformation. . Information about the *Agrobacterium*-mediated transformation method can be found in the document *Methods of plant genetic modification* available from the OGTR Risk Assessment References page.

24. Lines in Groups A, C and D contain constructs consisting of up to eight genes. In these groups, each construct has been introduced by transformation with a single plasmid. Lines in Group B contain constructs consisting of six to eight genes. These constructs have been introduced by co-transformation with two plasmids. The five constructs used in Group B contain one plasmid used in a line from Group A co-transformed with a second plasmid containing a single gene. Constructs introduced to lines in Group A and B contain multiple genes, while constructs introduced to lines in Group C and D each contain a single gene. There may be up to 20 lines containing each construct.

25. The introduced genes are derived from rye (1), bread wheat (4) or relatives of bread wheat - *Aegilops tauschii* (4), *Triticum monococcum* subsp. *aegilopoides*[4] (1), or *Triticum monococcum* (1). Candidate genes are shown in Table 2.

[4] Also designated *Triticum boeoticum* (and referred to as such in the application document). *Triticum monococcum* subsp. *aegilopoides* is the accepted name listed in the World Checklist of Selected Plant Species and the USDA Germplasm Resources Information Network (USDA GRIN) taxonomy searches (accessed February 2018).

Table 2: Genes and regulatory elements introduced to GM bread wheat and durum wheat lines

Genetic element	Gene Source	Description	Function
		Disease Resistance Genes	
Sr50	*Secale cereale*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr45	*Aegilops tauschii*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr22	*Triticum monococcum* subsp. *aegilopoides*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr35	*Triticum monococcum*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr46	*Aegilops tauschii*[a]	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr26	*Triticum aestivum*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr33	*Aegilops tauschii*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Sr33m	*Aegilops tauschii*	Nucleotide binding leucine rich repeat sequence	Stem rust resistance
Lr67	*Triticum aestivum*	Sugar transporter gene variant	Multi-pathogen resistance
Lr34	*Triticum aestivum*	ABC transporter variant	Multi-pathogen resistance
Lr34B	*Triticum aestivum*	Homologue of *Lr34*	Multi-pathogen resistance
		Promoters	
35S	Cauliflower mosaic virus	Promoter for resistance markers	
pSr50	*Secale cereale*	Native promoter from the *Sr50* gene	
pSr45	*Aegilops tauschii*	Native promoter from the *Sr45* gene	
pSr22	*Triticum monococcum* subsp. *aegilopoides*	Native promoter from the *Sr22* gene	
pSr35	*Triticum monococcum*	Native promoter from the *Sr35* gene	
pSr46	*Aegilops tauschii*[a]	Native promoter from the *Sr46* gene	
pSr26	*Triticum aestivum*	Native promoter from the *Sr26* gene	
pSr33	*Aegilops tauschii*	Native promoter from the *Sr33* gene	
pLr67	*Triticum aestivum*	Native promoter from the *Lr67* gene	
pLr34	*Triticum aestivum*	Native promoter from the *Lr34* gene	
		Introns	
Stls1 I2	*Solanum tuberosum*	Intron inserted in resistance marker sequence	
		Selectable Marker Genes	
hptII	*Streptomyces hygroscopicus*	Plant selectable marker - hygromycin resistance	
bar	*Streptomyces hygroscopicus*	Plant selectable marker - glufosinate herbicide resistance	
nptII	*Escherichia coli K12*	Plasmid selectable marker - kanamycin resistance	
		Terminators	
CaMVpolyA	Cauliflower mosaic virus	Terminator	
OCS	*Agrobacterium tumefaciens*	3' non-translated region of the octopine synthase gene	

[a] The source for this gene is listed as *Triticum aestivum* in the application for DIR 162 as the gene has been previously introgressed into wheat, however the original source is *Aegilops taschii*

26. Short regulatory sequences that control expression of the genes are also present in the GM wheat lines (Table 2). The regulatory sequences are derived from plants (potato) or microorganisms (Cauliflower mosaic virus or *Agrobacterium tumefaciens*).

27. The GM wheat plants also contain selectable marker genes (Table 2) that confer resistance to different classes of antibiotics or to herbicide. Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. The selectable marker genes are *hptII*, which codes for hygromycin phosphotransferase (HPH or HPT) enzymes; *nptII* (neomycin phosphototransferase II) which encodes an aminoglycoside 3'-phosphotransferase II enzyme that is also known as neomycin phosphototransferase II (NPTII) and the *bar* gene which encodes the phosphinothricin N-acetyltransferase (PAT) protein involved in glufosinate herbicide tolerance. The *hptII* (Zalacain et al., 1986) and *bar* (Thompson et al., 1987) genes are derived from *Streptomyces hygroscopicus*, a common saprophytic, soil-borne microorganism that is not considered to be a pathogen of plants, humans, or other animals (OECD, 1999).The *nptII* gene is derived from *Escherichia coli* (*E. coli*) strain K12. The *E. coli* bacterium is a common gut bacterium that is widespread in human and animal digestive systems and in the environment. More information on marker genes may be found in the document Marker Genes in GM Plants.

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 bread wheat and durum wheat lines. All the GMOs contain disease resistance genes, (either for stem rust resistance or for multi-pathogen resistance) derived from bread wheat, rye or bread wheat relatives. The lines have been grouped according to the combinations of genes inserted (Groups A and B), transformation using a single plasmid (Groups A, C, D) or co-transformation with two plasmids (Group B) and use of single genes (Groups C and D).

29. For wheat, there are a number of pests and diseases of concern, including a number of important fungal pathogens broadly grouped as necrotrophic leaf fungi, biotrophic leaf fungi and root and crown fungi (Murray and Brennan, 2009). The rust of interest in the current application is stem rust (*Puccinia graminis* f. sp. *tritici*), with the multi-pathogen resistance genes also providing resistance to leaf rust (*Puccinia triticina*), stripe rust (or yellow rust – *Puccinia striiformis*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*).

30. Two classes of resistance genes to rust diseases have been described in wheat: pathogen race specific resistance genes (R genes) and race nonspecific resistance genes, referred to as adult plant resistance genes (APR genes). Both R and APR wheat rust resistance genes are designated Lr, Sr, and Yr for leaf, stem and yellow rust (stripe rust) respectively (Ellis et al., 2014).

5.2.1 R genes

31. The majority of the resistance genes in this application belong to the nucleotide binding leucine rich repeat (NB-LRR) class. R genes were the first class of resistance genes to be genetically defined and introgressed into commercial varieties through conventional wheat breeding; single genes were found to confer high levels of resistance and this made selection of plants simple and economical (Ellis et al., 2014). A number of R genes are derived from wheat, other cereal crops and their relatives. Race-specific R genes are associated with a hypersensitive reaction in the host, resulting in incompatible host-pathogen interactions, based on a gene-for-gene system. However, R gene-mediated resistance is described as generally non-durable due to the high evolution rate of pathogens, leading to new virulent strains overcoming single resistance genes (McDonald and Linde, 2002). This often occurs within a few years (McDonald and Linde, 2002; Ellis et al., 2014; Herrera-Foessel et al., 2014).

32. It has been suggested that long-term success in controlling rusts using R genes would result from the release of varieties containing combinations - 'stacks' or 'pyramids' - of several effective R genes to minimise selection for virulence in rust pathogens (Singh et al., 2008; Ellis et al., 2014).

33. A number of R genes for wheat stem rust resistance are proposed for release in this application – *Sr22*, *Sr26*, *Sr33*, modified *Sr33*, *Sr35*, *Sr45*, *Sr46* and *Sr50* – and will be examined in lines containing individual genes or as combinations of three to eight genes per line. Some of these lines also include the APR gene *Lr67*. These *Sr* genes are listed among 39 genes that are effective against the known Ug99 wheat stem rust races, although field effectiveness may vary under differing disease pressure (Singh et al., 2011; Singh et al., 2015). It has been suggested that combinations of some of these genes with one another and in plant backgrounds containing other R or APR genes may be valuable to provide durable stem rust resistance (Periyannan et al., 2013; Saintenac et al., 2013; Mago et al., 2015; Steuernagel et al., 2016). The highly virulent Ug99 races are not present in Australia but have been detected in many other countries (Singh et al., 2011; Singh et al., 2015).

5.2.2 APR genes

34. APR genes are associated with partial rust resistance phenotypes, observed only in adult plants. The host-pathogen interaction is compatible, and resistance is characterised by less pathogen growth and slow disease development in the field, thus it may be described as 'slow rusting'. When several APR genes are accumulated, 'near immunity' can be achieved. APR gene-mediated resistance is described as broad and durable (Ellis et al., 2014; Herrera-Foessel et al., 2014; Mondal et al., 2016). APR genes may provide resistance to all isolates (or pathotypes) of a pathogen species or they may provide resistance to multiple pathogen species (Rinaldo et al., 2017). Functions of cloned APR genes are diverse, ranging from protein kinases, to transporters and transmembrane proteins (Ellis et al., 2014). There is also some evidence that the presence of some APR genes, including Lr34 may interact with R genes to boost rust resistance, although the exact mechanisms of these interactions are not fully understood (Vanegas et al., 2007; Ellis et al., 2014). The *Lr34* and *Lr67* genes are described as foundation APR genes, increasing the impact of other resistance genes, including NB-LRR class genes (Ellis et al., 2014).

35. The multi-pathogen resistance genes proposed for release in this application are APR genes that provide resistance to wheat stem rust (*P. graminis* f.sp. *tritici*), wheat leaf rust (*P. triticina*), wheat stripe rust (*P. striiformis*) and powdery mildew (*B. graminis*) diseases (Krattinger et al., 2009; Moore et al., 2015).

36. The *Lr67* gene encodes a predicted hexose transporter protein that differs from the form of the protein produced by the susceptible allele by two amino acids that are conserved in orthologous hexose transporters. The resistant transporter protein functions to reduce glucose uptake (Moore et al., 2015).

37. The *Lr34* gene has two mutations that enable the transporter protein encoded by *Lr34* to confer resistance, while *Lr34B* does not (Krattinger et al., 2011). The *Lr34B* gene is a homologue of *Lr34*, located on a different chromosome. The two mutations present in *Lr34* have been engineered into the *Lr34B* gene. The applicant proposes to assess the ability of the *Lr34B* gene containing the mutation to confer disease resistance in GM durum wheat lines and to compare the lines containing the modified *Lr34B* gene to GM durum wheat lines containing the known *Lr34* gene. It has been suggested that given the phenotypic similarities, *Lr34* and *Lr67* may encode similar proteins and that lines carrying genes encoding functionally redundant proteins may not perform better than single-gene lines (Spielmeyer et al., 2013). The *Lr34* gene is present in many bread wheat cultivars in Australia and in 2016 was effective against all known Australian stem rust pathotypes (Cuddy et al., 2016).

38. Each of the *Lr* genes will be examined in single gene GM lines (Group D) in bread wheat and durum wheat (*Lr67*) or in durum wheat only (*Lr34* and *Lr34B*). In addition, *Lr67* will be inserted in combination with *Sr* genes in lines of Groups A, B and C.

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

39. No toxicity or allergenicity studies have been performed on the GM wheat plants or purified proteins encoded by the genes, as the proposed trial is at preliminary research stage. However, a number of the genes are sourced from wheat or rye, which are routinely consumed by people and animals and the remaining genes are of the same classes as those sourced from these crop plants.

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

41. Three of the genes proposed for the current release - *Sr46*, *Lr34* and *Lr67* - have been previously approved under licence DIR 151, a limited and controlled release of GM wheat. No adverse effects have been reported for this release.

42. The *hptII* (*hph*) and *nptII* genes are the most commonly-used antibiotic resistance genes for selection of transformed plant cells (Breyer et al., 2014). There is no evidence that the *hptII* or *nptII* genes or the proteins they encode are toxic or allergenic (OGTR Risk Assessment documents and references therein). The *bar* gene and the protein it encodes (phosphinothricin N-acetyl transferase or PAT) has been assessed in other RARMPs most recently in DIR 120 and DIR 145, and in scientific literature. The environmental safety of the PAT protein present in biotechnology-derived crops, either alone or in combination with other GM traits, has also been extensively assessed by regulatory authorities worldwide (CERA, 2011). Crops containing these genes have been approved for release in a number of countries and for use in food and feed worldwide (Biosafety Clearing House website, ISAAA website, both accessed 27 February 2018). GM foods containing the *hptII, nptII* and *bar* genes have been assessed and approved for sale in Australia (FSANZ website, accessed 28 February 2018).

5.4 Characterisation of the GMOs

43. Although these lines are at an early stage of development, the applicant has provided preliminary information on expected phenotypes for some genes or groups of genes. Each of the *Sr* genes was introduced individually into wheat plants that were tested in the glasshouse and no effects other than rust disease resistance were observed. Transformed plants expressing the *Lr67* gene have also been tested in the glasshouse, with adult plants showing partial resistance to stem, leaf and stripe rusts and powdery mildew.

44. *Lr67* and *Lr34* are APR genes for rust resistance, providing resistance at the adult plant stage. However, the applicant has observed that elevated expression levels of *Lr34* can result in seedling resistance and suggest that this may also occur with *Lr67*, although they have not yet observed this effect in seedlings expressing *Lr67.* The applicant expects that the genes will provide rust disease resistance without associated pleiotropic effects, however high levels of resistance gene expression may result in dwarfed plants (Grant et al., 2003). The applicant has indicated that any transformed plants exhibiting this type of effect in the glasshouse would not be used in the field due to obvious pleiotropic effects.

