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Office of the Gene Technology Regulator

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Risk Assessment and Risk Management Plan for

DIR 151 - Limited and controlled release of
wheat genetically modified for disease
resistance, drought tolerance, altered oil
content and altered grain composition

Applicant - CSIRO

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 151

Decision

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

The application

Application number	DIR 151
Applicant	CSIRO
Project title	Limited and controlled release of wheat genetically modified for disease resistance, drought tolerance, altered oil content and altered grain composition
Parent organism	Wheat (<i>Triticum aestivum</i> L.)
Introduced genes and modified traits	Five groups of introduced genes are proposed: <ul style="list-style-type: none"> • <u>Group A</u>: nine genes (or gene fragments) involved in resistance to rust disease • <u>Group B</u>: thirteen genes involved in drought adaptation • <u>Group C</u>: three genes (or gene fragments) involved in altered starch metabolism • <u>Group D</u>: four genes involved in increased oil content • <u>Group E</u>: eight genes involved in altered grain dietary fibre content In addition, four genes are used as selectable markers across all groups
Proposed location	Ginninderra Experiment Station (ACT) and Boorowa Experiment Station, Shire of Boorowa (NSW)
Proposed release size	Up to 1 hectare (ha) per site per year
Proposed release dates	May 2017 – May 2022
Primary purpose	To evaluate the agronomic performance of all GM wheat lines under field conditions. For Group C and Group E, to generate flour for laboratory evaluation of food performance. For Group E, possibly to conduct animal and/or human feeding studies to assess nutritional value.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible.

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 animals to the GM plant material, increased potential for spread and persistence of the GMOs, and transfer of the introduced genetic material to sexually compatible plants. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to other desirable organisms, 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 apart from possible carefully controlled small scale animal and/or human nutritional trials, the proposed limits and controls effectively contain the GMOs and their genetic material and minimise exposure; and the GM wheat has limited ability to establish populations outside cultivation or transfer the introduced genetic material to other plants.

Risk management plan

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

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

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
<i>Avr</i>	Avirulence
bp	Base pair
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
<i>hptII</i>	Hygromycin phosphotransferase II gene
HR	Hypersensitive response
km	Kilometres
LGA	Local Government Area
m	Metres
NB-LRR	nucleotide binding site-leucine rich repeat
NLRD	Notifiable Low Risk Dealing
<i>nptII</i>	Neomycin phosphotransferase II gene
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
<i>R</i> gene	Gene conferring resistance to a particular pathogen
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
the Act	The <i>Gene Technology Act 2000</i>

Chapter 1 Risk assessment context

Section 1 Background

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

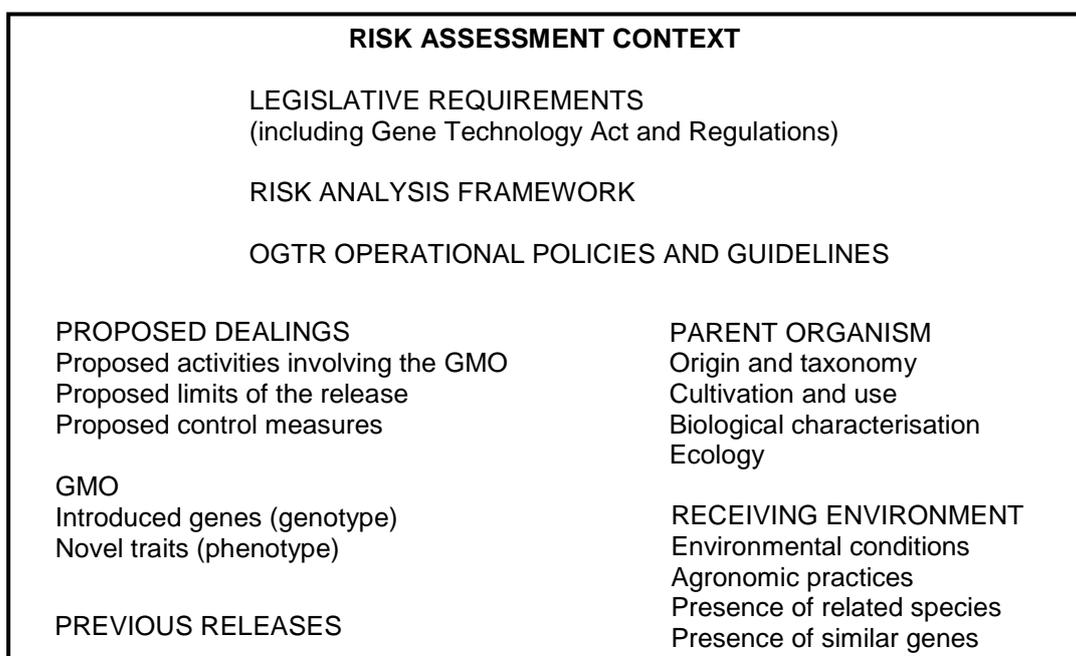


Figure 1. Summary of parameters used to establish the risk assessment context

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. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. One public submission was received and its consideration is summarised in Appendix B.

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. CSIRO proposes to release up to 1174 genetically modified wheat lines into the environment under limited and controlled conditions. The wheat lines have been genetically modified for

- resistance to leaf rust, stripe rust and stem rust (90 lines)
- tolerance to abiotic stresses (64 lines)
- altered starch metabolism (190 lines)
- increased oil content (30 lines)
- altered grain dietary fibre content (800 lines).

10. The purpose of the trial is to evaluate the agronomic performances of the GM wheat under Australian field conditions. For wheat lines with genetically modified grain composition, another purpose of this trial is to analyse changes in nutritional characteristics, dough making properties and end product quality. Flour derived from the grain of GM wheat lines with altered grain composition is proposed to be used for a range of carefully controlled, small scale animal and human nutritional trials under the oversight of CSIRO Human Nutrition Animal Ethics Committee and CSIRO Human Nutrition Research Ethics Committee, respectively. The GM wheat lines would not be permitted to enter the commercial human food or animal feed supply chains.

11. 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.

These dealings are detailed further below.

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

12. The release is proposed to take place on two sites at the Ginninderra Experiment Station, ACT and Boorowa Experiment Station, NSW on a maximum area of 1 ha per site per season over a five year period from May 2017 to May 2022.
13. Only trained and authorised staff would be permitted to deal with the GM wheat.

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

14. The applicant has proposed a number of controls to restrict the spread and persistence of the GM wheat and 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 sites with a 2 m buffer zone, a 10 m monitoring zone and a 190 m isolation zone in which no other wheat crop may be grown and where growth of related species is controlled
 - only permitting trained and authorised staff to access the trial sites
 - 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 the same as GM plants
 - inspecting all equipment for plant material, which will be destroyed prior to leaving the sites
 - transporting and storing GM plant material in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
 - destroying all plant material from the trial not required for testing or future trials
 - post-harvest monitoring of the trial sites at least once every 35 days for at least 2 years, with any wheat volunteers destroyed
 - promoting germination of any residual seed post-harvest by tillage and irrigation
 - not allowing the GM plant materials or products to be used in commercial human food or animal feed.

Section 4 The parent organism

15. The parent organism is bread wheat (*Triticum aestivum* L.) which is exotic to Australia. Commercial wheat cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, Tasmania, southern South Australia and southern Western Australia.
16. The cultivars used to generate the GM wheat lines are bread wheat cultivars Bobwhite, Chinese Spring, Chinese Spring Hope 3B, Fielder, Mace and the durum wheat cultivar Stewart. With the exception of Mace, these cultivars are not generally commercially grown in Australia. Bobwhite, Fielder and Stewart are commonly used to produce GM lines as these cultivars have been shown to be efficiently transformed using *Agrobacterium*-based transformation (Richardson et al. 2014). The cultivar Mace, offering resistance to wheat leaf and stem rust, is commercially cultivated in Australia.
17. Detailed information about the parent organism is contained in the reference document *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016) which was produced to inform the risk assessment process for licence applications involving GM wheat. Baseline information from this document will be used and referred to throughout the RARMP. Of particular interest for this RARMP are the characteristics of the parent plant which relate to spread and persistence and therefore to potential weediness. These are discussed in detail in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016) and references therein. The information included below summarises key points.
18. Wheat is not regarded as a weed of national significance ([National Weeds List](#)) and is described as a naturalised non-native species present in all Australian States and Territories with the exception of the Northern Territory (Groves et al. 2003). The Weed Risk Assessment included in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016) concludes that wheat possesses few attributes which would make it weedy. This supports the observation that there are very few weedy populations of

wheat in the Australian environment. Abiotic factors, such as water stress (drought or waterlogging), heat and cold stress, nutrient deficiencies as well as biotic stresses, limit the growth and survival of wheat outside of agricultural ecosystems.

19. Wheat is largely self-pollinating and pollen production is low by comparison with other cereals. Wheat pollen is considered to be heavy and short-lived and it is estimated that about 90% of pollen falls within 3 m of the plant. Wheat heads are described as non-shattering and seeds do not have high levels of dormancy. Any dormancy is generally easily broken due to climatic conditions in Australia. Wheat seeds are generally considered large and heavy, thus not easily transported by wind or water. They also lack physical characteristics to enable attachment to fur, feathers or clothing, although they may be transported in wool.

20. Wheat grains may be consumed by a number of animals, from livestock to rabbits, rodents and birds. Although whole seeds may survive digestion, there is limited information about the viability of seeds after consumption by livestock. Few or no viable seeds have been recorded in rabbit dung. Wheat seed germination rates after consumption by birds are either low (0.8 % - 2 % for seeds consumed by galahs or corellas) or there is no data on germination after consumption, for example, by emus.

21. Bread wheat cultivated in Australia is exclusively of white wheat varieties, which have low dormancy and a thin seed coat and are therefore expected to be easily broken down in the digestive system of animals.

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

5.1 Introduction to the GMOs

22. The applicant proposes the release of up to 1174 GM wheat lines into the environment under limited and controlled conditions. The GMOs are classified in five groups, designated Group A to Group E, on the basis of their genetic modifications and the respective desired traits (Table 1). Group A and Group C include lines that contain introduced gene silencing constructs (see below).

Table 1. Summary of the five groups of genetically modified wheat lines proposed in DIR 151

Group	Modified trait	Genes	Lines
A	Resistance to rust disease	9	Up to 90
B	Drought adaptation	13	Up to 64
C	Altered starch metabolism	3	Up to 190
D	Increased oil content	4	Up to 30
E	Altered grain dietary fibre content	8	Up to 800

23. GM wheat lines from Group A, Group B and Group D were all generated using *Agrobacterium tumefaciens*-mediated transformation. GM wheat lines from Group C and Group E were generated using either *Agrobacterium tumefaciens*-mediated transformation or biolistic transformation. Information about these transformation methods can be found in the document *Methods of plant genetic modification* available from the [Risk Assessment References](#) page on the OGTR website.

24. All GM wheat lines for Group B, Group D and Group E are overexpressing lines. Groups A and C comprise both overexpressing lines (i.e. enhanced expression of full length genes) and silencing lines. The silencing lines express introduced gene silencing constructs containing fragments of a putative rust resistance gene (Group A) or one of three alpha amylase genes (Group C). The function of the

silencing constructs is to suppress the expression of the corresponding (target) genes through RNA interference (RNAi) (see Section 5.2).

25. Candidate genes in Group C and Group E have been introduced as single genes. Candidate genes in Group A, Group B and Group D have been introduced either as single genes or in combinations of up to three genes.

26. The introduced genes were derived from wheat (21), maize (3), *Aegilops tauschii* (2), barley (2), *Brachypodium distachyon* (2), *Arabidopsis thaliana* (1), oats (1), rice (1), sesame (1), sorghum (1), *Triticum turgidum* (1) and from the common soil fungus *Umbelopsis ramanniana* (1).

27. The GM wheat lines also contain one of the four selectable marker genes: *bar*, *pat*, *hptII*, and *nptII*. *Bar* and *pat*, derived from the bacteria *Streptomyces hygrosopicus* and *S. viridochromogenes* respectively, encode the phosphinothricin acetyltransferase (PAT) enzyme which provide resistance to the herbicide glufosinate. *HptII* and *nptII*, derived from the bacteria *Escherichia coli*, encode the hygromycin phosphotransferase and neomycin phosphotransferase type II enzymes respectively, conferring antibiotic resistance. These selectable markers were used in the laboratory to select transformed GM plants during early stages of development.

28. Short regulatory sequences that control expression of the genes are also present in the GM wheat lines. The regulatory sequences are derived from plants (*Aegilops tauschii*, barley, *Brachypodium distachyon*, castor bean, *Flaveria trinervia*, maize, potato, rice and wheat) and microorganisms (*Agrobacterium tumefaciens*, *Cauliflower mosaic virus*, and *Cestrum yellow leaf clearing virus*) (Table 2).

Table 2. Regulatory genetic elements introduced into the GM wheat lines

Genetic element	Description	Source
Promoters (antibiotic resistance markers)		
<i>35S</i>	Promoter used for resistance markers	<i>Cauliflower mosaic virus</i>
<i>e-35S</i>	Promoter used for resistance markers	<i>Cauliflower mosaic virus</i>
<i>pCmYLCV</i>	Promoter used for resistance markers	<i>Cestrum yellow leaf clearing virus</i>
Terminators		
<i>Ocs 3'</i>	3' non translated region of the octopine synthase	<i>Agrobacterium tumefaciens</i>
<i>RbcS 3'</i>	3' non translated region of the Rubisco small subunit gene	<i>Triticum aestivum</i>
<i>Nos 3'</i>	3' non translated region of the nopaline synthase gene	<i>Agrobacterium tumefaciens</i>
<i>CaMVpolyA</i>	Terminator	<i>Cauliflower mosaic virus</i>
Introns		
<i>STLS1</i>	Intron inserted in resistance marker sequence	<i>Solanum tuberosum</i>
<i>Intron 1 cat</i>	Intron used in RNAi construct	<i>Ricinus communis</i>
<i>Intron 3 pdk</i>	Intron used in RNAi construct	<i>Flaveria trinervia</i>
<i>Rint 4</i>	Intron used in RNAi construct	<i>Oryza sativa</i>
<i>Rint 9</i>	Intron used in RNAi construct	<i>Oryza sativa</i>

29. The applicant has provided brief descriptions for each of the candidate genes. The applicant intends to gather more information on the effects of the introduced genes under this limited and controlled trial.

5.2 The introduced genes, encoded proteins and associated effects

30. The introduced genes and their encoded proteins are described to illustrate their potential function in the GM wheat lines. They have been grouped according to the trait associated with the introduced genes: resistance to rust disease, adaptation to drought, altered starch metabolism, increased oil content and altered grain dietary fibre content.

31. In addition to over-expression lines, Groups A and C contain introduced gene silencing constructs designed to suppress or reduce expression of the corresponding target gene(s). Suppression of the target genes is mediated by using a natural regulatory mechanism in plants known

as ribonucleic acid interference (RNAi) or gene silencing (Baykal & Zhang 2010). Using the RNAi pathway, an introduced silencing construct is transcribed into double-stranded RNA, which is processed by endogenous cellular machinery into short interfering RNAs (siRNAs). The siRNAs direct the degradation of messenger RNA (mRNA) molecules with matching sequence after the mRNAs are transcribed from genes and before they are translated into proteins. The efficiency of gene silencing is generally determined by the extent of homology between the silencing construct and the target gene (usually > 95% homology is required) and the length of the homologous region. In plants, introduced silencing constructs have been shown to effectively suppress expression of the target genes, but can also give rise to silencing of non-target genes with closely matching sequences.

5.2.1 Group A: resistance to rust disease

32. The three wheat diseases stem rust (or black rust, caused by *Puccinia graminis* f. sp. *tritici*), leaf rust (or brown rust, caused by *P. triticina*) and stripe rust (or yellow rust, caused by *P. striiformis* f. sp. *tritici*) are a major constraint in wheat growing regions, causing important losses to grain production (Ellis et al. 2014; Mondal et al. 2016).

33. 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 respectively (Ellis et al. 2014).

34. R genes are associated with a hypersensitive reaction in the host, resulting in incompatible host-pathogen interactions, based on a gene-for-gene system. The vast majority of R genes cloned belong to the nucleotide binding leucine rich repeat (NB-LRR) class. 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. This often occurs within a few years (Ellis et al. 2014; Herrera-Foessel et al. 2014).

35. 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. Therefore, it is sometimes 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). Functions of cloned APR genes are diverse, ranging from protein kinases to transporters and transmembrane proteins (Ellis et al. 2014).

36. The GM wheat lines included in Group A contain rust resistance genes, expressed singly or as a combination of up to three genes (see Table 3a and 3b for details). Four of the genes included in this group, *Lr34*, *Lr46*, *Lr67* and *Sr2*, are described as foundation APR genes, increasing the impact of other resistance genes, including NB-LRR class genes (Ellis et al. 2014).

Table 3a. Genes of interest introduced in Group A GM wheat lines

Gene	Full name and description	Source	Intended function	Type of expression
<i>Lr67*</i>	Sugar transporter gene variant	<i>Triticum aestivum</i>	Multi-pathogen resistance	Overexpression
<i>Lr46</i>	Slow anion channel like gene	<i>Triticum aestivum</i>	Multi pathogen resistance	Overexpression
<i>Lr34</i>	ABC transporter gene variant	<i>Triticum aestivum</i>	Multi pathogen resistance	Overexpression
<i>Yr36</i>	Kinase-lipid binding protein	<i>Triticum turgidum</i> <i>ssp dicoccoides</i>	Stripe rust resistance	Overexpression
<i>Lr21</i>	Nucleotide binding leucine rich repeat	<i>Aegilops tauschii</i>	Leaf rust resistance	Overexpression
<i>Sr46</i>	Nucleotide binding leucine rich repeat	<i>Aegilops tauschii</i>	Stem rust resistance	Overexpression
<i>Sr2-PMP3</i>	Putative transmembrane protein	<i>Triticum aestivum</i>	Potential stem rust resistance	Overexpression and silencing
<i>Sr2-D8LAL2</i>	Putative transmembrane protein	<i>Triticum aestivum</i>	Potential stem rust resistance	Silencing

Gene	Full name and description	Source	Intended function	Type of expression
<i>Sr2-GLP1_2</i>	Putative transmembrane protein	<i>Triticum aestivum</i>	Potential stem rust resistance	Silencing

* Note that this gene is used in two constructs, in two different cultivars

Table 3b. Promoters used in Group A GM wheat lines

Genetic element	Full name and description	Source	Used in GM wheat lines
<i>pLr67</i>	Native promoter from the Lr67 gene	<i>Triticum aestivum</i>	Lr67 Yr36 - Lr67
<i>pLr46</i>	Native promoter from the Lr46 gene	<i>Triticum aestivum</i>	Lr46
<i>pLr34</i>	Native promoter from the Lr34 gene	<i>Triticum aestivum</i>	Lr34
<i>pYr36</i>	Native promoter from the Yr36 gene	<i>Triticum turgidum</i> ssp <i>dicoccoides</i>	Yr36 - Lr21 Yr36 - Lr67
<i>pLr21</i>	Native promoter from the Lr21 gene	<i>Aegilops tauschii</i>	Yr36 - Lr21
<i>pSr46</i>	Native promoter from the Sr46 gene	<i>Aegilops tauschii</i>	Sr46
<i>pUbi1</i>	Promoter from ubiquitin 1 gene	<i>Zea mays</i>	Sr2-PMP3 (overexpression) Sr2-PMP3 - Sr2-D8LAL2 - Sr2- GLP1_2 (RNAi)

37. GM wheat lines included in Group A are either overexpressing or silencing lines, using either native or constitutive promoters (Table 3b). Expression of APR genes *Lr34* and *Lr67* has been demonstrated to confer resistance to leaf, stem and stripe rusts (Krattinger et al. 2009; Moore et al. 2015; Spielmeyer et al. 2013). GM wheat lines overexpressing resistance genes, singly or as a combination, are expected to display increased resistance to stem rust, leaf rust and/or stripe rust. Group A silencing lines contain partial gene fragments of *Sr2* genes for stem rust resistance; GM wheat lines in which the candidate genes have been silenced are expected to show decreased resistance to stem rust, leaf rust and/or stripe rust.