Section 6 The receiving environment

45. 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).

46. Information relevant to the commercial cultivation and distribution of bread wheat in Australia, including key biotic and abiotic interactions in the wheat-growing environment, is presented in the wheat biology document. Information relevant to the commercial cultivation and distribution of durum wheat in Australia is available in a number of industry publications (Hare, 2006; Kneipp, 2008; QDAF, 2012a, b; GRDC, 2016a, b, c; SARDI, 2017), with key factors discussed below in Sections 6.1 to 6.3. Durum wheat is cultivated in areas within the 'wheat belt' and as such is cultivated in a subset of the same environments as bread wheat in Australia.

6.1 Relevant biotic factors

47. While durum wheat can compete well with weeds, high weed pressure can affect yields. Weed control by integrated weed management is recommended for durum wheat, as for bread wheat crops

(GRDC, 2016a), and weed species relevant to durum production are similar to those for bread wheat (OGTR, 2017).

48. Important insect pests of durum wheat in northern areas are cutworms (*Agrostis* spp.) aphids, army worms (*Leucania* spp., *Spodoptera mauritia*) mites, including red-legged earth mite (RLEM – *Halotydeus destrcutor*), blue oat mite (*Penthaleus spp.*) and brown wheat mite (*Petrobia latens*) and in some seasons *Helicoverpa armigera*. Thresholds for insect control depend on the timing and severity of infestations and options for insect control. At some stages biopesticides may be suitable for insect pest control and a number of predatory insect species are valuable for pest insect control (GRDC, 2016a). Integrated pest management strategies are recommended in durum wheat production as for other cereal crops (GRDC, 2016c). Further information about insect pests in durum wheat and winter cereals can be found in grain industry publications (GRDC, 2016a, b, c).

49. A number of the nematode and other pathogen-related diseases of concern for bread wheat (OGTR, 2017) are also of concern for durum wheat. For northern regions, the root lesion nematodes *Pratelynchus thornei* and *Pratelynchus neglecta* are species with potential impact on durum wheat crops, although durum wheat is generally more resistant to *P. thornei* than bread wheat varieties (QDAF, 2012a, b; GRDC, 2016a). Resistance to and tolerance of nematodes varies between durum varieties (QDAF, 2012a, b; GRDC, 2016a) so variety selection, crop rotations and crop hygiene are important factors in managing nematodes in durum wheat (QDAF, 2012a, b; GRDC, 2016a, c). The most important disease for durum wheat is crown rot (*Fusarium pseudograminearum*) which can cause significant losses. Fusarium head blight (mainly caused by *Fusarium graminearum*) is also important and may be associated with mycotoxin production which results in downgrading of grain. Durum wheat is more susceptible to this pathogen than bread wheat. Other diseases of concern are common root rot (*Bipolaris sorokiniana*) which is often associated with crown rot, as well as leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis* f. sp. *tritici*) and stripe rust (or yellow rust – *Puccinia striiformis*), as well as yellow leaf spot (*Pyrenophora tritici-repentis*) (GRDC, 2016a). Resistance to these diseases varies between durum varieties, however all varieties are listed as susceptible or very susceptible to crown rot (GRDC, 2016c). Careful management of crop rotations, removal of crop residues that harbour disease inoculum and varietal selection are key tools for disease management in durum wheat, fungicide applications can be used but there are many considerations to ensure effectiveness (GRDC, 2016a).

50. Crop rotation, including non-cereal crops in particular, is recommended in durum wheat production for disease control, pest control, as well as weed control and to allow rotation of herbicides for weed control (Hare, 2006; Kneipp, 2008; GRDC, 2016a). This is also true for bread wheat production systems (OGTR, 2017).

6.2 Relevant abiotic factors

51. It is proposed that the GMOs will be grown at two locations. The release is proposed to take place at Ginninderra Experiment Station (GES), ACT and at Boorowa Agricultural Research Station (BARS), NSW, two dedicated fenced field trial sites. The GES site is approximately 2.3 ha and has been previously used for licenced field trials. The BARS site is a new proposed site, anticipated to be of approximately 2.3 ha. Both sites are on CSIRO controlled land. Access to the research stations is restricted to authorised staff and CSIRO has control over the management of the fields immediately surrounding the proposed trial sites.

52. The proposed sites are located at least 50 m away from the nearest natural waterways and the areas are not prone to flooding (information provided by the applicant).

53. Information regarding abiotic stresses for bread wheat can be found in the biology document. Briefly, these fit within the categories of nutrient stress, drought, waterlogging, heat stress, salinity, acidic soils and mineral toxicity. For durum wheat more information can be found in industry publications, however, key information is provided below.

54. Heat stress is regarded as the key abiotic stress for durum wheat in all growing regions, affecting grain yield, productivity with potential losses from heat stresses equal to or greater than those from drought and frost. Frost is also potentially damaging to durum wheat, as for other cereal crops (Hare, 2006;

GRDC, 2016a). Frost tolerant varieties are not available, however crop management to spread the flowering time within the crop may mitigate some frost risk (Hare, 2006). Waterlogging, particularly after flooding events, can also affect durum productivity (GRDC, 2016a). Availability of water is also key for durum cultivation, with production in northern regions reliant on soil moisture from rainfall prior to the growing season, while in southern areas rainfall during the production season is vital for adequate moisture levels (Kneipp, 2008). The key limiting nutrient for durum production is nitrogen (N) (Hare, 2006; GRDC, 2016a) and in some situations zinc (Zn) can also be a limiting factor as low Zn can also limit the uptake of other nutrients (Hare, 2006).

6.3 Relevant agricultural practices

55. 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 bread wheat and durum wheat by the applicant will not differ significantly from industry best practices used in Australia.

56. The GM wheat would either be hand sown in rows with a spacing of approximately 30 cm between rows, or planted with a small plot cone seeder in plots, 2 m wide and up to 10 m long, and maintained as a dryland crop, but with irrigation available if required.

57. The crop will be maintained using similar practices to those used in commercial wheat crops for management of weeds and disease. As the GM wheat lines within this trial are planted to assess the impact of candidate genes on disease resistance, there will be some differences in management of diseases such as rust.

58. Harvesting of the seeds would occur either by hand or using a plot harvester that is dedicated to GM trials. After harvest the land would be left fallow or planted with a break crop if approved.

6.4 Presence of related plants in the receiving environment

59. Both bread and durum wheats are commonly grown in Australia and are widely cultivated in the surrounding regions of the proposed field trial sites. The paddocks adjoining the GES and BARS trial sites are on CSIRO land. Those surrounding the GES are routinely used for GM and non-GM wheat trials and it is likely that a similar situation will occur for BARS.

60. In addition, the applicant has proposed that planting for the current application occur on areas of land previously approved for field trials DIR 111 and DIR 151, so other GM wheats may also present in the receiving environment. Under DIR 151, planting and growing of the GM wheat is permitted at GES and BARS until May 2022 and GM wheat approved under other licences may be grown in the monitoring zone. Therefore, it is possible that the GM wheat proposed for release under the current application could be grown immediately adjacent to GM wheat lines planted under DIR 151. At the GES site, there are also three planting areas currently under postharvest monitoring (PHM) from DIR 111. While no further planting can take place under this licence, it is possible that there may be volunteers present.

61. Gene flow can occur between cultivated varieties of bread wheat, although pollen flow is limited, generally occurring at low frequency and/or over short distances (Gatford et al. 2006). Bread wheat is considered a low-risk crop for both intraspecific and interspecific gene flow (Eastham & Sweet 2002). Although durum wheat has not been examined as closely as bread wheat, it is a closely related species and it is likely that pollen flow is limited and that the risks of intra- and inter-specific gene flow would be considered low for durum wheat as well. *Triticum turgidum* wheats – of which durum wheat is one subspecies – are self-fertilising, although natural hybridisation and introgression have been important in their diversification (Matsuoka, 2011). In general, pollen from the Graminae has a limited fertility period (Eastham and Sweet, 2002 and references therein), thus it is likely that pollen from durum wheat would not be viable for long.

6.4.1 Bread Wheat

62. Bread wheat (*Triticum aestivum* L.) is sexually compatible with a number of species within the tribe Triticeae that occur in Australia, including other cereal crops. Hybridisation with durum wheat occurs

readily (Wang et al., 2005), with field outcrossing between rates varying according a number of factors, including environmental conditions, particularly wind speed and direction (Matus-Cádiz et al., 2004; Loureiro et al., 2007), highlighting the need for testing in a range of environments (Loureiro et al., 2007). Field outcrossing from bread wheat to durum wheat in a smaller field trial was less than 1 % at a distance of 8 m (Loureiro et al., 2007). In a larger study in a semi-arid region, gene flow at 20 m was 0.01 to 0.02 %, with no pollen flow was recorded at or past 40 m. In the same study no long-distance pollen flow was recorded (340 – 580 m) (Matus-Cádiz et al., 2004).

63. Hybridisation of *T. aestivum* with rye (Meister, 1921; Leighty and Sando, 1928; Dorofeev, 1969) 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 bread 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).

64. Bread wheat readily hybridises with *Aegilops* species (goatgrasses). *Aegilops tauschii* is generally regarded as the donor for the D genome in hexaploid wheat, however, unlike bread wheat, durum wheat does not contain the D genome, thus could be regarded as being less closely related to *Aegilops* species than bread wheat (CFIA, 2006). However, 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, accessed 28 February 2018).

65. There has been one report of natural hybridisation between bread 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.

6.4.2 Durum Wheat

66. Durum wheat is regarded as self-fertile, although outcrossing of up to 5 % has been recorded (Bozzini, 1988). Pollen flow for tetraploid wheat (*T. turgidum*) landraces has been recorded from 1.6 % to 4.3 %, varying across years and locations and depending on whether pure stands or mixed stands of landraces were examined (Tsegaye, 1996). Outcrossing of durum wheat with other *T. turgidum* species, with related crop species and with weedy relatives have also been examined. Sterility barriers have been observed between durum wheat and *T. turgidum* subsp. *armeniacum* (= *T. timopheevii*) and *T. turgidum* subsp. *dicoccoides* (Feldman, 2001). Crosses with rye, maize, or pearl millet have been achieved, but only in glasshouses with embryo rescue (Inagaki and Hash, 1998; Cherkaoui et al., 2000; Klindworth et al., 2003; Garcia-Llamas et al., 2004).

67. There are reports of natural hybridisation of durum wheat with *Aegilops* species, however results were unconfirmed (CFIA, 2006). While crossing of durum wheat and *Aegilops spp*. has been documented, these crosses were generally achieved only under laboratory conditions (Jacot et al., 2004). Glasshouse crosses with non-cultivated or weedy relatives have been observed, including *Aegilops ovata* (Benavente et al., 2001), *Aegilops caudata* (Simeone et al., 1989) and *Thinopyrum bessarabicum* (Jauhar and Peterson, 2006), but these required embryo rescue. Glasshouse crosses with *Ae. tauschii* as the male parent have produced hybrids with durum wheat without embryo rescue. The crosses had a mean 'crossability' of 5.61 % across 15 crosses involving six durum lines, however 50 % of crosses yielded no hybrids (Zhang et al., 2008). This study provides some information about the potential location of the barriers to hybridisation between the two species on specific chromosomes of the durum parent. There are a number of weedy relatives of durum wheat present in cropping areas in North America, but no natural hybrids with weedy *Agropyron spp.* (wheatgrass), *Hordeum spp.* or *Elymus spp*. relatives have been reported (CFIA, 2006). Hybridisation between durum wheat and *Aegilops geniculata* (= *Ae. ovata*) have been observed in the field in Europe, but hybrids in the field were typically sterile, indicating sterility barriers between the two species (David et al., 2004). In addition, as previously noted, there are no naturalised populations of *Aegilops* species in Australia (Weeds in Australia, accessed 28 February 2018).

68. 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 *Elymus repens*[5] (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), but not as weeds of national significance (Weeds in Australia). There has been no concerted investigation on natural hybridisation of these native and introduced Triticeae species with wheat (bread or durum). 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 bread or durum wheats.

6.5 Presence of similar genes and encoded proteins in the environment

69. The introduced genes for rust disease resistance were isolated from bread wheat (*Sr26*, *Lr34*, *Lr34B* and *Lr67*) or rye (*Sr50*), both of which are commonly consumed cereal crops, or from bread wheat relatives. The *Sr46*, *Sr45*, *Sr33* and *Sr33mod* genes are sourced from *Ae. tauschii*, which is regarded as the D genome donor for *T. aestivum* (OGTR, 2017 and references therein). The *Sr35* gene is sourced from *T. monococcum* (Saintenac et al., 2013), a cultivated diploid wheat that has been suggested as the A genome donor for bread wheat (Kimber and Sears, 1987). The *Sr22* gene has been sourced from *T. monococcum* subsp. *aegilopoides* (Steuernagel et al., 2016), an A genome relative of bread wheat (Kimber and Sears, 1987).