38. The introduced genes are all derived from wheat, *Triticum turgidum* or *Aegilops tauschii*, and have been used for wheat germplasm improvement and introgression into commercial wheat varieties since the early 20th century (Ellis et al. 2014; Krattinger et al. 2009). A common phenotype observed for wheat cultivars expressing *Lr34* or *Lr67* resistance genes is for flag leaves to develop a necrotic leaf tip. GM wheat lines expressing these genes are expected to develop the same leaf tip necrosis phenotype. There is no expectation of unintended changes in the phenotype of the GM wheat lines expressing these genes.

5.2.2 Group B: drought adaptation

39. Soil water availability is one of the major abiotic stresses influencing wheat productivity. Cellular dehydration linked to drought leads to a series of biochemical and physiological changes. These changes result from up- or down-regulation of a large number of genes involved in abiotic stress responses and abiotic stress tolerance (Xiao & Xue 2001; Yamaguchi-Shinozaki & Shinozaki 2006).

40. Drought adaptation involves drought and heat tolerance and is, like tolerance to all abiotic stresses, a complex mechanism involving several biochemical pathways. Cross-talks between different stress signals and common signal transduction pathways have been described (Yamaguchi-Shinozaki & Shinozaki 2006 and references therein). Transcription factors¹ have been shown to be involved from the perception of stress signal to the expression of stress-related genes.

41. Adaptation to drought not only involves the regulation of abiotic stress-response genes: drought tolerance in wheat has also been linked to the regulation of enzymes involved in carbon fixation and

¹ Transcription factors are regulatory proteins that can up- or down-regulate transcription of target genes, by recognizing and binding to specific gene promoter sequences. Transcription regulation is a common form of gene expression regulation. It allows for fine-tuned expression of genes during development and in response to environmental conditions.

accumulation of water soluble carbohydrates, such as glucose, sucrose or fructose. Such accumulation is widely regarded as an adaptive response to drought stress (Xue et al. 2008). Stem carbon reserves are important sources for grain filling: variation in stem carbon reserves among genotypes has been shown to influence wheat yield and grain weight under water-limiting conditions (Xue et al. 2008). Drought tolerance in wheat and other cereals has also been linked to root architecture modification: increased root biomass, altered root angle and root depth have been shown to improve crop yield under water-limiting conditions (Chen et al. 2016; Meister et al. 2014).

42. The GM wheat lines included in Group B contain genes involved in tolerance to drought and heat, either directly, by regulating the expression of abiotic stress-response genes or indirectly, by regulating the accumulation of stem carbon reserves or modifying root architecture (see Table 4a and 4b for details). Twelve of the thirteen genes of interest are transcription factors. The last gene of interest, TaCAT1, encodes a calcium-binding protein, for which expression has been associated with stem carbon reserve levels in wheat (applicant's unpublished data).

Table 4a. Genes of interest introduced in Group B GM wheat lines

Gene	Full name and description	Source	Intended function	Type of expression
<i>TaCAT1</i>	Calcium binding protein	<i>Triticum aestivum</i>	Accumulation of stem carbon reserves	Overexpression
<i>TaNf-YA7</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaNAC69*</i>	Transcription factor	<i>Triticum aestivum</i>	Regulation of drought stress response genes, modification of root architecture	
<i>HvCBF1*</i>	Transcription factor	<i>Hordeum vulgare</i>		
<i>TaZFP34</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaHsfC2a</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaHfsA6f</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaRNAC1</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaNAC2</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaHsfC2d</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaHsfC1e</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaMYB20</i>	Transcription factor	<i>Triticum aestivum</i>		
<i>TaWRKY17²</i>	Transcription factor	<i>Triticum aestivum</i>		

*Note that these genes are used in two constructs with different promoters

² This gene has been referred to by the applicant as WRKY2 in DIR 100 and WRKY23 in the current application. However, sequence information provided by the applicant shows that this gene is more closely related to WRKY17 gene. Therefore, this gene will be referred to as WRKY17 in this document.

Table 4b. Promoters used in Group B GM wheat lines

Genetic element	Full name and description	Source	Used in GM wheat lines
<i>pRSP3</i>	Root specific promoter from RSP3 gene	<i>Oryza sativa</i>	CBF1 ZFP34 NAC69
<i>pUbi1</i>	Promoter from ubiquitin 1 gene	<i>Zea mays</i>	CAT1 NF-YA7 CAT1+NF-YA7
<i>pDhn8s</i>	Constitutive promoter with strong expression in roots and leaves, from Dhn8s gene	<i>Hordeum vulgare</i>	HsfC2a
<i>pPR1L2</i>	Root specific promoter, from PR1L2 gene	<i>Oryza sativa</i>	HsfA6f RNAC1
<i>pPIP2;3</i>	Root specific promoter, from PIP2;3 gene	<i>Oryza sativa</i>	NAC69 CBF1 NAC2 HsfC2d HsfC1e
<i>pGRP7</i>	Root specific promoter, from GRP7 gene	<i>Oryza sativa</i>	MYB20 WRKY17

43. All GM wheat lines included in Group B are overexpressing lines, using either root specific or constitutive promoters (Table 4b). Overexpression in root tissues of transcription factors involved in abiotic stress tolerance, such as *TaNAC69*, has been shown to increase root elongation and biomass, described as an adaptive response to drought (Chen et al. 2016). GM wheat lines overexpressing transcription factors are expected to display enhanced tolerance to drought and/or heat stress.

44. The introduced genes are all derived from wheat or barley and most likely function by regulating the expression of endogenous genes. The molecular action of most of these transcription factors is unknown.

45. Expression level of some genes and/or metabolic pathways may change as a result of the activity of the introduced genes. There is no expectation of unintended changes in the phenotype of the GM wheat lines expressing these genes.

5.2.3 Group C: altered starch metabolism

46. Starch is an insoluble polymer, classified in two categories: transitory starch and reserve starch. Transitory starch accumulates in leaves during the day and is hydrolysed at night. Reserve starch is stored in non-photosynthetic, storage organs such as tubers or seeds. In cereals such as wheat, starch accumulates during seed development and is metabolised during germination to ensure early seedling growth (Whan et al. 2014).

47. Alpha-amylases are the main enzymes involved in wheat starch degradation during germination. Three isoforms of alpha-amylases, AMY1, AMY2 and AMY3, have been described in wheat. AMY1 and AMY2 have been extensively characterised and their expression has been linked to two quality defects, pre-harvest sprouting and late maturity alpha-amylase (Mares & Mrva 2014). Pre-harvest sprouting (PHS) is defined as the germination of grain in the ear prior to harvest, generally in response to rain, while late maturity alpha-amylase (LMA) refers to the synthesis of AMY1 during the middle stages of grain development, in the absence of sprouting or rain (Mares & Mrva 2014). The enzyme is retained in the grain through to harvest (Barrero et al. 2013; Mares & Mrva 2014). Both PHS and LMA result in lower prices for growers: high levels of alpha-amylase in harvested grain has been considered to reduce flour quality and to decrease baking properties (Ral et al. 2016).

48. Studies by Whan *et al* (2014) and Ral *et al* (2016) showed that overexpressing AMY3 in the endosperm during grain development did not significantly impact grain morphology, weight or starch content. Moreover, flour from grain overexpressing AMY3 showed a marked increase in baking

quality, despite a Falling Number³ value corresponding to a severely sprouted grain, unfit for milling or baking (Ral et al. 2016).

49. The GM wheat lines included in Group C contain the alpha-amylase genes AMY1, AMY2 and AMY3 (see Table 5a and 5b for details). The introduced genes are all derived from wheat and are under the control of constitutive or grain-specific promoters, targeting the grain endosperm and the aleurone layer, respectively (Table 5b). The candidate genes will be expressed singly.

Table 5a. Genes of interest introduced in Group C GM wheat lines

Gene	Full name and description	Source	Intended function	Type of expression
AMY1	Alpha-amylase 1	<i>Triticum aestivum</i>	Altered starch metabolism	Overexpression and silencing
AMY2	Alpha-amylase 2	<i>Triticum aestivum</i>		
AMY3	Alpha-amylase 3	<i>Triticum aestivum</i>		

Table 5b. Promoters used in Group C GM wheat lines

Genetic element	Full name and description	Source	Used in GM wheat lines
<i>pBx17</i>	Grain endosperm specific promoter from glutenin gene Bx17	<i>Triticum aestivum</i>	Overexpression and silencing of AMY1, AMY2 and AMY3
<i>pLPT2</i>	Aleurone specific promoter from LPT2 gene	<i>Triticum aestivum</i>	
<i>pUbi1</i>	Promoter from ubiquitin 1 gene	<i>Zea mays</i>	

50. GM wheat lines included in Group C are either overexpressing or silencing lines. Overexpressing lines are expected to display high alpha-amylase activity and altered starch metabolism in the target tissues and/or organs. GM wheat lines in which candidate genes have been silenced are expected to display low alpha-amylase activity and altered starch metabolism.

51. There is no expectation of unintended changes in the phenotype of the GM wheat lines expressing these genes, as none of the candidate genes are known to impact other metabolic pathways.

5.2.4 Group D: increased oil content

52. Yield of oil crops needs to improve to meet with increasing worldwide demand, linked to food, fuel and industrial requirements. Due to the increased pressure on arable land, new, engineered crops accumulating lipids in vegetative tissues have been proposed as a mean for meeting global production needs (Chapman et al. 2013; Vanhercke et al. 2014).

53. Strategies to increase lipid production pathways in vegetative tissues have focused on three key steps: increasing fatty acid biosynthesis, increasing triacylglycerol assembly and/or decreasing triacylglycerol breakdown (Vanhercke et al. 2013). These three steps are referred to as “push”, “pull” and “protect”, respectively. Co-expression of three genes involved in each of these push, pull, protect steps in tobacco resulted in a 75-fold increase in leaf triacylglycerol levels compared to wild type (Vanhercke et al. 2014).

54. The three genes expressed in tobacco by Vanhercke et al. (2014) are the transcription factor Wrinkled 1 (WRI1), the enzyme diacylglycerol acyltransferase 1 (DGAT1), both derived from *Arabidopsis thaliana* and the oleosin oil-body protein from *Sesamum indicum*. WRI1 regulates glycolysis and fatty acid synthesis, the “push” step (Chapman et al. 2013). DGAT1 is responsible for the “pull” step, catalysing the last step of triacylglycerol biosynthesis, by adding an acyl group to diacylglycerol molecules (Lardizabal et al. 2008). Oleosins coat and stabilise cytoplasmic oil droplets in oilseeds, the “protect” step (Vanhercke et al. 2014).

55. The GM wheat lines included in Group D contain the three genes included in the study by Vanhercke et al. (2014), as well as the enzyme DGAT2a (see Table 6a and 6b for details). DGAT1 and

³ Falling Number test gives an indication of alpha-amylase and protease activity in wheat grain. A low Falling Number value indicates significant alpha-amylase activity, which is assumed to be linked to the presence of sprouted grain (Ral et al. 2016).

DGAT2a are from two different gene families but both encode proteins with diacylglycerol acyltransferase activity (Lardizabal et al. 2008). The introduced genes are derived from *Arabidopsis thaliana*, corn, sesame and from the common soil fungus *Umbelopsis ramanniana*.

Table 6a. Genes of interest introduced in Group D GM wheat lines

Gene	Full name and description	Source	Intended function	Type of expression
<i>ZmWRI1</i>	Transcription factor (Wrinkled 1)	<i>Zea mays</i>	Enhanced oil accumulation	Overexpression
<i>UrDGAT2a</i>	Diacylglycerol acyltransferase	<i>Umbelopsis ramanniana</i>		
<i>AtDGAT1</i>	Diacylglycerol acyltransferase 1	<i>Arabidopsis thaliana</i>		
<i>SinOLEOSIN</i>	Oleosin	<i>Sesamum indicum</i>		

Table 6b. Promoters used in Group D GM wheat lines

Genetic element	Full name and description	Source	Used in GM wheat lines
<i>pOsAct1</i>	Promoter from Actin 1 gene	<i>Oryza sativa</i>	<i>ZmWRI1</i> - <i>UrDGAT2a</i>
<i>pUbi1</i>	Promoter from ubiquitin 1 gene	<i>Zea mays</i>	<i>ZmWRI1</i> - <i>UrDGAT2a</i>
<i>pZmSSU</i>	Promoter from Rubisco small subunit gene	<i>Zea mays</i>	<i>ZmWRI1</i> - <i>UrDGAT2a</i>
<i>pBx17</i>	Grain specific promoter from glutenin gene Bx17	<i>Triticum aestivum</i>	<i>AtDGAT1</i> - <i>ZmWRI1</i> - <i>SinOLEOSIN</i>
<i>pBdGLU1</i>	Promoter from glutenin gene GLU1	<i>Brachypodium distachyon</i>	<i>AtDGAT1</i> - <i>ZmWRI1</i> - <i>SinOLEOSIN</i>
<i>pOsGLU4</i>	Promoter from glutenin gene GLU4	<i>Oryza sativa</i>	<i>AtDGAT1</i> - <i>ZmWRI1</i> - <i>SinOLEOSIN</i>

56. All GM wheat lines included in Group D are overexpressing lines, using either grain specific or constitutive promoters (Table 6b). Candidate genes will be expressed as combination of two, “pull”-“push” genes, WRI1 and DGAT2a, or three, “pull”-“push”-“protect” genes, WRI1, DGAT1 and Oleosin. GM wheat lines overexpressing “pull” and “push” genes in vegetative tissues are expected to show increased oil content in the leaves, up to 10% dry weight. GM wheat lines overexpressing “pull”, “push” and “protect” genes in the grain are expected to show increased oil content in the grain, up to 5% dry weight.

57. Overexpression of “pull”, “push” and/or “protect” genes has been shown to impact the phenotype of some GM wheat lines in the glasshouse, with plants growing more slowly than the control and developing necrosis in older leaves. Leaves overproducing oils may become more palatable to some insects and less to others. There is no expectation of unintended changes in the phenotype of the GM wheat lines expressing these genes.

5.2.5 Group E: altered grain dietary fibre content

58. Intake of dietary fibre has been shown to be highly beneficial in prevention and treatment of diseases such as colorectal cancer, high serum cholesterol, cardiovascular diseases, obesity and non-insulin-dependent diabetes (Burton & Fincher 2012; Burton et al. 2006). (1-3,1-4)- β -D-glucan, a linear polymer of glucose also referred to as β -glucan, is an important component of dietary fibre, and is found only in the cell walls of grasses (Poaceae) and related families from the Poales (Doblin et al. 2009).

59. Glucose molecules within the β -glucan polymer are linked either by (1-3) or (1-4) bonds, arranged in an irregular but not random sequence (Jobling 2015). The (1-3)/(1-4) linkage ratio varies between cereals and within tissue types. This impacts polymer solubility and viscosity in solution, which, in turn, affects its health benefits. For example, oat and barley grain β -glucans are highly soluble while wheat β -glucans are insoluble (Burton & Fincher 2012; Jobling 2015). β -glucan content also varies between cereals: barley grain contains from 2.5 to 11.3% β -glucan, while wheat contains from 0.4 to 1.4% (Izydorczyk & Dexter 2008).

60. The β -glucan biosynthesis pathway is still largely unknown (Jobling 2015). Identified biosynthesis genes in grasses belong to the two cellulose synthase-like *CsIF* and *CsLH* protein families (Burton et al.

2006; Doblin et al. 2009; Jobling 2015). *CsIF* and *CsIH* are thought to be independent, as no significant transcriptional correlation was observed between the genes in barley tissues (Doblin et al. 2009).

61. The GM wheat lines included in Group E contain the *CsIF6* gene, derived from barley, *Brachypodium distachyon*, maize, oats, rice and sorghum or *CsIH* gene, derived from *B. distachyon* (see Table 7a and 7b for details). *CsIF6* genes have been shown to be critical for β -glucan synthesis: barley mutants that do not express *CsIF6* have very low amounts of β -glucans (Burton & Fincher 2012). *CsIF6* proteins have recently been shown to control the (1-3)/(1-4) linkage ratio in β -glucans (Jobling 2015). *CsIH* gene has been shown to be highly expressed in *B. distachyon*, compared to barley and wheat (Christensen et al. 2010).

Table 7a. Genes of interest introduced in Group E GM wheat lines

Gene	Full name and description	Source	Intended function	Type of expression
<i>AsCsIF6</i>	Cellulose synthase like F6 gene	<i>Avena sativa</i>	Altered dietary fibre content	Overexpression
<i>BdCsIF6</i>	Cellulose synthase like F6 gene	<i>Brachypodium distachyon</i>		
<i>HvCsIF6</i>	Cellulose synthase like F6 gene	<i>Hordeum vulgare</i>		
<i>ZmCsIF6-1</i>	Cellulose synthase like F6 gene 1	<i>Zea mays</i>		
<i>ZmCsIF6-2</i>	Cellulose synthase like F6 gene 2	<i>Zea mays</i>		
<i>OsCsIF6</i>	Cellulose synthase like F6 gene	<i>Oryza sativa</i>		
<i>SbCsIF6</i>	Cellulose synthase like F6 gene	<i>Sorghum bicolor</i>		
<i>BdCsIH</i>	Cellulose synthase like H gene	<i>Brachypodium distachyon</i>		

Table 7b. Promoters used in Group E GM wheat lines

Genetic element	Full name and description	Source	Used in GM wheat lines
<i>pBx17</i>	Grain specific promoter from glutenin gene Bx17	<i>Triticum aestivum</i>	Every promoter-candidate gene combination will be tested
<i>pOsGLUB</i>	Promoter from glutenin gene GLUB5	<i>Oryza sativa</i>	
<i>pOsGLUC</i>	Promoter from glutenin gene GLUC	<i>Oryza sativa</i>	
<i>pTaPinA</i>	Promoter from the purindoline A gene	<i>Triticum aestivum</i>	
<i>pTaPinB</i>	Promoter from the purindoline B gene	<i>Triticum aestivum</i>	

62. All GM wheat included in Group E are overexpressing lines, using grain specific promoters (Table 7b). Each gene will be expressed under the control of each of the promoters. Candidate genes will be expressed singly. GM wheat lines overexpressing *CsIF6* or *CsIH* genes are expected to accumulate more fibre in grain.

63. Overexpression of *CsIF6* or *CsIH* genes has resulted, in some GM wheat lines, in the production of smaller, wrinkled grains. Homologues of the candidate genes are expressed in wild type wheat grain. There is no expectation of unintended changes in the phenotype of the GM wheat lines expressing these genes.

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

64. The genes and partial genes used in this application were obtained from eleven plants and one fungus (see Table 8 for details). Twenty one of the 37 genes used in this application are present in many commercial varieties of wheat. They are regularly consumed by humans and livestock without adverse effects.

Table 8. Organisms used as source of candidate genes

Organism	# of genes derived	Status in Australia	Source of exposure
Wheat	21	Cultivated	Food and feed
Maize	3	Cultivated	Food and feed
<i>Aegilops tauschii</i>	2	Not present, declared pest	Utilised in wheat breeding
Barley	2	Cultivated	Food and feed
<i>Brachypodium distachyon</i>	2	Weed of natural ecosystems	Present (common) in the environment
<i>Arabidopsis thaliana</i>	1	Weed of natural ecosystems	Present (common) in the environment
Oats	1	Cultivated	Food and feed
Rice	1	Cultivated	Food and feed
Sesame	1	Cultivated Weed of natural and agricultural ecosystems	Food and feed
Sorghum	1	Cultivated	Food and feed
<i>Triticum turgidum</i>	1	Cultivated	Food
<i>Umbelopsis ramanniana</i>	1	Present	Present (common) in the environment

65. No toxicity/allergenicity studies have been performed on the GM wheat plants or purified proteins produced by the full-length genes, as the proposed trial is at preliminary research stage. However, proteins encoded by the genes used in this application have homologues that occur naturally in a range of organisms, including plants consumed by people and animals. On this basis, people and other organisms have a long history of exposure to homologues of the introduced proteins.

66. The selectable markers used in this application have been used extensively. A number of GM crops, including food crops, containing the *pat*, *bar*, *hptII* or *nptII* genes have been approved for commercial release both in Australia and overseas. No adverse effects on humans, animals or the environment have been reported from any such releases (CERA 2011).