70. The wheat relative *Ae. tauschii* is not present in Australia (Weeds in Australia), however it has been widely used as a genetic resource for wheat germplasm improvement since the early 20th century (Krattinger et al., 2009). Neither *T. monococcum* nor *T. monococcum* subsp. *aegilopoides* are recorded as present in the natural environment in Australia (Atlas of Living Australia; The Australasian Virtual Herbarium; Global Biodiversity Information Facility; accessed 12 February 2018).

71. The *Lr34* gene has been present in commercial bread wheat varieties for at least 50 years (Krattinger et al., 2011; Ellis et al., 2014) and was incorporated into over 50 % of commercial wheat varieties (Hoisington et al., 1999). In a review of rust resistance and rust genotypes in commercial cereal crops in Australia, 30 of the 87 bread wheat lines tested had the *Lr34* genotype (Cuddy et al., 2016), thus the *Lr34* gene is likely to be common in the environment. The *Sr22* gene is present in the Australian commercially-released Schomburgk bread wheat cultivar (Khan et al., 2005). A number of other R genes and APR genes occur in wheat or have been introgressed into wheat from other crop plants or from wild relatives. Thus as well as the specific genes mentioned, the classes of genes and the classes of proteins encoded by the genes proposed for this application are common in the environment and humans and other beneficial organisms have a long history of exposure to these genes and their encoded proteins.

72. The *hptII, nptII* and *bar* genes are derived common bacteria that are widespread in human and animal digestive systems and/or in the environment. Both humans and animals are routinely exposed to these genes and their encoded proteins in the environment.

73. All promoters used to drive expression of the introduced genes are the native promoters for the inserted genes, which were derived from wheat or wheat relatives. Humans and animals have been safely consuming these plants for centuries. The CaMV35S promoter, derived from a common plant virus, is used to drive marker gene expression. Other regulatory sequences are from common organisms including Cauliflower mosaic virus, *A. tumefaciens* and potato.

[5] This species is listed in the Atlas of living Australia as *Elytrigia repens*, however in USDA GRIN and WCSP databases the accepted name is *Elymus repens*.

Section 7 **Relevant Australian and international approvals**

7.1 Australian approvals

74. Wheat lines containing three of the genes – *Sr46*, *Lr34* and *Lr67* – have been approved for limited and controlled release under the licence for DIR 151.

75. There have been no approvals for the commercial release of GM bread wheat or durum wheat in Australia.

7.2 International approvals

76. Field trials of GM wheat have been approved in a number of countries including the United States, Canada, the United Kingdom and a number of European countries. These approvals are for a range of modified traits, including improved yield and tolerance to abiotic stresses (USDA APHIS Biotechnology Permits, EU GM Register; accessed March 2018).

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

Chapter 2 Risk assessment

Section 1 **Introduction**

78. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of, gene technology (Figure 2).

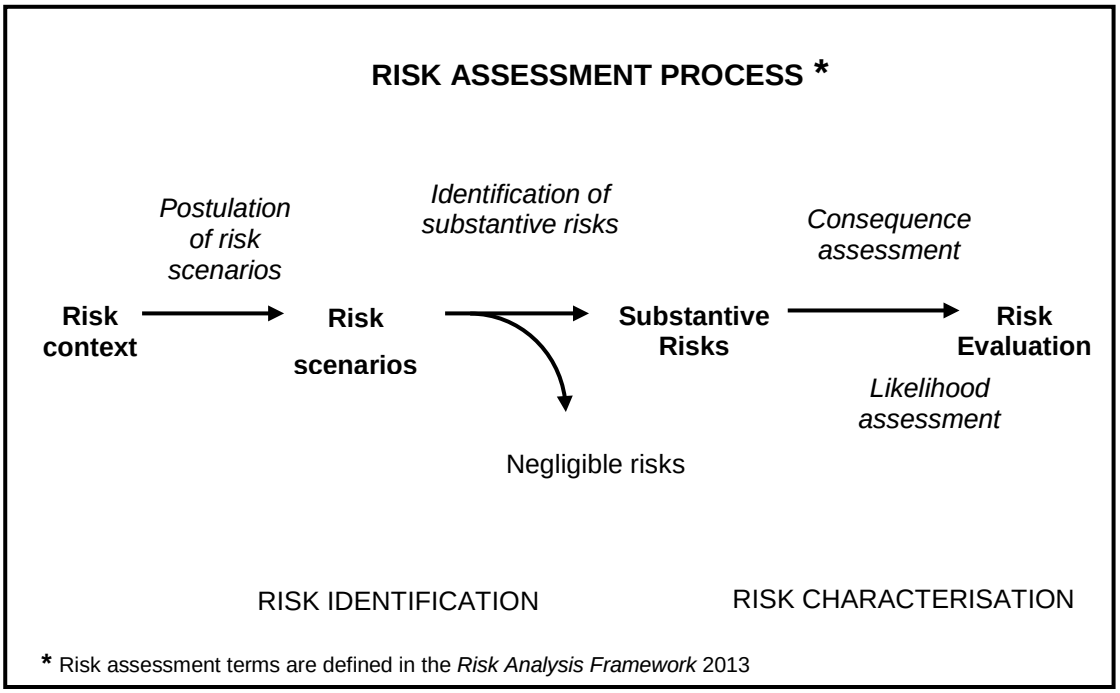

Figure 2 The risk assessment process

79. 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.

80. 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.

81. 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.

82. 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**

83. 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).

84. 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

85. 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.

The introduced genetic elements

86. As discussed in Chapter 1 (Table 1 and Table 2), the GM bread wheat lines have been modified by the introduction of between one and seven genes for stem rust resistance and/or one gene for multi-pathogen resistance. Each of the GM durum wheat lines has been modified by the introduction of one of three genes for multi-pathogen resistance. The introduced genes will be considered further as potential sources of risk.

87. The GM wheat and durum wheat lines also contain one or two marker genes selected from *hptII, nptII, or bar.* The *hptII* and *nptII* genes confer antibiotic resistance, while *bar* provides tolerance to phosphonithricin-containing herbicides. These genes were used as selectable markers during development of the GM plants. The *hptII, nptII* and *bar* genes and their 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* and *nptII* is available in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. The *bar* gene and its protein product, PAT, have been assessed in other RARMPs as well as in scientific literature, as detailed in Chapter 1 (5.2). The environmental safety of the PAT protein present in biotechnology-derived crops has also been extensively assessed worldwide (CERA, 2011). As the marker genes have not been found to pose a substantive risk to either people or the environment, they will not be further considered for this application.

88. The introduced genes are controlled by introduced regulatory sequences. These are derived from a number of common sources including potato (*Solanum tuberosum*), a bacterium (*Agrobacterium tumefaciens*) and a plant virus (Cauliflower mosaic virus - CaMV). Each of the rust resistance or multi-pathogen resistance genes has a native promoter and terminator sequence, from the same source as the genes i.e. bread wheat and related species. Table 2 in Chapter 1 provides more information about the regulatory sequences. 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.

Unintended effects

89. 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). Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further in this application.

2.2 Causal pathway

90. 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.

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

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

93. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR 117. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

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

95. These harms are based on those used to assess risk from weeds (Virtue, 2004; Keese et al., 2014). 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

96. 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 bread wheat and GM durum wheat 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.

97. 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 bread wheat and durum wheat

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes conferring enhanced rust disease resistance and multi-pathogen resistance	Growing GM wheat 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 introduced genes are sourced from bread wheat, rye and wheat relatives that are routinely used for food or feed, or have been the source of genes introduced into wheat through conventional breeding • 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 small size and short duration of the proposed trial would 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 conferring enhanced rust disease resistance and multi-pathogen resistance	Growing GM wheat plants at the field trial sites ⬇ Expression of the introduced genes in GM plants ⬇ Pollen flow between GM wheat plants and other GM wheat plants growing at the sites ⬇ Hybridisation of different GM wheat 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	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 have been the source of genes introduced into wheat through conventional breeding • 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 expect that novel proteins would be expressed in hybrids nor that the expressed proteins would behave differently in a hybrid background • The small size and short duration of the proposed trial would minimise exposure of people and other desirable organisms to the GM plant material
3	Introduced genes conferring enhanced rust disease resistance and multi-pathogen resistance	Growing GM wheat at the field trial sites ⬇ Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Establishment of GM wheat 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	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 • Bread wheat and durum wheat grains have limited dispersal by animals • Bread wheat and durum wheat have limited ability to survive outside agricultural settings • The GM wheat 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 conferring enhanced rust disease resistance and multi-pathogen resistance	Growing GM wheat at the field trial sites ⬇ Fertilisation of sexually compatible plants outside the trial site by pollen from GM wheat plants ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid 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	No	• The proposed limits and controls minimise the likelihood of pollen flow from the trial site to sexually compatible plants • Bread wheat and durum wheat have limited ability to outcross • There is no indication that hybrid plants would have increased ability to survive outside agricultural settings • Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.

2.4.1 Risk scenario 1

Risk Source	Introduced genes conferring enhanced rust disease resistance and multi-pathogen resistance
Causal Pathway	⬇ Growing GM wheat 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 for humans or increased toxicity to other desirable organisms

Risk source

98. The source of potential harm for this postulated risk scenario is the introduced genes for rust disease resistance and multi-pathogen disease resistance in GM wheat lines.

Causal pathway

99. GM wheat is planted at the trial sites and the genes for rust disease resistance and/or multi-pathogen resistance are expressed. The proteins encoded by these genes are expressed either at seedling and adult plant stages (R genes) or only at adult stages. The encoded proteins may be expressed in a range of tissues at all developmental stages.

100. 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 at the trial site, but may also occur during transport and handling of GM plant material. Other organisms such as livestock, rodents, marsupials, birds or invertebrates may be exposed at the trial site through contact with, or ingestion of GM plant material. A range of birds, stock and wildlife may consume cereals (Hill et al., 1988; AGRI-FACTS, 2002; OGTR, 2017), thus, they may have direct contact with the GM plant material during ingestion.

101. Limits and controls are proposed for the trial that would minimise 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 would be used for human food or animal feed. The trial sites would be located on land owned and controlled by CSIRO, and would only be accessed by authorised people.

102. The trial is proposed for a maximum of five growing seasons during the period from September 2018 until September 2023. The potential for exposure is limited to a short period when GMOs are present at the trial sites during these growing seasons. In addition, the areas proposed are small, thus further limiting exposure. The total area of the two sites is 4 ha, but of this a maximum planting area of 20 m^2 per site per season is proposed, thus a total of 40 m^2 per season if both sites are planted.

Potential harm

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

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

105. Although no toxicity or allergenicity studies have been performed on the GM plant material, the introduced genes were isolated from wheat, rye or relatives of wheat that have been the source of genes

introduced into wheat through conventional breeding (Chapter 1, Section 5.1). Thus, people and other beneficial organisms are exposed to the same or similar proteins through their diet and in the environment. The *Lr34* gene has been present in commercial bread wheat varieties for at least 50 years (Krattinger et al., 2011; Ellis et al., 2014). It was incorporated into over half of commercial wheat varieties worldwide (Hoisington et al., 1999), including Australian commercial bread wheat lines (Cuddy et al., 2016). Three genes proposed for the current release - *Lr34*, *Lr67* and *Sr46* - have been approved for planting under the licence for DIR 151. No adverse effects have been reported from this release and 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.

106. Non-GM wheat is not regarded as toxic to humans or other desirable organisms. However, it can produce allergic and autoimmune responses in susceptible individuals by inhalation of flour (for example baker's asthma) or ingestion (coeliac disease) (OGTR, 2017). There is no reasonable expectation that any of the genes proposed for this trial would influence the pathways producing known allergens in wheat. Additionally, plant material from this trial may not be used for food or feed.

Conclusion

107. 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 genes conferring enhanced rust disease resistance and multi-pathogen resistance
Causal Pathway	⬇ Growing GM wheat plants at the field trial sites ⬇ Expression of the introduced genes in GM plants ⬇ Pollen flow between GM wheat plants and other GM wheat plants growing at the sites ⬇ Hybridisation of different GM wheat 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

108. The source of potential harm for this postulated risk scenario is the introduced genes for rust disease resistance and multi-pathogen disease resistance in GM wheat lines.

Causal pathway

109. Due to the small size of the planting areas proposed for this field trial, different lines grown under DIR 162 would be planted in close proximity to one another. Given that different GM lines from both bread wheat and durum wheat are sexually compatible and that they may have similar flowering times, pollen flow between the GMOs is likely and could potentially result in hybrid plants containing additional genes.

110. In addition, as set out in Chapter 1, Section 6.4, the GM wheat grown at the proposed release sites may be grown immediately adjacent to GM wheat lines planted under the licence for DIR 151. Some of the rust resistance genes proposed for release under DIR 162 are the same as those approved for release in GM wheat approved under DIR 151. However, other GMOs licenced under DIR 151 contain different rust resistance or multi-pathogen resistance genes, or have been modified for other trait classes (enhanced drought tolerance, altered oil content and altered grain composition) with genes not included in the

current application. Thus, there is potential for the production of hybrid GM wheat plants containing additional introduced genes for these traits.

111. At the GES site, there is also one planting area from DIR 111 currently under postharvest monitoring (PHM). While no further planting can take place under this licence, the site is under post-harvest monitoring, so it is possible that there may be volunteers present at the site from this release. The introduced genes for DIR 111 conferred traits including altered grain composition, altered nutrient use efficiency, enhanced rust disease resistance or improved drought tolerance. The *Lr34* rust resistance gene proposed for the current application is also approved for DIR 111, however, other genes and traits are not included in the current application, so any volunteers could potentially create different stacked lines through hybridisation with plants released under DIR 162.

112. Wheat is largely self-pollinating, with less than one percent cross pollination reported beyond distances of 0.5 to 20 m with results depending on the size and conditions of the reported studies (Matus-Cádiz et al., 2004; Gatford et al., 2006; Loureiro et al., 2007; Rieben et al., 2011). Nonetheless, under the licence conditions described above, adjacent plantings of sexually compatible GM plants are permitted, so pollen flow between planting areas may occur at low levels. This could lead to pollination and seed set, resulting in hybrid seeds produced in the DIR 111, 151 or 162 trials. However, existing licence conditions for DIRs 111 and 151 and proposed conditions for DIR 162 should effectively manage hybrid seeds or plants. The licence requirements for post-harvest monitoring of planting areas under the licence for DIR 111, require that any volunteers from this trial must be destroyed prior to flowering. Thus, the likelihood of any hybrids occurring between plants from this trial and those proposed for release under DIR 162 is minimal. If any hybrid seed was produced on plants grown under DIR151 and DIR162 it would be harvested with other trial material. Any hybrid seed that was dispersed onto the trial site during harvest and germinated would also be destroyed prior to flowering under the licence conditions for DIR 151 and proposed conditions for DIR162, minimising the likelihood of any further hybridisation and persistence. Thus, exposure of people or other desirable organisms to hybrids would be minimal.

Potential Harm

113. If hybrids between lines from DIR 162, or between lines from DIR 162 and DIR 151 or DIR 111 were to occur, such lines may contain additional proteins produced as a result of expression of the stacked introduced genes. People and other desirable organisms may thus be exposed to hybrid GM bread wheat and/or durum wheat plants containing proteins encoded by the stacked genes and these proteins may be toxic or allergenic to humans or toxic to other desirable organisms.

114. However, Risk Scenario 1 did not identify toxicity or allergenicity of any of the individual genes as a substantive risk. Likewise, the RARMPs for DIR 111 and DIR 151 concluded that there were no substantive risks associated with toxicity or allergenicity of the genes and their products, nor for potential hybrids of the lines in these releases. There is no evidence to suggest that combinations of genes from DIR 162 with those from either DIR 111 or DIR 151 would result in the production of novel proteins, or that their expression would 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, or from organisms that have been the source for genes for conventional wheat breeding, suggesting that humans and other desirable organisms have a long history of exposure to the genes and their products.

115. The licence for DIR 151 has a condition that prohibits the use of any seed from the trial for development of cultivars for commercial release if any other GMOs approved under a separate licence or any sexually compatible species have been planted at the site concurrently with the GMOs under DIR 151. Thus, if GMOs from DIR 151 and DIR 162 were planted adjacent to one another, any hybrid seed produced and harvested from DIR 151 could only be planted within a limited and controlled trial. DIR 151 has limits and controls to minimise the exposure of people and other desirable organisms to the genes and their expressed proteins and the spread of seed outside the trial site, as would any future trial that used seed from DIR 151.

116. 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

117. Risk scenario 2 is not identified as a substantive risk due to limited exposure and the lack of toxicity or allergenicity to humans of the introduced genes and their encoded proteins or those from hybrid plants containing combinations of these proteins. These genes, or combinations of genes, and their encoded proteins also lack 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 genes conferring enhanced rust disease resistance and multi-pathogen resistance
Causal Pathway	⬇ Growing GM wheat plants at the field trial sites ⬇ Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Establishment of GM wheat 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

Risk source

118. The source of potential harm for this postulated risk scenario is the introduced genes for rust disease resistance and multi-pathogen disease resistance in GM wheat lines.

Causal pathway

119. If GM wheat seed was dispersed outside the trial sites, 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, expressing genes for enhanced rust disease resistance and/or multi-pathogen resistance. This could increase the likelihood of exposure of people or other desirable organisms to the proteins expressed in the GM plants.

120. Similarly, if hybrids occurred at the trial site between different GM wheat lines, seed from such hybrids could also be spread outside the trial site. While 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, (discussed in Risk Scenario 2), there is a small possibility that such hybrid GM wheat seed could also be dispersed from the trial site.

121. Morphological and physiological characteristics of wheat would generally limit the likelihood of spread and persistence in the environment. These are summarised in Chapter 1, Section 4 and in the biology document for bread wheat. Both bread wheat and durum wheat have been selected during domestication for reduced shattering of seed heads - a mechanism for seed dispersal in ancestral wheat plants (Li and Gill, 2006). If the GM wheat persisted at trial sites through dormancy of seeds in the seed bank, the number of volunteers may be increased after the trial, providing seeds for spread to other areas. Bread wheat does not show a high degree of dormancy or a persistent seed bank under Australian conditions (OGTR, 2017). Although durum wheat has been noted as maintaining good germinability for three to five years or longer under very cold, dry, low oxygen conditions (Bozzini, 1988), it is unlikely that such a combination of conditions would occur in the Australian wheat-growing areas. While durum wheats are classed as hard wheats, studies examining preharvest sprouting susceptibility found that durum wheat varieties showed cultivar variability and that preharvest sprouting tolerance that was in the middle of the range for white coated wheats tested in the same study (McCaig and DePauw, 1992). This suggests that it is unlikely that durum wheat seeds would show greater dormancy than bread wheat seeds. Thus although a

range of factors in the environment can influence dormancy in both bread wheat and durum wheat, it is unlikely that either species would persist to provide significant seed banks in the Australian environment.

122. Dispersal of GMOs outside the limits of the trial sites is most likely to occur through the activity of people or animals and through extreme weather events.

Dispersal through human activity

123. Although human activity is generally one of the main mechanisms for seed dispersal from wheat crops (OGTR, 2017), the applicant has proposed limits and controls to prevent the spread of GM wheat 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 would be cleaned in a designated clean-down area before leaving the site or being used for any other purpose. All GM plant material would 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

124. The activity of animals such as rodents, herbivores and birds could lead to dispersal of the GMOs outside the limits of the trial sites. 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.

125. Wheat seeds can be dispersed in sheep wool (Ryves, 1988), however wheat lacks seed dispersal characteristics such as stickiness, burrs and hooks (Howe and Smallwood, 1982). The intended introduced traits of the GM plants are not expected to alter these characteristics of seeds. Dispersal on animal hooves is probable but not well reported. Durum wheat seeds share many morphological characteristics with bread wheat seeds, including those that would influence the likelihood of transport by animals. Although studies are not available specifically for durum wheat, it is highly likely that durum wheat seeds have similar probability of spread in these ways by animals.

126. Reports on seed dispersal for wheat through ingestion are rare. Intact wheat seed may make up to 30% 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 and Suárez, 1995). Seeds which survive chewing and digestion by animals are typically small and dormant (Malo and Suárez, 1995). Therefore rabbits are unlikely to disperse the GM wheat seeds.

127. Rodents are opportunistic feeders and their diet includes seeds and other plant material (Caughley et al., 1998). They may not only eat and destroy seed at the seed source, but may also hoard seeds (AGRI-FACTS, 2002), which increases the possibility of seed dispersal. However, the applicant proposes an area around the GM planting area, maintained in a manner that does not attract or harbour rodents and the implementation of rodent control measures if rodents are detected. These measures would minimise the potential for seed dispersal by rodents. Furthermore, if dispersal did inadvertently occur, the GM wheat lines are susceptible to standard weed control measures.

128. A variety of birds may feed on cereal crops, including wheat, however a search of the literature found little evidence of extensive spread of seed via birds. Birds such as cockatoos do most damage to wheat during germination (Temby and Marshall, 2003) and while they can cause damage to cereal crops during germination and seed ripening, only a small proportion of intact wheat seed can be excreted by corellas and galahs, with varying germination rates (Woodgate et al., 2011). Even under controlled conditions germination rates of seed were very low, ranging from 0.8 % to 2 % (Woodgate et al., 2011). Emus may feed on wheat seed but generally prefer other foods (Davies, 1978), however it is likely that germination rates of seed after digestion are low and experimental evidence is sparse. The majority of wheat varieties grown in Australia are white wheat varieties (Blakeney et al., 2009) which have thin seed coats and are easily broken down during digestion (Temby and Marshall, 2003; Yasar, 2003). One study has

shown that durum seed is rapidly digested after feeding to mallard ducks, however the study did not indicate whether any intact seed survived passage through the gut and therefore whether any viable seed survived digestion (Clark and Gentle, 1990). Although durum wheat is classed as a hard wheat variety, the lack of weedy populations of durum wheat in the environment indicates that viable seeds are not easily spread by birds or by other means.

129. 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, 2017).

130. 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. In addition to the proposed management of vegetation in the buffer zone and monitoring zone to allow detection of volunteers and assist in rodent control. 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 assessment for bread wheat considers a range of factors with respect to the spread of wheat seeds. The likelihood of dispersal of viable plant parts by land-based animals is rated as 'unlikely to occasional' for wheat. It is expected that a similar rating could be made for durum wheat, considering their similar characteristics. The limited size and duration of the current trial further limits the availability of viable seed for spread and there are also a number of factors which limit the survival of wheat plants outside cultivation if seeds were spread from the trial site (OGTR, 2017). Considering the similar requirements for durum wheat cultivation and survival (Hoisington et al., 1999; Hare, 2006; GRDC, 2016a), it is likely that the same factors would limit the survival of durum wheat seed spread outside the trial site.

Dispersal in extreme weather

131. 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 short distances by extreme winds, but it is not clear that this could move plant material outside the trial site. It is unlikely that either bread wheat or durum wheat seed would be spread by wind as they have non-shattering seed heads, seeds are heavy and they lack specific structures associated with wind transport. Dispersal by water is possible, but is unlikely as bread and durum wheat 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

132. If GM plants were able to establish outside the trial site they could potentially cause increased toxicity or allergenicity to humans or increased toxicity to other desirable organisms 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 bread wheat and durum wheat and their products, alone or in combination through hybridisation, would be any more toxic or allergenic than non-GM bread wheat or durum wheat.

133. Establishment of GM wheat 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.

134. Although both bread wheat and durum wheat 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). Bread wheat is listed as a naturalised, non-native species present in agricultural ecosystems in all Australian states and territories except the Northern Territory (NT), while durum wheat is not listed as naturalised or weedy in agricultural ecosystems (Groves et al., 2003). Large weedy populations of bread wheat and durum wheat 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 persist, disperse and establish outside an agricultural setting.

135. The introduced genes may provide the GM plants an increased ability to survive under high disease pressures and under those conditions their competitive ability may be increased. However, in order to increase weediness, these characteristics would need to be coupled with other mechanisms that increase 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, either in individual lines or in a hybrid background.

136. There is some suggestion that the introduced genes for rust disease or multi-pathogen resistance may have pleiotropic effects, as high levels of disease resistance gene expression may be associated with impaired plant growth (Grant et al., 2003) or have fitness costs (Tian et al., 2003). The applicant has stated that any plants that show such effects in the glasshouse would not be selected for testing in the field. In addition, if any such effects occurred in the field, they are likely to impair fitness of the GMOs rather than increasing the likelihood of spread and persistence in the environment.

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

138. Risk Scenarios 1 and 2 did not identify toxicity or allergenicity of any of the individual genes or combinations of the introduced genes in a hybrid background, 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

139. 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 bread wheat or durum wheat 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 genes conferring enhanced rust disease resistance and multi-pathogen resistance
Causal Pathway	⬇ Growing GM wheat plants at the field trial sites ⬇ Fertilisation of sexually compatible plants outside the trial site by pollen from GM wheat 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

Risk source

140. The source of potential harm for this postulated risk scenario is the introduced genes for rust disease resistance and multi-pathogen disease resistance in GM wheat lines.

Causal pathway

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

142. 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 bread wheat plants can be found in the bread wheat biology document. This information is also summarised in Chapter 1, Section 6.4, together with information about durum wheat.

143. 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.

144. Bread wheat is mainly self-pollinating and where pollen dispersal does occur, the main method is wind. Cross-pollination rates are influenced by the genotype of the variety and by environmental conditions, such as wind direction and humidity. Bread wheat outcrossing is generally less than 1 %, but rates of up to 6 % or higher have been observed. Wheat pollen is heavy and short-lived, with most pollen falling within the first few metres. Durum wheat is also regarded as being largely self-pollinating, although as with bread wheat, outcrossing rates can reach up to 5 %, again influenced by cultivar and environmental conditions (Bozzini, 1988). Field trials conducted in 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). Given the similarities between the two species, it is likely that outcrossing rates and pollen movement for GM durum wheat would be similar to that for GM bread wheat and the introduced genes for disease resistance are unlikely to increase the likelihood of wheat outcrossing.

145. Bread wheat and durum wheat are sexually compatible with one another and hybrids between the two have been observed in the field (Wang et al., 2005). Both species are sexually compatible with a number of other 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, for example hybrids with *H. marinum* have not been reported in Australia for bread wheat and *Aegilops* species (goatgrasses) are not considered to be naturalised in Australia.