67. No adverse health effect or impact on the environment was reported by the staff handling the GM wheat lines during the screening. There has been no report of harm to human health and safety to the environment resulting from glasshouse or field trials.

5.4 Characterisation of the GMOs

68. No information regarding the phenotypic characterisation of GM wheat lines from Group A and B has been provided. The applicant has reported that Group D plants in the glasshouse may sometimes be smaller with some necrosis in older leaves.

69. Initial assessment of baking properties, oil content and β -glucan content for Group C, Group D and Group E, respectively has been conducted.

70. One GM wheat line from Group E, expressing the gene *AsCs1F6*, was previously trialled under DIR 111. No information regarding the phenotypic characterisation of this line has been provided.

Section 6 The receiving environment

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

72. Information relevant to the growth and distribution of commercial wheat in Australia is discussed in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016).

6.1 Relevant abiotic factors

73. The release is proposed to take place at Ginninderra Experiment Station (GES), ACT and at Boorowa Experiment Station (BES), NSW, two dedicated fenced field trial sites. The GES site is of approximately 2.3 ha and has been previously used for licenced field trials. The BES site is a new proposed site, anticipated to be of approximately 2.3 ha⁴. Both sites are on CSIRO controlled land. Access to the research stations is restricted to authorised staff. CSIRO has control over the management of the fields immediately surrounding the proposed trial sites.

74. 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).

6.2 Relevant biotic factors

75. Wheat plants are susceptible to a wide range of pests and diseases that can severely reduce grain harvest and quality. Wheat rusts are among the major pathogens of concern in wheat, due to their ability to cause devastating losses. Potential yield losses due to stem, leaf and stripe rusts in years suitable for disease development has been described by Murray and Brennan (2009) to be of 46, 24 and 35% respectively. Losses recorded in 2009 were much lower, with a maximum yield loss of 2.6% recorded for stripe rust (Murray & Brennan 2009).

76. However, the emergence and spread of broadly virulent strains such as the stem rust strain Ug99 in other countries, is of concern because of their potential introduction in the Australian environment. If uncontrolled, potential losses due to stripe and stem rusts only would reach AUD 1472 million per year (Murray & Brennan 2009).

77. The potential impact of stem and stripe rust has become higher over the last twenty years, possibly linked to temperature increases that have occurred throughout much of the Australian wheatbelt (Murray & Brennan 2009). The most effective means of protection for both stem and stripe rust is the use of wheat cultivars containing durable resistance genes (Ellis et al. 2014).

6.3 Relevant agricultural practices

78. The limits and controls of the proposed release are outlined in Section 3.1 and Section 3.2 of this Chapter. It is anticipated that the agronomic practices for the cultivation of the GM wheat by the applicant will not differ significantly from industry best practices used in Australia.

79. It is proposed to grow the GM wheat as a dryland crop, with irrigation available if necessary. GM wheat lines and reference wheat are proposed to be grown. The GM wheat will be either hand sown in rows with 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.

⁴The BES site was described in the current application as of approximately 3 ha. However, the size of the planned facility for GM trials was later reduced to 2.3 ha.

80. The crop will be maintained in a similar fashion to commercial wheat crops, for management of weeds and disease. As some GM wheat lines within this trial are to assess impact of candidate genes on disease resistance, there will be some differences in management of diseases such as rust.

81. Seeds will be harvested either by hand or with a plot harvester dedicated for use on GM plants. It is proposed that after harvest, the land will be left to fallow or planted with a break crop such as lucerne, forage brassica, canola or field peas.

6.4 Presence of related plants in the receiving environment

82. 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 BES trial sites are on CSIRO land and are routinely used for GM and non-GM wheat trials.

83. Wheat (*Triticum aestivum* L.) is sexually compatible with a number of species within the tribe Triticeae that occur in Australia. Of particular importance are durum wheat (*Triticum turgidum* ssp. *durum*), rye (*Secale cereale*), and Triticale (X *Triticosecale*). Hybridisation with durum wheat occurs readily (Wang et al. 2005), whereas that with rye (Dorofeev 1969; Leighty & Sando 1928; Meister 1921) and Triticale (Ammar et al. 2004; Kavanagh et al. 2010) is rarer. Wheat also readily hybridises with *Aegilops* species (goatgrasses), but no *Aegilops* species are considered to be naturalised in Australia. Any specimens of *Aegilops* that have been collected in Australia presumably originate from seed accidentally introduced amongst wheat seed, or straying from that brought in for breeding programs (AVH 2010).

84. One case of natural hybridisation between wheat and the weedy species *Hordeum marinum* has been reported in Europe, and described as likely to be a rare event (Guadagnuolo et al. 2001). *H. marinum* is found in wheat growing areas of Australia however no natural hybridisation has been reported under Australian conditions.

85. Australasia possesses four native Triticeae genera – *Anthosachne*, *Australopyrum*, *Erenochloa* and *Ophiorus* – as well as a number of introduced species of Triticeae, such as *Elytrigia repens* (couch grass) and at least four *Thinopyrum* species (Bell et al. 2010). *Thinopyrum ponticum* (tall wheatgrass) has been used as a saltland pasture plant in Australia, and in some regions has come to be classified as a weed (Barrett-Lennard 2003; NYNRMP 2011). There has been no concerted investigation on natural hybridisation of these native and introduced Triticeae species with wheat. Factors such as genome incompatibilities, the necessity for the parent plants to be in close proximity, concurrent flowering, and the ability of the hybrid progeny to set viable seed, combine to make it extremely unlikely that any of these Triticeae would ever naturally cross with wheat.

6.5 Presence of similar genes and encoded proteins in the environment

86. The 37 introduced genes are derived from the plants *Aegilops tauschii*, *Arabidopsis thaliana*, barley, *Brachypodium distachyon*, maize, oats, rice, sesame, sorghum, *Triticum turgidum*, wheat, and from the common soil fungus *Umbelopsis ramanniana* (see Table 8 for details). Barley, maize, oats, rice, sorghum, *Triticum turgidum* and wheat are grown commercially in Australia and the other species from which introduced genes have been derived are common in the Australian environment, with the exception of *Aegilops tauschii*. However, *Aegilops tauschii* has been widely used as a genetic resource for wheat germplasm improvement since the early 20th century (Krattinger et al. 2009).

87. Information provided in the application indicates that the introduced genes are homologous to genes present in the Australian environment. Therefore, people are naturally exposed to these genes.

88. The *pat*, *bar*, *nptII* and *hptII* selectable marker genes are derived from bacteria that are widespread in the environment.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Approvals by the Regulator

89. One GM wheat line included in this application has previously been approved by the Regulator for limited and controlled release in Australia under licences DIR 111. There have been no reports of adverse effects on human health or the environment resulting from this release. The other lines have not been field trialled.

90. Information on previous DIR licences for GM wheat is available from the [OGTR GMO Record](#). The Regulator has previously approved 19 field trial releases of GM wheat. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.

7.1.2 Approvals by other government agencies

91. There are no approvals of these GM wheat lines, including pending approvals, from other Australian authorities.

7.2 International approvals

92. None of the GM wheat lines covered in this application has been approved for release in any other countries.

Chapter 2 Risk assessment

Section 1 Introduction

93. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of, gene technology (Figure 3). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

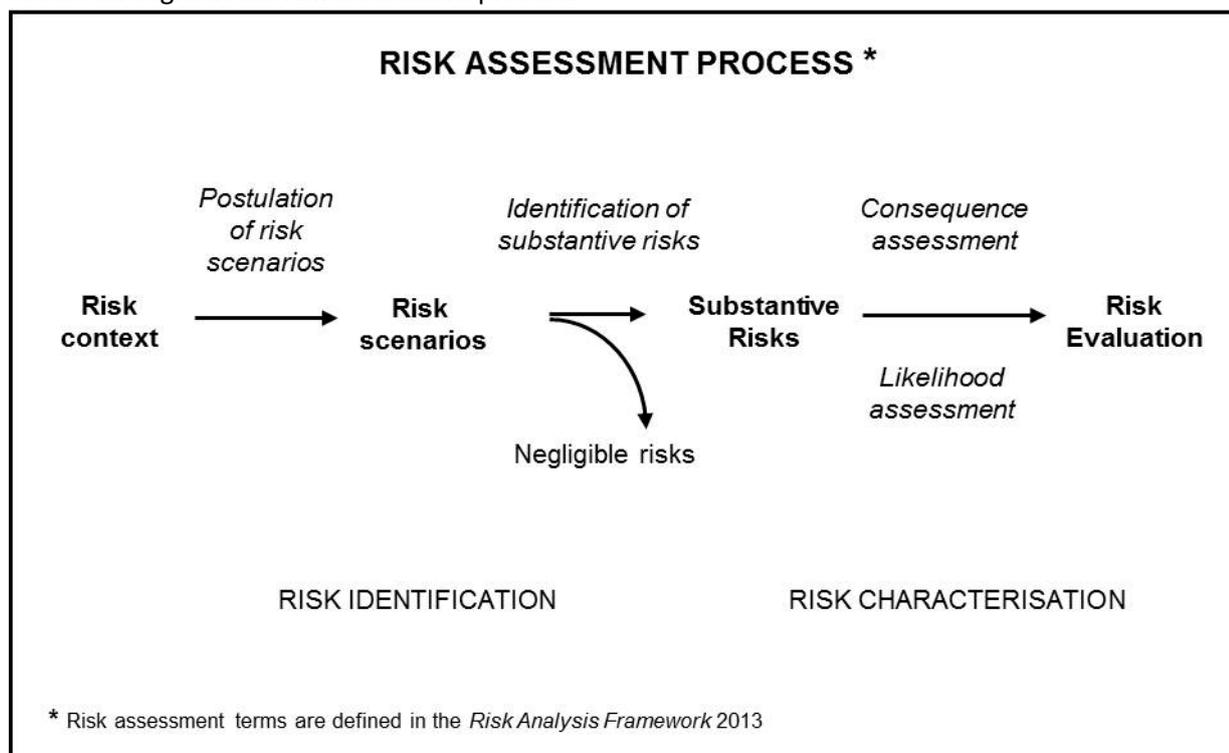


Figure 2. The risk assessment process

94. 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.

95. 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.

96. 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.