146. There has been no concerted investigation of natural hybridisation of the native and introduced Triticeae with bread wheat or durum 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.

147. The proposed limits and controls for this trial would minimise the likelihood of pollen flow from the trial to related species outside the trial site. Under these conditions, no bread wheat, durum wheat or related species may be planted within at least 200 m of a planting area while GM wheat lines are being cultivated and any related species must be controlled within this distance during flowering. This would greatly reduce the potential for pollen flow from the trial to related species planted outside the trial sites, including cultivated wheat. In addition to this, the applicant proposes postharvest monitoring of the sites for any volunteer GM wheat to prevent production of plants that could hybridise with related species through pollen flow.

Potential Harm

148. If pollen from GM wheat 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.

149. 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 plants, or with sexually compatible species. Bread wheat and durum wheat are the only related species present in Australia with which the GM wheat lines can readily hybridise. 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.

150. As discussed in Risk scenario 1 and Risk scenario 2, the introduced gene products, or combinations of these, are not expected to be toxic to humans or other organisms. Properties of these genes and their products are not expected to differ in a hybrid background. Therefore, in the rare event of vertical transfer from the GM bread wheat or durum wheat lines to non-GM bread wheat or durum wheat 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.

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

152. Additionally, as mentioned, 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

153. Risk scenario 4 is not identified as a substantive risk due to the limited occurrence of long distance pollen flow for bread wheat and durum wheat and the very low likelihood of viable hybrids. 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**

154. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis[6].

155. 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.

156. 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.

[6] 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.

157. 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.

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

- 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

159. 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.

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

Section 4 **Risk evaluation**

161. 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.

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

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

163. 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
- 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

164. 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 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.[7]

[7] 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

165. 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.

166. 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.

167. 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.

168. 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

169. 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 bread wheat and durum wheat. 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

170. 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 Draft licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by CSIRO

171. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by CSIRO 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.

172. The applicant proposes two sites for the release of GM wheat lines lines. These are located on research stations in the ACT (Ginninderra) and in NSW (Boorowa). CSIRO owns the land on which the trials

are proposed and the land surrounding the trial sites. For each site, more than one planting area may be used. The field trial would run for five years, which includes up to five planting seasons. The maximum total area planted would be 40 m^2 per season – 20 m^2 per site per season.

173. The applicant has proposed that the sites will have lockable gates. They also propose that only authorised personnel would be permitted to deal with the GMOs. Standard licence conditions have been included in the draft licence that requires that only authorised people are permitted to undertake any activity authorised by the licence and that all people dealing with the GMOs must be informed of relevant licence conditions. These measures are considered appropriate to limit the potential exposure of humans to the GMOs (Risk Scenario 1).

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

175. As discussed in Risk Scenario 2, there is potential for gene stacking between DIR 162 lines within the trial, as well as the possibility of hybridisation with GMOs from DIR 151 planted at the site, or volunteers from DIR 111 at GES. Pollen transfer between GMOs grown under individual licences for DIR 111, DIR 151, or DIR 162 has been considered for each licence separately, as has the possibility of gene flow between lines from DIR 111, DIR 151 and DIR 162 (Risk Scenarios 1 and 2).

176. From that discussion, there is no expectation that combinations of genes from DIR 162 with those from either DIR 111 or DIR 151 would result in the production of novel proteins, or that their expression would be altered in a hybrid background (Risk scenario 1). Nor is there expectation that the hybrid GMOs would possess increased weedy characteristics (Risk Scenario 2). Nonetheless, if seed from DIR 162 trial was used to develop future GM bread wheat or durum wheat lines, there is a possibility that other genes could be unintentionally present. Therefore, a draft licence condition has been proposed for DIR 162 to require that from trials where such gene flow, and resulting hybrids, could have occurred, the licence holder must report the details of all lines grown concurrently from other licences to the Regulator. This would ensure that relevant genotypic information would be available for any seed produced from this trial, that may be used for future licence applications (Risk Scenarios 1 and 2). This would avoid the possibility that seed from unintended hybrids could be used to develop lines for future commercial release.

177. The applicant proposed the presence of a fence surrounding the field trial site to prevent livestock accessing the trial. The requirement to exclude livestock may be achieved in a number of ways, including (but not limited to) fencing the trial site. A standard licence condition has been included in the draft licence that prohibits the use of plant material from this trial for food or feed, thus livestock cannot be allowed to feed on the GM wheat (Risk Scenarios 1, 2 and 3). As discussed in the RARMP for DIR 152, this may be achieved in a number of ways, including, but not limited to, the presence of a livestock-proof fence. Therefore, the draft licence is not prescriptive in this regard.

178. The potential for seed dispersal by other animals has also been discussed in Risk Scenario 3 and, for the reasons discussed there, it is considered unnecessary to propose additional measures to control large animals or measures to control access of birds to the planting areas (Risk Scenario 3). In addition, there is no evidence that the GM bread wheat and GM durum wheat lines or hybrid GM wheat lines would be more toxic to birds than the non-GM parental wheat lines. Hence, there is no proposal to control access of birds to the GM wheat lines with respect to Risk Scenario 1 and Risk Scenario 2.

179. 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 baits.

180. Recent licences for GM wheat or for GM wheat and barley include a condition stating that rodent control measures must be used within the planting area while the GMOs are being grown and until the planting areas have been cleaned. The measures include, but are not limited to, trapping or baiting. In addition, these licences contain conditions requiring that the monitoring zone must be maintained in a manner that does not attract or harbour rodents while the GMOs are being grown and until the planting areas have been cleaned. Examples of this maintenance include keeping the monitoring zone either free of

vegetation or planted with vegetation mown to a height of less than 10 cm. These conditions are proposed in the draft licence to minimise risk associated with rodent activity and to facilitate detection of GM plant material that has been dispersed during dealings with the GMOs (Risk Scenario 3). This is discussed later in Chapter 3.

181. The applicant has proposed containment measures for the GM wheat that include a monitoring zone surrounding the trial site with a 2 m buffer zone, a 10 m monitoring zone and a 190 m isolation zone in which no bread wheat, durum wheat or sexually compatible plants will be grown. The proposed monitoring and isolation zones provide a combined separation distance of 200 m between the GMOs and any other wheat. The potential for outcrossing in wheat has been discussed in detail in the biology document for bread wheat and in a number of RARMPs. The most recent detailed discussion is in DIR 112 and DIR 102 (wheat and barley), with summaries in DIR 152 (wheat and barley) and in DIR 151 (wheat).

182. Based on the evidence available, including scientific literature on gene flow, international containment measures for GM wheat trials and guidelines for producing basic and certified seed, an isolation distance of 200 m between the GMOs and any other intentionally planted crops of bread wheat, durum wheat or related species is considered suitable. Conditions in earlier licences specify that the entire 200 m isolation zone must be inspected during flowering of the GMOs for the presence of volunteers and related species. This condition was reviewed for DIR 152, a limited and controlled release of GM wheat and barley.

183. As discussed in DIR 152, a total of 60 m was considered sufficient to manage gene flow to volunteers or sexually related species outside the planting area. This was achieved by the requirement for a monitoring zone of at least 10 m surrounded by an inspection zone of at least 50 m extending from the outer edge of the Monitoring Zone. These zones have conditions restricting planting of wheat and related species and requiring monitoring for and destruction of volunteers and related species. The 140 m extending from the outer edge of the inspection zone is the isolation zone in which no wheat (barley) or related species could be planted, but which does not require inspection during flowering of the GMOs. Thus an overall isolation distance of 200 m was achieved.

184. Many seed certification schemes do not distinguish between bread wheat and durum wheat - or indeed between different cereal crops - with respect to their requirements (California Crop Improvement Association, 2003; Seed Services Australia, 2013; OECD, 2016). The Canadian scheme does include conditions for individual cereal crop species with regard to the recent cropping history of land used to grow pedigreed seed crops, separation distances from other crops and weed control requirements. However, the requirements are the same for bread wheat and durum wheat (Canadian Seed Growers' Association, 2005). This information, when considered in addition to the scientific literature (Risk Scenario 4) and the similarities between bread wheat and durum wheat discussed in earlier sections, indicates the same isolation distance is suitable for both bread wheat and durum wheat.

185. Thus, the draft licence includes conditions requiring a 10 m monitoring zone and a 50 m inspection zone to manage any risk of gene flow from wheat as well as a 140 m isolation zone, thus providing an isolation distance of 200 m. The presence of a 2 m buffer zone surrounding the planting area[8] proposed by the applicant has not been included as, based on the available evidence, there were no plausible risks that would warrant the use of this control measure. Other measures were deemed adequate to manage potential risk associated with rodent activity or GMO dispersal and have been included as conditions in the draft licence.

186. The applicant has proposed the use of multiple planting areas at the trial sites. Under the conditions proposed in the draft licence, where more than one planting area is established at a field trial site, no planting area may be less than 10 m from the outer edge of the surrounding monitoring zone (See Figure 1,

[8] The applicant used the term 'location' to indicate the area in which GMOs are planted at a field trial site.

Chapter 4). Where multiple planting areas are established, any land between planting areas is included in the monitoring zone and would need to be maintained as such.

187. 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) and has been included as a licence condition. A condition has also been included in the draft licence requiring immediate notification of any extreme weather event affecting the properties during the release to allow assessment and management of any risks.

188. The applicant has proposed a number of measures to minimise the persistence of GM wheat 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 at least two years after harvest, including a 6 month volunteer-free period before signoff.

189. Germination rates differ between buried grain and grain lying on the surface; grains remaining near the surface, e.g. following shallow tillage after harvest, can generally easily germinate and become established (Ogg and Parker, 2000). Shallow tillage after harvest, combined with irrigation, will germinate much of the seed lying on the surface (Ogg and Parker, 2000). However, deep cultivation in certain soil types can reduce seed viability, but can also encourage prolonged dormancy in seeds as a result of a cool, moist low oxygen environment (Pickett, 1989; Ogg and Parker, 2000).

190. 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 immediately prior to the end of the monitoring period, would effectively manage survival and persistence of viable wheat seeds in the soil. Therefore, the draft licence includes conditions requiring that after harvest, the trial sites should receive at least three irrigations at intervals of at least 28 days, with the last required irrigation occurring at a time that would promote germination of volunteers within the final volunteer-free period. These measures would minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4).

191. The applicant proposes that rainfall events of greater than 20 mm in a 24 h period would be deemed to be equivalent to an irrigation event. A condition in the draft licence 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 would ensure that any remaining 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).

192. As noted earlier, a 2 m buffer zone surrounding each planting area is not a requirement under the draft licence. However, draft licence conditions do require inspection of any areas where GM material has been dispersed (including during planting, growing or harvest) and that volunteers and related species must be destroyed or prevented from flowering. A further condition in the draft licence requires that harvest of GM wheat be conducted separately from other crops. These conditions are proposed to manage any potential for spread and persistence of the GMOs due to mechanical dispersal of grain during sowing and harvesting (Risk Scenario 3). The applicant has stated that seed from the GMOs will be harvested either by hand or by plot harvester and that all wheat planted at the trial sites, including non-GM control varieties, would be treated as GM. These measures would minimise the spread of GM seed from the trial site during harvest.

193. No information has been provided regarding the handling of seed following harvest, although the applicant proposes that seed may be used for experimental analysis in PC2 laboratories, under appropriate NLRDs and may be used to plant further trials. Draft licence conditions specify that if seed harvested from the GMOs is threshed other than in accordance with Notifiable Low Risk Dealings (NLRD) requirements, it

must be threshed separately from any other crop, and threshing must take place on a planting area or in a facility approved in writing by the Regulator.

194. The applicant has proposed that any GM plant material would be transported to approved facilities for analysis or destruction 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 which have been included in the draft licence 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 (Risk Scenario 3 and 4).

195. The applicant has proposed that all equipment; including machinery, storage and transport equipment, tools, footwear and clothing; would be inspected for GM seeds and cleaned before removal from the site or use for any other purpose. Equipment would be cleaned at the planting area, then moved to a concrete inspection/cleaning pad located at the entrance to each site, inspected and re-cleaned if required. Any seeds would be collected from the concrete pad and returned to the planting area or transported to a PC2 facility for destruction using an approved method. They have proposed that areas used to clean equipment, that are not on the planting area or the inspection pad, would be monitored bimonthly for at least 12 months. If volunteers were identified in the final six months of monitoring, the length of monitoring would be extended such that no volunteers were identified in the last six months of monitoring. Conditions in the draft licence require that any area where GMOs have dispersed as a result of dealings under the licence, including any area used to clean equipment, must be cleaned as soon as practical and inspected at least every 35 days from the date of cleaning for a minimum of 24 months prior to sign-off. Any volunteers detected must be destroyed before flowering. These measures will minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4).

196. The applicant has proposed that GM plants and other wheat within each planting area would be disposed of using one or more of the following methods: uprooting, tilling, treatment with herbicide, burning/incineration, autoclaving, crushing, milling, burial of seed or other plant material under 1m of soil in an area immediately adjacent to the trial site, or a method approved in writing by the Regulator. Autoclaving, crushing, milling and deep burial are considered effective methods for seed destruction as they render seed non-viable, therefore minimising the risk of germination and/or spread.

197. The applicant has proposed that areas used to bury seed would be monitored bimonthly for at least 12 months. If volunteers were identified in the final six months of monitoring, the length of monitoring would be extended such that no volunteers were identified in the last six months of monitoring. However, as burial is defined as a means of destroying the GMOs, the burial site is not considered an area that requires cleaning under the conditions of the draft licence. Thus under draft licence conditions there is no specific requirement to inspect the burial site. However, draft conditions do require that if any disturbance of the burial site is observed by the licence holder, the Regulator must be notified and remedial action must be taken. Likewise, if any volunteers are observed at the burial site, this area would require cleaning as an area in which the GMOs have been dispersed in the course of dealings under the licence, and post-cleaning conditions would apply (see paragraph 195, above).

198. 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 Scenarios 1 and 2).

199. After harvest the land will be left fallow or planted with a break crop if approved in writing by the Regulator. The location may be re-sown with GM wheat in the following season.

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

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

- limit the duration of the release to a maximum of five planting seasons, until September 2023

- limit the release to a maximum of two locations, in the ACT (Ginninderrra - GES) and NSW (Boorowa – BARS)
- limit the release to a maximum total area of 40 m^2 per season across both sites
- 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 bread wheat or durum wheat 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 bread wheat, durum wheat 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 separately from other crops
- harvest the GM wheat 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
- apply measures to promote the germination of any wheat 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 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

201. 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

202. 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.

203. 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.

204. 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

205. If a licence were issued, CSIRO 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.

206. CSIRO 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

207. 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, CSIRO 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

208. 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.

209. 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

210. 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.

211. 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.

212. 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**

213. Additional information has been identified that may be required to assess an application for a commercial release of these GM bread wheat and GM durum wheat lines, or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM bread wheat and durum wheat lines, particularly with respect to potential for increased toxicity and allergenicity
- additional phenotypic characterisation of the GM bread wheat and durum wheat lines, particularly with respect to traits that may contribute to weediness

Section 5 **Conclusions of the consultation RARMP**

214. The RARMP concludes that the proposed limited and controlled release of GM bread wheat and GM durum wheat 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.

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

Chapter 4 Proposed licence conditions

Section 1 **Interpretations and Definitions**

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

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

'Clean' (or **'Cleaned'**) means, as the case requires:

a. in relation to an area specified in this licence as requiring Cleaning, the Destruction of the GMOs in that area, to the reasonable satisfaction of the Regulator; or
b. in relation to Equipment, the removal and/or Destruction of the GMOs, to the reasonable satisfaction of the Regulator.

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

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

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

a. uprooting;
b. tilling, but only subject to the conditions of this licence;
c. treatment with herbicide;
d. burning/incineration;
e. autoclaving;
f. milling;

g. crushing;

h. burial, but only subject to the conditions of this licence; or

i. a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of plants with mature seed heads still attached, Tilling would not be appropriate due to the possible introduction of large numbers of viable seeds into the seedbank.

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

'Facility' a facility approved in writing by the Regulator.

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

'GM' means genetically modified.

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

'Inspection Zone' means an area of land extending 50 m in all directions from the outer edge of the Monitoring Zone as indicated in Figure 1.

'Isolation Zone' means an area of land extending 140 metres in all directions from the outer edge of the Inspection Zone as indicated in Figure 1.

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

'Monitoring Zone' means an area of land extending outwards at least 10 m from the outer edge of the Planting Area, as indicated in Figure 1.

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

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

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

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

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

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

'Regulator' means the Gene Technology Regulator.

'Related Species' means plants from the genus *Triticum*, excluding bread wheat and durum wheat.

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

'Site' means the area of land within which one or more Planting Areas and associated Monitoring Zone may be established.

'Tillage' (or **'Tilled'** or **'Tilling'**) means the use of any technique to disturb the soil.

'Volunteers' means GM or non-GM Wheat plants which have not been intentionally grown.

'Waterways' means all permanent natural waterways and man-made waterways that flow into natural waterways.

Wheat means plants of *Triticum aestivum* L. em Thell (bread wheat) or *Triticum turgidum* subsp. *durum* (Desf.) Husn (durum wheat).

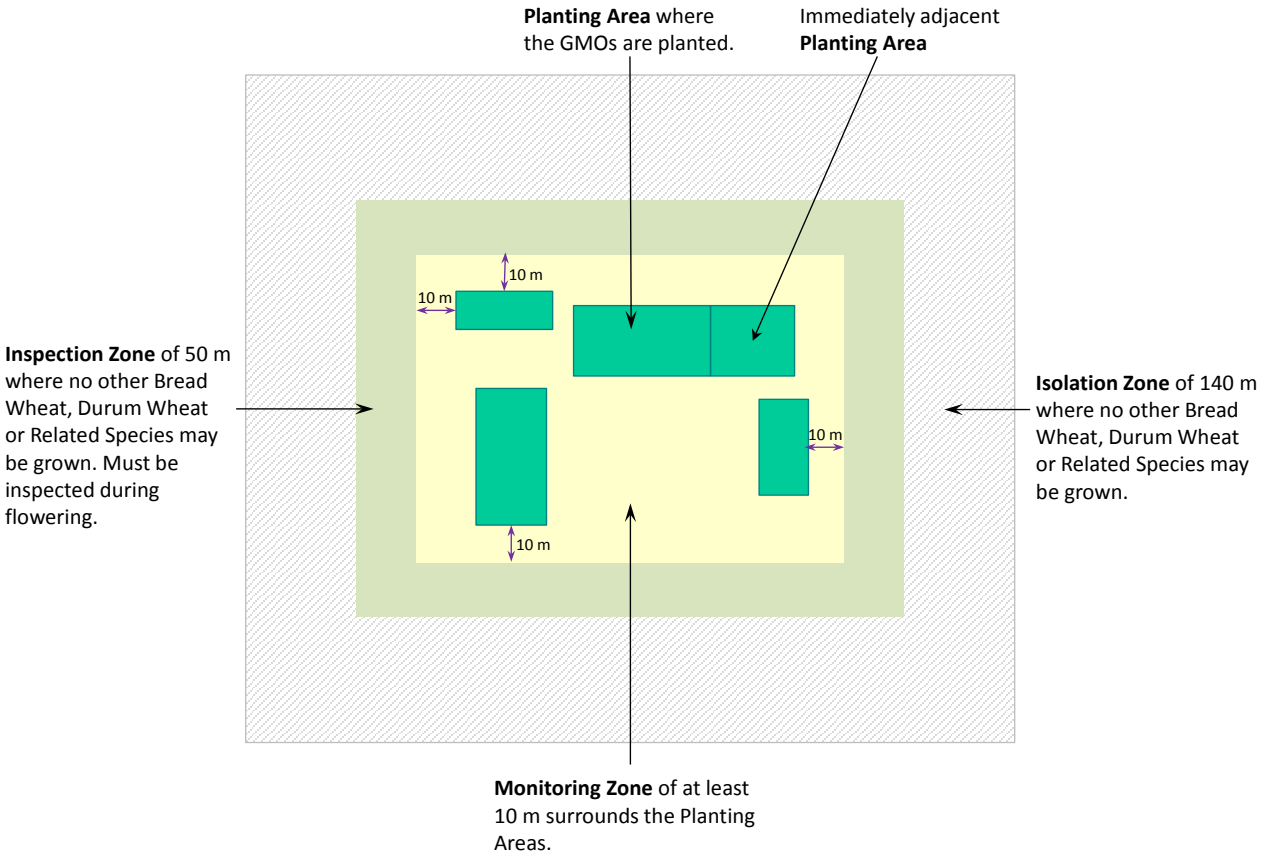

Figure 1 Diagram (not to scale) showing the relationship between Planting Area, Monitoring Zone, Inspection Zone and Isolation Zone.

Section 2 General conditions and obligations

3. This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

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

5. The holder of this licence ('the licence holder') is CSIRO.

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

7. The dealings authorised by this licence are to conduct experiments with the GMOs, breed, propagate, grow, transport and dispose of the GMOs, and possession, supply or use of the GMOs in the course of any of these dealings.

Obligations of the Licence Holder

8. The licence holder must notify the Regulator in writing as soon as practically possible if any of the contact details of the project supervisor change from that notified in the licence application or subsequently.

Note: please address correspondence to ogtr.applications@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following conditions address ongoing suitability of the licence holder.

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

10. The licence holder must:

    a. inform the Regulator immediately in writing, of:

        i. any relevant conviction of the licence holder occurring after the commencement of this licence; and

        ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; and

        iii. any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it; and

    b. provide any information related to the licence holder's ongoing suitability to hold a licence, if requested, within the stipulated timeframe.

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

The following conditions seek to ensure that persons conducting the dealings are aware of the licence conditions and appropriate processes are in place to inform people of their obligations.

12. Prior to conducting any dealings with the GMOs, the licence holder must provide to the Regulator:

    a. names of all organisations and persons or functions or positions of the persons who will be covered by the licence, with a description of their responsibilities; and

        Note: Examples of functions or positions are 'project supervisor', site manager', 'farm labourer' etc.

    b. detail of how the persons covered by the licence will be informed of licence conditions; and

    c. detail of how the licence holder will access and control the Planting Area, Monitoring, Inspection Zones, Isolation Zones and approved Facilities, for the duration of the licence; and

        Note: this may include a description of any contracts, agreements, or other enforceable arrangements.

    d. written methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism, and to distinguish between categories of GMOs approved for release; and

    e. a Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected.

13. Any changes to the information provided under the immediately preceding condition must be communicated in writing to the Regulator within 14 days of the changes occurring.

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

    a. the particular condition (including any variations of it); and

    b. the cancellation or suspension of the licence; and

    c. the surrender of the licence.

15. The licence holder must not permit a person covered by this licence to conduct any dealing unless:

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

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

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

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

16. The licence holder must:

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

    b. provide the Regulator, if requested, with copies of the signed and dated statements referred to in the immediately preceding condition.

Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment is communicated to the Regulator.

17. The licence holder must inform the Regulator if the licence holder becomes aware of:

    a. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    b. any contraventions of the licence by a person covered by the licence; or

    c. any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

a. *the licence holder will be taken to have become aware of additional information of a kind mentioned in paragraph 17.a if he or she was reckless as to whether such information existed; and*

b. *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in paragraph 17.b and 17.c, if he or she was reckless as to whether such contraventions had occurred, or such unintended* effects existed.

Note: Contraventions of the licence may occur through the action or inaction of a person. For example if it is a condition of the licence that volunteers are destroyed prior to flowering and a volunteer flowers, then the person responsible for controlling volunteers will have contravened that licence condition.

18. If the licence holder is required to inform the Regulator under the immediately preceding condition, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of the incident via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location if required.

19. If the licence holder informs the Regulator under the immediately preceding condition and the Regulator requests further information, such information must be provided in a manner, and within the time period, stipulated by the Regulator.

Obligations of persons covered by the licence

20. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

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

Section 3 **Limits and Control Measures**

Limits on the release

The following licence conditions maintain the risk assessment context within which the application was assessed, by imposing limits on where and when the GMOs may be grown, and on other activities that can be undertaken.

22. The only plants that may be intentionally grown at a Planting Area are:

    a. the GMOs covered by this licence as described in Attachment A of the licence;

    b. non-GM Wheat plants; and

    c. plants approved in writing by the Regulator.

Note: Attachment A is not included in the draft licence as the plants are described in the Risk Assessment and Risk Management Plan.

23. Planting and growing of the GMOs may only occur within the following limits:

    a. up to two Sites per season for the release of GM Wheat lines, as listed in the table below:

Location	Maximum size of total Planting Areas per growing season	Duration
Ginninderra Experiment Station (ACT)	40 m^2	August 2018 – September 2023 inclusive
Boorowa Experiment Station (NSW)		August 2018 – September 2023 inclusive

    b. for each Site, more than one planting area may be established each season

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

25. If GM plants, other than the GMOs authorised by this licence , are:

    a. grown under another licence within the Site at a time when the GMOs authorised by this licence are also being grown; and

    b. sexually compatible with the GMOs authorised by this licence;

then information about all lines grown concurrently must be reported to the Regulator in accordance with condition 61.b.iii.

Note: This information may also be required to be provided for related future licence applications.

26. If experimentation, analysis or storage of the GMOs is not conducted in accordance with Notifiable Low Risk Dealings (NLRD) requirements, then such activities may only be undertaken within:

    a. a Planting Area; or

    b. a Facility approved in writing by the Regulator.

Note: Dealings conducted in accordance with NLRD requirements must be assessed by an IBC before commencement, must comply with the requirements of the Gene Technology Regulations 2001, and are not subject to the conditions of this licence.

Containment measures

The following licence conditions maintain the risk assessment context within which the application was assessed by restricting spread and persistence of the GMOs.

27. The outer edge of the Planting Area must be at least 50 m away from Waterways.

3.1.1 Cultivation of the GMOs

28. The outer edge of the Planting Area must be surrounded by a Monitoring Zone of at least 10 m. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and/or destruction of Volunteers and Related Species whilst the GMO is growing in the Planting Area until the Planting Area is Cleaned.

Note: Measures to achieve this could include areas of land free of any vegetation and/or vegetation kept mown to a height of less than 10 cm.