97. 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

98. 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)
99. 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

100. The source of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

2.1.1 The introduced genes

101. As discussed in Chapter 1, the GM wheat lines have been modified by the introduction of one to three of 37 candidate genes involved in disease resistance, drought tolerance, starch metabolism, oil and dietary fibre biosynthesis. The genes were sourced from the plants *Aegilops tauschii*, *Arabidopsis thaliana*, barley, *Brachypodium distachyon*, maize, oats, rice, sesame, sorghum, *Triticum turgidum*, wheat, and from the common soil fungus *Umbelopsis ramanniana*. These introduced genes are considered further as potential sources of risk.

2.1.2 The introduced marker genes

102. The GM wheat lines contain the *bar*, *pat*, *hptII*, or *nptII* gene, which confer herbicide tolerance (*bar*, *pat*) or antibiotic resistance (*hptII*, *nptII*) and were used as selectable marker genes. These 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* can be found in the document Marker genes in GM plants available from the [Risk Assessment References page](#) on the OGTR website. Further information about *pat* and *bar* can be found in the report published by the Centre for Environmental Risk Assessment (CERA) (CERA 2011).

103. As the genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

2.1.3 The introduced regulatory sequences

104. The introduced genes are controlled by introduced regulatory sequences. These were derived from plants, bacteria and plant viruses (see Chapter 1, Table 2). Regulatory sequences are naturally present in plants and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, risks from these regulatory sequences will not be further assessed for this application.

2.1.4 Unintended effects

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

2.2 Causal pathway

106. 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.

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

2.2.1 Horizontal gene transfer

108. The potential for horizontal gene transfer and any possible adverse outcomes has been reviewed in the literature (Keese 2008) and has been assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108. No risk greater than negligible was identified due to the rarity of these events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, horizontal gene transfer will **not** be assessed further.

2.2.2 Unauthorised activities

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

2.3 Potential harm

110. Potential harms from GM plants include:

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

111. These harms are based on those used to assess risk from weeds (Standards Australia Ltd et al. 2006). Judgements of what is considered harm depend on the management objectives of the land into which the GM plant is expected to spread and persist. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

112. Three risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 9, and discussed individually below. Postulation of risk scenarios considers impacts of the GM wheat or its 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.

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

Table 9. Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition	Growing GM wheat plants at the field trial sites ↓ Expression of the gene constructs in GM plants ↓ Exposure of humans and other desirable organisms at the trial sites by ingestion of, or contact with GM plant material	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms	No	<ul style="list-style-type: none"> The genes of interest are derived from organisms that are routinely used in food and feed or that are widely present in the environment. The encoded proteins and similar proteins occur naturally in the environment and are not known to be toxic or allergenic to people and other organisms. Insertion of the silencing constructs does not lead to expression of a protein GM plant material would not be used in human food or animal feed, with the exception of proposed human/animal nutritional studies described in the application. The limited scale, short duration and other proposed limits and controls minimise exposure of people and other organisms to the GM plant material.
2	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition	Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM wheat plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants OR Reduced utility or quality of the environment	No	<ul style="list-style-type: none"> The proposed limits and controls would minimise the likelihood that GM plant material would leave a trial site. There is no expectation the introduced gene constructs confer other characteristics to enhance the spread and persistence of the GM wheat lines. Wheat grains have limited ability to be dispersed by animals. Wheat has limited ability to survive outside agricultural settings. The GM wheat lines used in this trial are susceptible to standard weed control measures.
3	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition	Fertilisation of sexually compatible plants outside the trial sites by pollen from GM wheat plants ↓ Germination of GM hybrid seeds ↓ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants	No	<ul style="list-style-type: none"> The proposed limits and controls would minimise the likelihood of pollen flow to sexually compatible plants outside the trial sites. Wheat has limited ability to outcross. Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.

Risk scenario 1

<i>Risk source</i>	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Growing GM wheat plants at the field trial sites</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of the gene constructs in GM plants</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of humans and other desirable organisms at the trial sites by ingestion of, or contact with the GM plant material</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms

Risk source

114. The source of potential harm for this postulated risk scenario is the introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition.

Causal pathway

115. The overexpression constructs used for Groups A to E are designed to increase expression of the genes of interest, thus altering disease resistance, drought tolerance, oil content and grain composition. The silencing constructs containing wheat gene fragments are designed to produce siRNAs that suppress the expression of putative rust resistance genes (Group A) or alpha-amylase genes (Group C), thus altering resistance to leaf, stem and/or stripe rust or grain composition, respectively.

116. People may be exposed to the GM wheat or its products through contact or inhalation of pollen. This would be expected to occur mainly at the trial sites but could also occur during transportation and handling of the GM wheat lines. Organisms that may be present at the trial sites, including birds, rodents and invertebrates, may be exposed to the GM wheat lines through contact or consumption.

117. The proposed limits and controls of the trial (Chapter 1, Sections 3.1 and 3.2) would minimise the likelihood of exposure of people and other organisms to GM plant material. The GM wheat grains will not be used for commercial human food or animal feed. The trial sites would be located on a land owned and controlled by CSIRO, and would only be accessed by authorised people. Furthermore, as the proposed trials are limited to a total of 1 ha per site per season, only a small number of people would deal with the GM wheat lines and a small number of organisms would be exposed to them.

118. Small scale controlled animal studies are proposed with non-viable material derived from the GM wheat lines included in Group E – altered grain dietary fibre content. These might include studies involving rats or pigs. Human nutrition trials may also be conducted, involving human volunteers in controlled nutritional experiments. The applicant has not proposed any means for segregating the GM wheat lines from each other while growing in the field, so the potential exists for gene stacking or mixing of harvested seed over the course of the trial. Animals and volunteers participating in nutritional studies could ingest GM wheat products prepared from more than one group and containing several introduced gene constructs, gene products and associated compositional changes. This could potentially lead to increased toxicity or allergenicity.

119. However, as outlined above, the introduced genes are derived from wheat or other organisms that are routinely used in food and feed or that are widely present in the environment, and exposure to GM plant materials carrying RNA or proteins encoded by the individual genes is unlikely to lead to toxic or allergenic effects. Similarly, it is unlikely that combining a number of gene constructs or traits will increase toxicity or allergenicity.

120. Approval from an Animal Ethics Committee operating in accordance with State and Territory legislation and National Health and Medical Research Council (NHMRC) code of practice for animal experimentation would be obtained before conducting any animal experiments. Approval from an Ethical Conduct in Human Research Committee operating in accordance with State and Territory legislation and

following values, principles, governance and review processes specified in NHMRC [National Statement on Ethical Conduct in Human Research](#) would be obtained before conducting any nutritional trial.

121. These nutritional trials would be conducted under CSIRO supervision, and could include determination of the impact of the GM wheat on mineral bioavailability, bone, cardiovascular and metabolic health, satiety, and weight management.

Potential harm

122. 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).

123. 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).

124. Expression of the introduced candidate genes could result in production of novel toxic or allergenic compounds. The expression of the introduced candidate genes, or gene silencing constructs could alter the production of endogenous compounds of wheat that are toxic or allergenic. The potential for the production of novel toxins or allergens and for altered production of endogenous wheat toxins and allergens will be considered further.

125. Potentially, people exposed to the proteins expressed by the introduced genes may show increased toxic reactions or increased allergenicity. From consideration of the causal pathways, these are limited to staff involved in handling and harvesting the GM wheat plants during the course of the field trial, and to volunteers involved in nutritional trials. Similarly, exposure to the proteins expressed by the introduced genes may lead to increased toxicity to other desirable organisms.

126. The introduced gene silencing constructs could lead to the production of potentially toxic or allergenic substances in the GM wheat lines. Transcription of the gene fragments in the silencing constructs forms hairpin RNA. This double-stranded RNA enters the RNAi pathway, leading to suppression of expression of the genes of interest, rather than being translated into a protein. Therefore the introduction of the silencing construct does not lead to expression of a novel protein that could potentially be toxic or allergenic. In these circumstances, there is no reasonable expectation that the introduced constructs will lead to an increase in the level of any endogenous compound in the GM wheat that has toxic or allergenic properties.

127. The potential for toxicity or allergenicity of introduced gene silencing constructs has been addressed comprehensively in previous RARMPs, the latest being DIR 131. On the basis of the evidence detailed there, the expression of the introduced gene silencing constructs is highly unlikely to result in the production (directly or indirectly) of a novel toxin or allergen.

128. Non-GM wheat is not known to be toxic to humans or other organisms. However, non-GM wheat flour can produce allergic and autoimmune responses in susceptible individuals on inhalation (such as baker's asthma) or ingestion (such as coeliac disease). These undesirable properties are not expected to be altered in the wheat proposed for release.

129. Although no toxicity or allergenicity studies have been performed on the GM wheat plant material, the introduced genes were isolated from wheat or other naturally occurring organisms that are already widespread and prevalent in the environment, including common food sources (barley, oats, rice, sesame or maize) and a soil organism (the fungus *Umbelopsis ramanniana*) (Chapter 1, Section 5.1). Thus, people and other organisms are exposed to the same or similar proteins through their diet and the environment.

130. Therefore, the allergenic and toxic properties are not expected to be altered in the GM wheat lines proposed for release. In addition, exposure of staff to the GM plant material either in the glasshouse or, in the case of GM wheat lines containing the gene *AsCs1F6*, through previous field trials, did not result in adverse reactions.

Conclusion

131. Risk scenario 1 is not identified as a substantive risk, due to limited exposure and the lack of toxicity or allergenicity of the introduced proteins and partial genes to humans or other desirable organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Risk scenario 2

<i>Risk source</i>	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition
<i>Causal pathway</i>	↓ Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM plants ↓
<i>Potential harm</i>	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants OR Reduced utility or quality of the environment

Risk source

132. The source of potential harm for this postulated risk scenario is the introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition.

Causal pathway

133. If GM wheat seed was dispersed outside the trial sites or persisted at the sites after completion of the trial, this seed could germinate and give rise to plants expressing the introduced gene constructs. These plants could spread and persist in the environment outside the trial limits and people and other organisms may be exposed to GM plant materials.