29. Non-GM Wheat plants grown in a Planting Area must be handled as if they were the GMOs.

30. Multiple Planting Areas may be contained within a single Monitoring Zone. No Planting Area may be less than 10 m from the outer edge of the Monitoring Zone (as indicated in Figure 1).

31. The only plants which may be grown in the Monitoring Zone are GM plants authorised under this licence, under another licence issued by the Regulator, or plants approved in writing by the Regulator.

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

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

34. The GMOs must not be grown in a Planting Area if any crop of Wheat or a Related Species is present within the Inspection Zone or Isolation Zone.

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

Area	Period of inspection	Inspection frequency	Inspect for	Action
a. Monitoring Zone	**From** 14 days prior to the expected commencement of Flowering of any GMOs* **until** 28 days after all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Volunteers & Related Species	Destroy before Flowering or prevent from Flowering
b. Inspection Zone	**From** 14 days prior to the expected commencement of Flowering of any GMOs* **until** 28 days after all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Volunteers & Related Species	Destroy before Flowering or prevent from Flowering

**Condition 61.a.iv requires the licence holder to provide information to the Regulator on the expected flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to flowering of any GMOs.*
Note: Details of any inspection activity must be recorded in a Logbook as detailed in Condition 61.f.

Dispersal of GMOs

36. Measures must be implemented to control rodents within the Planting Area while GMOs are being grown and until the Planting Area(s) have been Cleaned.

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

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

Note: Measures to achieve this could include areas of land free of any vegetation and/or vegetation kept mown to a height of less than 10 centimetres.

38. The GMOs must be harvested separately from any other crop.

39. Harvesting must be conducted in a manner that avoids dispersal of GMOs outside the Planting Area.

40. If the GMOs are Destroyed, they are taken to have been harvested for the purposes of this licence.

41. If seed harvested from the GMOs is threshed other than in accordance with Notifiable Low Risk Dealings (NLRD) requirements, it must be threshed separately from any other crop, and threshing must take place on the Planting Areas or in a Facility approved in writing by the Regulator.

42. Any extreme weather event that is expected to affect or has already affected a Planting Area or associated areas, while the GMOs are growing or while the Planting Area is subject to inspection requirements, must be notified in writing to the Regulator as soon as practically and reasonably possible.

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

Processing or experimentation with GMOs

43. If processing of GM seed or experimentation or analysis with the GMOs is not conducted under a Notifiable Low Risk Dealings (NLRD) authorisation, such activities may only be undertaken within:

a. a Planting Area; or

b. a Facility approved in writing by the Regulator.

Note: Dealings conducted under a NLRD authorisation must be assessed by an Institutional Biosafety Committee before commencement, must comply with the requirements of the Regulations, and are not subject to the conditions of this licence.

44. Within a Facility approved under the preceding conditions, any area that is used for threshing, processing, experimentation or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

45. GMOs not required to conduct experiments or for planting under this licence must be Destroyed as soon as practicable.

Dispersal of the GMOs during transport or storage

46. If GMOs are stored prior to experimentation, they must be stored in a Facility within an unbreakable container labelled as containing GMOs or Destroyed as soon as practicable after use.

47. If transport or storage of the GMOs is not conducted in accordance with NLRD requirements, such activities must:

a. only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation; and

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

c. comply with all other conditions of this licence.

Note: Dealings conducted in accordance with NLRD requirements must be assessed by an IBC before commencement, must comply with the requirements of the Gene Technology Regulations 2001, and are not subject to the conditions of this licence.

Note: Condition 15 requires signed statements for persons transporting or disposing of the GMOs.

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

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

Cleaning

49. The Planting Area must be Cleaned before the end of the first May following harvesting of the GMOs.

50. If all the GMOs have been Destroyed in the Planting Area, then the area is taken to have been Cleaned for the purposes of this licence and all post-Cleaning conditions will apply.

51. While post-Cleaning inspection requirements apply to the Planting Area:

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

b. any Tillage of the area must be to a depth no greater than the depth of sowing of the GMOs; and

Note: delaying the Tillage for at least 28 days following the harvest of the GMOs may promote after-ripening of grain remaining on the soil surface and thereby reduce persistence of seed in the soil, however if conditions are conducive to germination Tillage may be carried out earlier.

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

i. the GMOs or non-GM Wheat planted in accordance with the conditions of this licence; or

ii. agreed to in writing by the Regulator.

d. prior to an application for Sign-off, the area must receive at least three irrigations, at intervals of at least 28 days, with the last required irrigation occurring at a time that would promote the germination of Volunteers within the volunteer-free period immediately prior to the Sign-off application; and

Note: A period of natural rainfall (as recorded in condition 58.f) may be taken as irrigation only with the agreement of the Regulator. Evidence (such as rainfall measurements, photos of germinating plants etc.) that the rainfall has been sufficient to promote germination should be provided.

e. prior to the final irrigation referred to in the immediately preceding condition, the area must be Tilled.

52. Any area outside the Planting Area where the GMOs have been dispersed in the course of dealings under this licence, must be Cleaned as soon as practicable and before use for any other purpose.

Notes: This would include, but is not limited to approved Facilities, areas used to Clean Equipment.

Notes: Areas of land that have been Cleaned are subject to inspections (Condition 57). Cleaning activities must be recorded and provided to the Regulator (Condition 61.d).

53. For a Facility, once Cleaning has been completed, the licence holder must send a notification to the Regulator that the Facility has been Cleaned.

54. Cleaning of Equipment must occur as soon as practicable after use and before use for any other purpose.

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

Areas/Equipment to be Cleaned	When
a. Planting Area	Before the end of the first May following harvesting of the GMOs
b. any area where GMOs have dispersed during planting, growing or harvesting c. any Equipment used in connection with the GMOs d. any area used to Clean any Equipment used in connection with the GMOs e. any area used to experiment with, analyse or store GMOs	As soon as practicable and before use for any other purpose

Notes: If Tillage is used as a means of Cleaning, it must be conducted in accordance with Condition 51.b. Areas of land that have been Cleaned, or from which the GMOs have been harvested, are also subject to Inspections (Condition 57). Cleaning activities must be recorded and provided to the Regulator (Condition 61.d).

Conditions relating to Destruction by burial

56. If Destruction of Plant Material occurs by burial, the licence holder must:

    a. bury Plant Material in a pit into the ground at Ginninderra Experiment Station or Boorowa Agricultural Research Station under this licence ; and

    b. Plant Material must be buried in such a way that it is covered by a layer of soil at least 1 metre in depth, the top of which is no higher than the soil surface surrounding the burial site; and

    c. within 14 days of burial, provide the Regulator a written notice indicating the precise location of the burial site (GPS coordinates and either a street address or other directions), the date on which burial occurred and broad description of the Plant Material buried (Planting Area and year the GMOs were planted); and

    d. if disturbance of the burial site is identified, take appropriate remedial action and notify the Regulator of the disturbance and the remedial action taken; and

    e. if Volunteers are identified, Destroy before Flowering.

Persistence of the GMOs post-Cleaning

57. Post-Cleaning areas of land must be inspected by people trained to recognise Bread Wheat or Durum Wheat. Inspections must cover the entirety of the areas to be inspected. Actions must be taken as follows:

Area of land	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area or other areas that have been Cleaned	From the day of completion of harvest of the last Wheat plant in the Planting Area, until: i. the area is replanted with the GMOs; or ii. the Regulator has issued a Sign-off for the area.	At least once every 35 days	Volunteers	Destroy before Flowering

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

    a. date of the inspections;

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

    c. details of the experience, training or qualification that enables the person(s) to recognise Volunteers, if not already recorded in the logbook;

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

    Note: this may also include spraying or maintenance measures used to facilitate inspections for Volunteers

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

    f. details of any post-harvest rainfall events including measurements at or near the area, or any irrigation events;

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

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

    i. details of rodent control methods used and any evidence of rodent activity.

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

Note: Details of Inspection activities must be provided to the Regulator (Condition 61).The Regulator has developed a standardised proforma for recording inspection activities. This can be made available on request.

Contingency plan

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

Section 4 **Sign off**

60. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and other areas requiring Cleaning if:

a. all post-Cleaning inspection activities have been conducted for at least 24 months on the area

b. conditions have been conducive for germination and detection; and

c. no Volunteers have been detected on this area for at least six months of the inspection period immediately prior to the Sign off request.

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

Section 5 **Reporting and Documentation**

The following licence conditions are imposed to demonstrate compliance with other conditions, facilitate monitoring of compliance by staff of the OGTR, and emphasise appropriate selection of the Planting Area.

61. Notifications must be sent to the Regulator as follows:

Notice	Content of notice	Timeframe
a. Intention to Plant	i. Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the trial sites (e.g. Google Maps) and any other descriptions. If the Planting Area is on an area from DIR 151 that is not signed off, you must state this in the notification ii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details) iii. Date on which the GMOs will be planted iv. Period when the GMOs are expected to Flower v. Period when harvesting is expected to commence vi. How all areas requiring post-Cleaning inspections are intended to be used until sign-off, including the proposed post-harvest crop(s) (if any) vii. Details of how you propose to manage inspection activities, including strategies for the detection and destruction of volunteer GMOs viii. History of how the site has been used for the previous two years	At least 7 days prior to each planting (to be updated immediately if the notified details change)
b. Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition iii. Details of all sexually compatible lines being grown	Within 7 days of any planting

Notice	Content of notice	Timeframe
	concurrently under any other licence.	
c. Harvest	i. Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting
d. Cleaning	i. Actual date(s) on which any areas needing Cleaning were Cleaned ii. Method of Cleaning	Within 7 days of completion of any Cleaning
e. Burial	i. Actual date(s) and precise location of Burial ii. Broad description of the GMOs buried (Condition 56.c) iii. Record of any disturbance to the Burial Site and remedial actions taken iv. Record of any Volunteers observed at the Burial site and details of Destruction	Within 14 days of any burial As soon as practicable Within 7 days of completion of any Cleaning
f. Inspection activities	i. Information recorded in a Logbook as per the inspection requirements (Conditions 35, 57 and 58).	Within 35 days of Inspection

References

ABARES (2017). Australian crop report. Report No. 184. (Canberra: Australian Bureau of Agricultural and Resource Economics and Sciences).

ABS (2013). 7124.0 - Historical selected agriculture commodities, by state (1861 to present), 2010-2011. (Australian Bureau of Statistics).

AGRI-FACTS (2002). Mice and their control. Report No. Agdex 683. (Alberta Agriculture, Food and Rural Development).

Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. Critical Reviews in Toxicology *36*, 219-251.

Beauchemin, K.A., McAllister, T.A., Dong, Y., Farr, B.I., and Cheng, K.J. (1994). Effects of mastication on digestion of whole cereal grains by cattle. Journal of Animal Science *72*, 236-246.

Bell, L.W., Wade, L.J., and Ewing, M.A. (2010). Perennial wheat: a review of environmental and agronomic prospects for development in Australia. Crop and Pasture Science *61*, 679-690.

Benavente, E., Alix, K., Dusautoir, J.-C., Orellana, J., and David, J.L. (2001). Early evolution of the chromosomal structure of *Triticum turgidum–Aegilops ovata* amphiploids carrying and lacking the Ph1 gene. Theoretical and Applied Genetics *103*, 1123-1128.

Blakeney, A.B., Cracknell, R.L., Crosbie, G.B., Jefferies, S.P., Miskelly, D.M., O'Brien, L., Panozzo, J.F.*, et al.* (2009). Understanding Australian wheat quality. A basic introduction to Australian wheat quality. (Grains Research and Development Corporation).

Bozzini, A. (1988). Origin, distribution, and production of durum wheat in the world. In Durum: Chemistry and technology, G. Fabriani, and C. Lintas, eds. (Minnesota, USA: American Association of Cereal Chemists), pp. 1-16.

Breyer, D., Kopertekh, L., and Reheul, D. (2014). Alternatives to antibiotic resistance marker genes for *in vitro* selection of genetically modified plants – Scientific developments, current use, operational access and biosafety considerations. Critical Reviews in Plant Sciences *33*, 286-330.

California Crop Improvement Association (2003). Seed certification standards in California - Grain. (University of California, Davis).

Canadian Seed Growers' Association (2005). Canadian Regulations and Procedures for Pedigreed Seed Crop Production - Circular 6. (Canadian Seed Growers' Association, Ottawa, Canada).

Caughley, J., Bomford, M., Parker, B., Sinclair, R., Griffiths, J., and Kelly, D. (1998). Managing vertebrate pests: rodents (Canberra: Bureau of Rural Sciences; Grains Research and Development Corporation).

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

CFIA (2006). The biology of *Triticum turgidum* ssp. (subspecies) *durum* (Durum Wheat). (Canadian Food Inspection Agency).

Cherkaoui, S., Lamsaouri, O., Chlyah, A., and Chlyah, H. (2000). Durum wheat × maize crosses for haploid wheat production: Influence of parental genotypes and various experimental factors. Plant Breeding *119*, 31-36.

Clark, R.G., and Gentle, G.C. (1990). Estimates of grain passage time in captive mallards. Canadian Journal of Zoology *68*, 2275-2279.

Cuddy, W., Park, R.F., Bariana, H., Bansal, U., Singh, D., Roake, J., and Platz, G. (2016). Expected responses of Australian wheat, triticale and barley varieties to the cereal rust diseases and genotypic data for oat varieties. (Sydney: The University of Sydney, Plant Breeding Institute).

David, J.L., Benavente, E., Brès-Patry, C., Dusautoir, J.-C., and Echaide, M. (2004). Are neopolyploids a likely route for a transgene walk to the wild? The *Aegilops ovata* x *Triticum turgidum durum* case. Biological Journal of the Linnean Society *82*, 503-510.

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

Dorofeev, V.F. (1969). Spontaneous hybridization in wheat populations of Transcaucasia. Euphytica *18*, 406-416.

Eastham, K., and Sweet, J. (2002). Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. Report No. 28. (Copenhagen, Denmark: European Environment Agency).

Ellis, J.G., Lagudah, E.S., Spielmeyer, W., and Dodds, P.N. (2014). The past, present and future of breeding rust resistant wheat. Frontiers in Plant Science *5*, Article 641.

Feldman, M. (1976). Wheats. *Triticum* spp. (Graminae - Triticinae). In Evolution of Crop Plants, N.W. Simmonds, ed. (New York: Longman Group Limited), pp. 120-128.

Feldman, M. (2001). Origin of cultivated wheat. In The World Wheat Book A History of Wheat Breeding, A.P. Bonjean, and W.J. Angus, eds. (London: Intercept Ltd), pp. 3-56.

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

Garcia-Llamas, C., Ramirez, M.C., and Ballesteros, J. (2004). Effect of pollinator on haploid production in durum wheat crossed with maize and pearl millet. Plant Breeding *123*, 201-203.

Gatford, K.T., Basri, Z., Edlington, J., Lloyd, J., Qureshi, J.A., Brettell, R., and Fincher, G.B. (2006). Gene flow from transgenic wheat and barley under field conditions. Euphytica *151*, 383-391.

Grant, J.J., Chini, A., Basu, D., and Loake, G.J. (2003). Targeted activation tagging of the *Arabidopsis* NBS-LRR gene, ADR1, conveys resistance to virulent pathogens. Molecular Plant-Microbe Interactions *16*, 669-680.

GRDC (2016a). Durum Northern. (Grains Research and Development Corporation).

GRDC (2016b). Wheat Northern Region. (Grains Research and Development Corporation).

GRDC (2016c). Wheat Southern Region. (Grains Research and Development Corporation).

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

Hare, R. (2006). Agronomy of the durum wheats Kamilaroi, Yallaroi, Wallaroi and EGA Bellaroi (New South Wales Department of Primary Industries).

Herrera-Foessel, S.A., Singh, R.P., Lillemo, M., Huerta-Espino, J., Bhavani, S., Singh, S., Lan, C*., et al.* (2014). *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. Theoretical and Applied Genetics, 781-789.

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

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.-M., Skovmand, B., Taba, S., and Warburton, M. (1999). Plant genetic resources: What can they contribute toward increased crop productivity? Proceedings of the National Academy of Sciences *96*, 5937-5943.

Howe, H.F., and Smallwood, J. (1982). Ecology of seed dispersal. Annual Review of Ecology and Systematics *13*, 201-228.

Inagaki, M.N., and Hash, C.T. (1998). Production of haploids in bread wheat, durum wheat and hexaploid triticale crossed with pearl millet. Plant Breeding *117*, 485-487.

Jacot, Y., Ammann, K., Rufener, P., Mazyad, A., Chueca, C., David, J., Gressel, J.*, et al.* (2004). Hybridization between wheat and wild relatives, a European Union Research Programme. In Introgression from genetically modified plants into wild relatives, H.C.M. den Nijs, D. Bartsch, and J. Sweet, eds. (UK: CAB International), pp. 63-73.

Jauhar, P.P., and Peterson, T.S. (2006). Cytological analyses of hybrids and derivatives of hybrids between durum wheat and *Thinopyrum bessarabicum*, using multicolour fluorescent GISH. Plant Breeding *125*, 19-26.

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

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

Khan, R.R., Bariana, H.S., Dholakia, B.B., Naik, S.V., Lagu, M.D., Rathjen, A.J., Bhavani, S.*, et al.* (2005). Molecular mapping of stem and leaf rust resistance in wheat. Theoretical and Applied Genetics *111*, 846-850.

Kimber, G.K., and Sears, E.R. (1987). Evolution of the genus *Triticeae* and origin of cultivated wheat. In Wheat and wheat improvement, E.G. Heyne, ed. (Madison, Wisconsin, USA: American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.), pp. 154-164.

Klindworth, D.L., Williams, N.D., and McIntosh, R.A. (2003). Interspecific hybridization of a multiploid mutant of durum wheat with rye and *Triticum monococcum* L. results in pentaploid hybrids. Plant Breeding *122*, 213-216.

Kneipp, J. (2008). Durum wheat production. (Orange, NSW: NSW Department of Primary Industries).

Krattinger, S.G., Lagudah, E.S., Spielmeyer, W., Singh, R.P., Huerta-Espino, J., McFadden, H., Bossolini, E.*, et al.* (2009). A Putative ABC Transporter Confers Durable Resistance to Multiple Fungal Pathogens in Wheat. Science *323*, 1360-1363.

Krattinger, S.G., Lagudah, E.S., Wicker, T., Risk, J.M., Ashton, A.R., Selter, L.L., Matsumoto, T.*, et al.* (2011). Lr34 multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. The Plant Journal *65*, 392-403.

Leighty, C.E., and Sando, W.J. (1928). Natural and artificial hybrids of a Chinese wheat and rye. Journal of Heredity *19*, 23-27.

Li, W., and Gill, B.S. (2006). Multiple genetic pathways for seed shattering in the grasses. Functional and Integrative Genomics *6*, 300-309.

Loureiro, I., Escorial, M.C., Gonzalez-Andujar, J.L., Garcia-Baudin, J.M., and Chueca, M.C. (2007). Wheat pollen dispersal under semiarid field conditions: potential outcrossing with *Triticum aestivum* and *Triticum turgidum*. Euphytica *156*, 25-37.

Mago, R., Zhang, P., Vautrin, S., Simkova, H., Bansal, U., Luo, M.C., Rouse, M.*, et al.* (2015). The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus. Nature Plants *1*, 15186.

Malo, J.E., and Suárez, F. (1995). Herbivorous mammals as seed dispersers in a Mediterranean dehesa. Oecologia *104*, 246-255.

Matsuoka, Y. (2011). Evolution of polyploid *Triticum* wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant and Cell Physiology *52*, 750-764.

Matus-Cádiz, M.A., Hucl, P., Horak, M.J., and Blomquist, L.K. (2004). Gene flow in wheat at the field scale. Crop Science *44*, 718-727.

McCaig, T.N., and DePauw, R.M. (1992). Breeding for preharvest sprouting tolerance in white-seed-coat spring wheat. Crop Science, 19-23.

McDonald, B.A., and Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology *40*, 349-379.

Meister, G.K. (1921). Natural hybridization of wheat and rye in Russia. Journal of Heredity *12*, 467-470.

Mondal, S., Rutkoski, J.E., Velu, G., Singh, P.K., Crespo-Herrera, L.A., Guzman, C., Bhavani, S*., et al.* (2016). Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. Frontiers in Plant Science *7*, Article 991.

Moore, J.W., Herrera-Foessel, S., Lan, C., Schnippenkoetter, W., Ayliffe, M., Huerta-Espino, J., Lillemo, M*., et al.* (2015). A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. Nature Genetics *47*, 1494-1498.

Murray, G.M., and Brennan, J.P. (2009). The current and potential costs from diseases of wheat in Australia. (Canberra: Grains Research and Development Corporation).

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

OECD (2016). OECD seed schemes 2016: OECD seed schemes for the varietal certification or the control of seed moving in international trade. (Paris: The Organisation for Economic Co-operation and Development).

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

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

OGTR (2017). The biology of *Triticum aestivum* L. (Bread Wheat) Version 3.1. (Office of the Gene Technology Regulator).

Periyannan, S., Moore, J., Ayliffe, M., Bansal, U., Wang, X., Huang, L., Deal, K*., et al.* (2013). The gene *Sr33*, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. Science *341*, 786-788.

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

QDAF (2012a). Durum wheat in Queensland. (State of Queensland (Queensland Department of Agriculture and Fisheries)) Accessed: 16/02/2018. Available online at: QDAF 2012 durum wheat.

QDAF (2012b). Fusarium head blight. (State of Queensland (Queensland Department of Agriculture and Fisheries)) Accessed: 30/01/2018. Available online at: QDAF 2012 Fusarium head blight.

Ranieri, R. (2015). Geography of durum wheat. In Pastaria International (Italy).

Rieben, S., Kalinina, O., Schmid, B., and Zeller, S.L. (2011). Gene flow in genetically modified wheat. PLoS ONE *6*, 1-12.

Rinaldo, A., Gilbert, B., Boni, R., Krattinger, S.G., Singh, D., Park, R.F., Lagudah, E*., et al.* (2017). The *Lr34* adult plant rust resistance gene provides seedling resistance in durum wheat without senescence. Plant Biotechnology Journal *15*, 894-905.

Ryves, T.B. (1988). Supplementary list of wool-alien grasses recorded from Blackmoor, North Hants., 1959-1976. Watsonia *17*, 73-79.

Saintenac, C., Zhang, W., Salcedo, A., Rouse, M.N., Trick, H.N., Akhunov, E., and Dubcovsky, J. (2013). Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science *341*, 783-786.

SARDI (2017). 2018 Sowing guide South Australia. (Adelaide: South Australian Research and Development Institute).

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C*., et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Research *24*, 1-17.

Seed Services Australia (2013). Seed Certification Manual. (Adelaide: Primary Industries South Australia).

Simeone, R., Pignoni, D., Blanco, A., and Attolico, M. (1989). Cytology and fertility of hybrids and amphiploids between *Aegilops caudata* L. ×*Triticum turgidum* (L.) Thell. Plant Breeding *103*, 189-195.

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S*., et al.* (2011). The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annual Review of Phytopatholy *49*, 465-481.

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S.A*., et al.* (2008). Will stem rust destroy the world's wheat crop? Advances in Agronomy *98*, 271-309.

Singh, R.P., Hodson, D.P., Jin, Y., Lagudah, E.S., Ayliffe, M.A., Bhavani, S., Rouse, M.N*., et al.* (2015). Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. Phytopathology *105*, 872-884.

Spielmeyer, W., Mago, R., Wellings, C., and Ayliffe, M. (2013). *Lr67* and *Lr34* rust resistance genes have much in common - they confer broad spectrum resistance to multiple pathogens in wheat. BMC Plant Biology *13*.

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N*., et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiology *161*, 1587-1594.

Steuernagel, B., Periyannan, S.K., Hernandez-Pinzon, I., Witek, K., Rouse, M.N., Yu, G., Hatta, A*., et al.* (2016). Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nature Biotechnology *34*, 652-655.

Taylor, R.D., and Koo, W.W. (2015). 2015 Outlook of US & world wheat industires 2015 - 2024. (Center for Agricultural Policy and Trade Studies. North Dakota State University).

Temby, I., and Marshall, D. (2003). Reducing cockatoo damage to crops. Landcare Notes. Report No. LC0009. (State of Victoria, Department of Sustainability and Environment).

Thompson, C.J., Movva, N.R., Tizard, R., Crameri, R., Davies, J., Lauwereys, M., and Botterman, J. (1987). Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO Journal *6*, 2519-2523.

Tian, D., Traw, M.B., Chen, J.Q., Kreitman, M., and Bergelson, J. (2003). Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. Nature *423*, 74.

Tsegaye, S. (1996). Estimation of outcrossing rate in landraces of tetraploid wheat (*Triticum turgidum* L.). Plant Breeding *115*, 195-197.

Vanegas, C.D.G., Garvin, D.F., and Kolmer, J.A. (2007). Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by Lr34. Euphytica *159*, 391-401.

Virtue, J.G. (2004). SA weed risk management guide. (Adelaide, South Australia: Department of Water, Land and Biodiversity Conservation).

Wang, H.Y., Liu, D.C., Yan, Z.H., Wei, Y.M., and Zheng, Y.L. (2005). Cytological characteristics of F2 hybrids between *Triticum aestivum* L. and *T. durum* Desf. with reference to wheat breeding. Journal of Applied Genetics *46*, 365-369.

Woodgate, J.L., Steadman, K.J., and Buchanan, K.L. (2011). A study of seed viability following consumption by birds. (Unpublished final report submitted to the OGTR.).

WQA (2017). 2017/18 WQA Wheat variety master list. (Wheat Quality Australia) Accessed: 2/14/2018. Available online at: WQA Masterlist.

Yasar, S. (2003). Performance of broiler chickens on commercial diets mixed with whole or ground wheat of different varieties. International Journal of Poultry Science *2*, 62-70.

Zalacain, M., Gonzalez, A., Guerrero, M.C., Mattaliano, R.J., Malpartida, F., and Jimenez, A. (1986). Nucleotide sequence of the hygromycin B phosphotransferase gene from *Streptomyces hygroscopicus*. Nucleic Acids Research *14*, 1565-1581.

Zhang, L., Yan, Z., Dai, S., Chen, Q., Yuan, Z., Zheng, Y., and Liu, D. (2008). The crossability of *Triticum turgidum* with *Aegilops tauschii*. Cereal Research Communications *36*, 417-427.