134. Similarly, pollen from GM plants could fertilise other GM plants within the trial sites leading to GM plants with combined GM traits. If such hybridisation occurred, the progeny could have up to two stacked traits from rust resistance, drought tolerance, altered starch composition, altered oil content and/or altered dietary fibre content. It is unlikely that hybrid progeny could persist at the sites from one season to the next, due to post-harvest control measures to ensure removal of GM volunteers. However, there is a small possibility that hybrid GM wheat seed possessing multiple stress tolerances and/or altered characteristics could also disperse from the sites.

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

Dispersal through human activity

136. Dispersal of GMOs outside the limits of the trial sites could occur through the activity of people, including the use of agricultural equipment. The proposed trial sites would be surrounded by a fence with lockable gates and only approved staff with appropriate training would have access to the sites. This would reduce inadvertent access by humans thus minimising dispersal of GM plant material. Dispersal of GM plant material by authorised people entering the proposed trial sites would be minimised as the applicant proposes harvesting by hand or by using a dedicated plot harvester, and cleaning of all equipment used at the trial sites. 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 its dispersal.

Dispersal through animal activity

137. The activity of animals such as rodents, herbivores and birds could lead to dispersal of the GMOs outside the limits of the trial sites. Wheat lacks seed dispersal characteristics such as stickiness, burrs and hooks (Howe & Smallwood 1982). The intended introduced traits of the GM plants are not expected to alter these characteristics of seeds. Wheat seeds could be dispersed and germinate after passage through the digestive system of some mammals or birds. For example, viable wheat seeds have been detected in cattle dung (Kaiser 1999).

138. However, reports on seed dispersal for wheat through ingestion are rare. Seeds which survive chewing and digestion by animals are typically small and dormant (Malo & Suárez 1995). Though birds can cause damage to cereal crops during germination and seed ripening, only a small proportion intact wheat seed can be excreted by corellas and galahs, with varying germination rates (Woodgate et al. 2011). Wheat seed may be dispersed by emus (Calvino-Cancela et al. 2006), however germination rates were very low (McGrath & Bass 1999; Rogers et al. 1993), or in some cases not provided (Davies 1978).

139. Kangaroos, rabbits and rodents are known pests of wheat crops, and cattle or sheep may also graze cereals. The proportion of viable wheat seeds excreted by cattle or other livestock is extremely low and unlikely to constitute a significant means of dispersal for the GM wheat (Kaiser 1999). Although rabbits are known pests of wheat crops, viable wheat seeds have not been found in rabbit dung (Malo & Suárez 1995). Therefore rabbits are unlikely to disperse the GM wheat seeds. In addition, there is only a relatively short period during which viable wheat grains could be eaten or removed from the planting area by large animals (during sowing and prior to harvest). The applicant has proposed to surround the planting area with areas that would be inspected for volunteers at least every 14 days while the GMOs are flowering. In the event of any spread of viable seeds by livestock and other large animals, this measure would allow identification and removal of volunteers and further limit the already low potential for dispersal through animal activity.

140. 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 also hoard seeds (AGRI-FACTS 2002), which increases the possibility of seed dispersal. In addition, Group D includes GM wheat lines with up to 5% of oil content (dry weight) in the grain, compared to 2.5 to 3.5% in non GM wheat lines (OGTR 2016). It is possible that higher oil content in the grain could result in increased palatability, leading to increased consumption and dispersal by animals such as rodents. 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.

Dispersal through dormancy

141. Persistence of GMOs at the trial sites could occur through dormancy of seeds in the seed bank. However, wheat cultivars grown in Australia have little seed dormancy and do not produce a persisting seed bank (OGTR 2016). Although wheat is the most widely grown crop in Australia, and so has had extensive opportunity to naturalise, it has not become a significant weed in any Australian ecosystem (Groves et al. 2003). See Chapter 1, Section 4 for more details regarding wheat spread and persistence characteristics.

142. Group C includes GM wheat lines in which *TaAMY1*, *TaAMY2* or *TaAMY3* have been silenced. It is possible that a low expression of alpha-amylase genes in the grain could lead to delayed germination and/or increased dormancy. This could lead to an increased likelihood of establishment for these particular lines. Information provided by the applicant shows a 24 to 48 hours delay in emergence compared to the untransformed parent line. However no difference in the number of seeds that underwent germination could be detected.

143. The applicant proposes to promote germination of any residual GM wheat seed by post-harvest tillage and irrigation, and to monitor the trial sites and destroy wheat volunteers for at least two years. These measures are considered to minimise the likelihood of persistence of GMOs after completion of the trial. Furthermore, the GM wheat lines included in Group C are susceptible to standard weed control measures.

144. For groups A and B, the expected phenotypic differences between the GM wheat lines and their non-GM progenitors include increased disease resistance or drought tolerance. These introduced traits are not expected to alter the reproductive or dispersal characteristics of the GM plants. The introduced traits may provide a survival advantage, as there is the potential for the GM plants to have an increased distribution in the natural environment and agricultural settings, particularly in areas with increased disease prevalence or lower availability of water. There is some uncertainty regarding increased survival, as the performance of the GM plants in the field is yet to be determined. However, wheat lacks other weedy characteristics, as outlined in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016), which provides baseline information on the weediness of wheat, including factors limiting the spread and persistence of non-GM plants of the species (see Chapter 1, Section 4 for more details). Thus, the GM wheat would be unlikely to successfully compete with established weedy species when not under cultivation. In addition, a large number of herbicides are registered to control volunteer wheat ([APVMA website](#)), and these herbicides or non-chemical weed management methods would be as effective on GM wheat as on non-GM wheat.

Dispersal through extreme weather events

145. Extreme weather events could lead to spread of the GMOs. The applicant has proposed to conduct the trial at least 50 m from the nearest natural waterway. This is considered to minimise the potential for seed dispersal during flooding. It is unlikely that high winds could lead to dispersal of GM wheat seed as they lack structures which will aid windborne dispersal, however, this could be possible in the event of a severe storm.

Potential harm

146. If GM wheat plants were to establish beyond the trial limits, they could potentially cause increased toxicity or allergenicity in people or toxicity to desirable organisms, or reduced establishment or yield of desirable plants. However, as discussed in Risk scenario 1, the introduced gene products are not expected to be toxic or allergenic to people or to other organisms. This would apply even if the GM wheat plants established beyond the trial limits and would similarly apply in the case of GM plants with stacked traits.

147. If GM wheat plants were to establish and persist beyond the trial limits, this could potentially impact the environment, e.g. it could reduce establishment or yield of desirable agricultural crops; reduce establishment of desirable native vegetation; reduce utility of roadsides, drains, channels and other intensive use areas; or reduce the quality of the biotic environment by providing a reservoir for pathogens or pests.

148. The potential of these harms can be evaluated against the experience of conventional breeding. Wheat germplasm improvement using traditional breeding methods has, in part, focused since the early 20th century on producing cultivars with tolerance and/or resistance to abiotic and biotic stresses. Release of these modern cultivars has not led to increased wheat dispersal or persistence, either in agricultural or natural ecosystems. No commercially released variety of wheat that is the product of any form of conventional breeding has been reported to have negatively impacted the environment beyond the levels normally associated with the cereal, and subsequently been flagged as an environmental weed ([Department of Environment National Weed List](#)). In this context, it is relevant to note that a number of these non-GM varieties have been bred for disease resistance or abiotic stress tolerance, and none have been recorded as causing harm to the environment.

149. Therefore, the presence in wheat of any of the introduced gene constructs, or a combination of these constructs through hybridisation, is unlikely to result in these GM plants being classified as weeds.

150. As discussed above, the causal pathways which may lead to increased spread and persistence of the GM wheats are unlikely to occur. Therefore, the presence in wheat of any of the introduced genes is unlikely to lead to any of the potential harms listed above.

Conclusion

151. Risk scenario 2 is not identified as a substantive risk due to the extremely limited ability of the GM wheat to spread and persist outside cultivation, the proposed limits and controls designed to restrict

dispersal, and the susceptibility of the GM wheat to standard weed control measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Risk scenario 3

<i>Risk source</i>	Introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> Fertilisation of sexually compatible plants outside the trial sites by pollen from GM wheat plants <p style="text-align: center;">↓</p> Germination of GM hybrid seeds <p style="text-align: center;">↓</p> Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas <p style="text-align: center;">↓</p>
<i>Potential harm</i>	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms <p style="text-align: center;">OR</p> Reduced establishment or yield of desirable plants

Risk source

152. The source of potential harm for this postulated risk scenario is the introduced gene constructs for abiotic or biotic stress tolerance, and for altered grain or leaf composition.

Causal pathway

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

154. People and other organisms could then be exposed to the proteins expressed from the introduced genes through contact with (including inhalation of pollen) or consumption of hybrid plants.

155. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. Baseline information on vertical gene transfer associated with non-GM wheat plants can be found in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR 2016).

156. Wheat is predominantly self-pollinating, and the chances of cross-pollination with commercial crops or other sexually compatible plants are low and decrease with distance from the GM plants (OGTR 2016). Due to these characteristics, wheat has been described as a low risk crop for both intra- and interspecific gene flow (Eastham & Sweet 2002).

157. The main method of wheat pollen dispersal is wind, with the role of insects considered minimal. Wheat pollen is heavy and short-lived, with most pollen falling within the first few metres. Field trials conducted in ACT investigating gene flow from GM lines to non-GM crops have shown a cross-pollination frequency of 0.012% to 0.055%, over a distance of less than 12 m (Gatford et al. 2006). Cross-pollination rates are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity. The genetic modifications for disease resistance, drought tolerance, altered starch composition, altered oil content or altered dietary fibre content are unlikely to increase the propensity of the GM wheat to outcross.

158. Wheat is sexually compatible with many species within the genus *Triticum*. Durum wheat (the only other *Triticum* species present in Australia) can cross with wheat, although gene flow from bread wheat beyond 40 m is very unlikely (Matus-Cadiz et al. 2004). Wheat is also sexually compatible with closely related genera such as *Aegilops*. However, no *Aegilops* species are considered to be naturalised in Australia.

159. Wheat can be used as a pollinator for crosses with related genera *Secale* (rye), *Elytrigia*, *Agropyron* or *Roegneria* under controlled conditions (Eastham & Sweet 2002; Jacot et al. 2004). Hybrids obtained from crosses between wheat and *Secale cereale* are sterile but treatment with colchicine to double the

chromosome number results in a fertile plant, the commercialised Triticale (Knupffer 2009). Wheat x Triticale crosses have been performed under controlled conditions, producing sterile hybrids with low fitness (Bizimungu et al. 1997). These crosses are highly unlikely under field conditions.

160. *Elytrigia repens* does occur as an introduced plant in Australia, but a review of possible means of pollen-mediated gene flow from GM wheat to wild relatives in Europe concluded that there was a minimal possibility of gene flow from wheat to *Elytrigia* spp., with natural hybrids described as highly sterile (Eastham & Sweet 2002). As discussed in Chapter 1, Section 6.4, the introduced weedy species *H. marinum* is found in wheat growing areas of Australia but no natural hybridisation has been reported under Australian conditions.

161. Although there is some uncertainty due to data gaps, it is unlikely that hybridisation of wheat with the four native Australasian *Triticeae* genera, *Anthosachne*, *Australopyrum*, *Erenochloa* and *Ophiorus*, occurs under natural conditions due to genetic incompatibility and low fitness (OGTR 2016).

162. The proposed limits and controls of the trial would minimise the likelihood of the dispersal of pollen and potential for gene flow to plants outside the trial sites. For example, the applicant proposes to control related species within 200 m of the trial sites. Isolation from related species and other wheat cultivation will greatly restrict the potential for pollen flow and gene transfer. In addition, the applicant proposes to perform post-harvest monitoring and to destroy any volunteer plants found at the sites to ensure that no GM wheat remains that could then hybridise with sexually compatible plants.

Potential harm

163. If pollen from GM wheat lines was to be dispersed, resulting hybrid plants could spread and persist in the environment, leading to increased toxic reactions or allergenicity in people and/or other desirable organisms. Hybrids expressing the introduced genes could also reduce the establishment and yield of desired plants and subsequently reduce biodiversity.

164. 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 other related species. 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, or a level of weediness greater than that of either parent.

165. As discussed in Risk scenario 1, the introduced gene products are not expected to be toxic to humans or other organisms. Properties of these genes are not expected to differ in a hybrid background. Therefore, in the rare event of the vertical transfer from the GM wheat lines to non-GM wheat plants or sexually compatible species, it is expected that the introduced genes in the subsequent hybrid will have the same properties as in the GM wheat parent.

166. As discussed in Risk scenario 2, the introduced genes are unlikely to make the GM wheat lines weedier. As above, the properties of the introduced genes are expected not to change in a hybrid background resulting from cross-pollination.

Conclusion

167. Risk scenario 3 is not identified as a substantive risk due to the limited ability of wheat pollen to be dispersed at long distances, and to the proposed limits and controls designed to restrict pollen flow from the GM wheat. Further, Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

168. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis⁵.

⁵ A more detailed discussion of uncertainty is contained in the Regulator's [Risk Analysis Framework](#) available from the OGTR website or via Free call 1800 181 030.

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

170. 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.

171. 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.

For DIR 151, 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 in land uses outside of agriculture

172. 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.

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

Section 4 Risk Evaluation

174. 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.

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

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

176. Three 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, neither of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- none of the GM plant material or products will be used for human food or animal feed, with the exception of the nutritional studies described in the application

- the balance of the evidence indicates that the introduced proteins are unlikely to be toxic or allergenic
- limited ability of the GM wheat plants to establish populations outside cultivation
- limited ability of the GM wheat plants to transfer the introduced genetic material to other plants
- limits on the size, location and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the spread and persistence of the GM wheat plants and their genetic material.

177. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM wheat plants into the environment are considered to be negligible. The Risk Analysis Framework, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment⁶.

⁶ 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 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

178. 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.

179. 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.

180. 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.

181. 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

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

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

3.1 Licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by CSIRO

184. 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 three risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

185. As stated above, limits and controls proposed by the applicant to limit the spread and persistence of the GMOs were taken into account while postulating risk scenarios. However, a control proposed by the applicant (the presence of a 2 m buffer zone surrounding the planting area) has not been included as licence conditions. Based on the available evidence, there were no plausible risks that would warrant the

use of this control measure. Other measures were held to be adequate to manage potential risk associated with rodent activity or GMO dispersal and have been included as licence conditions (see below).

186. The applicant proposes that the release will be limited to a maximum of 1 ha per site per year at the Boorowa Experiment Station (NSW) and the Ginninderra Experiment Station (ACT) and the duration of the release will be limited to five years. These measures will limit the potential exposure of humans and other organisms to the GMOs (Risk Scenario 1) and the potential for the GM wheat lines to disperse and establish outside the proposed release sites (Risk Scenario 2).

187. GM wheat has previously been planted under another DIR licence, DIR 111, at the Ginninderra Experiment Station. At the time of proposed release, the areas planted under DIR 111 will be in the post-trial monitoring phase with licence conditions that ensure volunteer wheat plants are destroyed prior to flowering. As the planting areas and associated zones of DIR 111 will be routinely monitored for volunteer wheat plants and any volunteers destroyed prior to flowering, pollen transfer between GM plants of the two trials is highly unlikely. This will limit the potential for the GM wheat lines to hybridise with other lines (Risk Scenario 3).

188. The proposed trial sites are located within the Boorowa and Ginninderra Experiment Stations, on CSIRO owned and operated land. The applicant has stated that both sites would be surrounded by a livestock-proof fence. This would minimise the potential exposure of desirable animals to the GMOs (Risk Scenario 1). A standard licence condition has been included in the licence which requires that no food or feed may be produced from plant material in this trial, thus livestock cannot be allowed to feed on the GM wheat.

189. The applicant has proposed that the sites will have lockable gates. The applicant also proposes that only authorised personnel would be permitted to deal with the GMOs. A standard licence condition requires all people dealing with the GMOs to be informed of relevant licence conditions. These measures would limit the potential exposure of humans to the GMOs (Risk Scenario 1). Since restricting the dealings to only authorised personnel is considered appropriate for limiting exposure of humans to the GMOs, it is not considered necessary to have the gates locked and hence is not a licence condition.

190. There is a possibility that seed might be moved by small animals such as rodents or birds. The applicant states that in previous GM wheat trials (DIR 054, 092, 093, 094, 099, 111 and 112) there was only limited evidence of rodent activity at Ginninderra Experiment Station. On this basis, the applicant did not propose to use rodent baits. The only rodent control measures proposed by the applicant are a 2 m buffer zone immediately around each planting area maintained as bare fallow and a 10 m monitoring zone surrounding the buffer zone where vegetation is kept below a height of 10 cm, so that this area does not attract or harbour rodents.

191. However, as Boorowa Experiment Station is a new trial site, evidence regarding rodent presence needs to be gathered. Therefore, a licence condition requires that the presence of rodents must be monitored by placing rodent baits in the planting area. Rodents must be controlled if present. Rodent baiting, combined with the use of a 10 m monitoring zone where vegetation is kept below a height of 10 cm, should minimise potential dispersal of GMOs outside the trial sites by rodents (Risk Scenario 2). The presence of a 2 m bare buffer zone was not relied on as a rodent control measure in the risk assessment (Risk Scenario 2), given that a 10 m monitoring zone (managed as described) will minimise the likelihood of rodent activity. Therefore, the 2 m buffer zone has not been imposed as a licence condition.

192. Birds are known to cause damage to cereal crops mostly during germination in autumn, but may feed on the crop at different times, including during grain ripening (Temby & Marshall 2003). An extensive search of the literature did not identify any report of birds other than emus transporting and dispersing wheat seed (through the digestive track or taking panicles containing viable seeds) or seedlings from wheat crops. The presence of a fence surrounding the trials sites, as proposed by the applicant, would exclude emus from the planting areas. However, the risk of seed dispersal by emus is considered as being very low (Risk Scenario 2).

193. White wheat varieties are the only bread wheat varieties grown in Australia (Blakeney et al. 2009). These varieties have a thin seed coat and are readily digested by birds (Yasar 2003). The varieties Bobwhite,

Chinese Spring, Chinese Spring Hope 3B, Fielder and Mace used to generate the GM wheat lines are all white wheat varieties (Jackson 2011). One durum wheat cultivar, Stewart is also used to generate some GM wheat lines. Durum wheat varieties have been described as sharing the same seed coat characteristics as white wheat varieties (McCaig & DePauw 1992). Therefore it is considered appropriate that no measures are needed to restrict the access of birds to the trial sites.

194. As stated above, the applicant proposed to surround the planting areas with a 2 m buffer zone maintained as bare fallow and a 10 m monitoring zone with vegetation kept to 10cm high. However, the presence of a 2m buffer zone is not regarded as necessary, given that a 10 m monitoring zone will allow identification of volunteers and related species. Therefore, the 2 m buffer zone has not been imposed as a licence condition. The applicant proposed that the monitoring zone would be surrounded with a 190 m isolation zone where no sexually compatible species will be grown. In addition, a licence condition would require that the planting area, buffer zone, monitoring zone and isolation zone be inspected for presence of related (sexually compatible) species while the GMOs are flowering, and any volunteers or related species found must be destroyed prior to flowering. This would minimise potential gene flow to related species (Risk Scenario 3).

195. Under previous wheat licences, monitoring of flowering is to be conducted fortnightly, from two weeks prior to flowering until cleaning of the sites. The applicant has proposed, for this application, to fortnightly monitor flowering until the completion of flowering. The applicant has provided information regarding flowering patterns for plants grown under controlled conditions in the glasshouse, showing no difference between the GM wheat lines and corresponding untransformed parent lines. However, as plant behaviour can vary between controlled and field conditions, and as six different wheat cultivars are proposed as the parent organisms, flowering may not be synchronised. Therefore, a licence condition requires that fortnightly monitoring of flowering must be conducted from two weeks prior to flowering until four weeks after the end of flowering. This will ensure that all wheat plants have finished flowering.

196. The potential for pollen movement and gene flow between GM wheat and other sexually compatible species has been addressed at some length in previous RARMPs, the latest being DIR 112. On the basis of the evidence detailed there, including scientific literature on gene flow, international containment measures for GM wheat trials, and the standards for producing basic and certified seed, an isolation distance of 200 m is considered adequate to minimise gene flow from the GM wheat plants to other wheat plants or other sexually compatible species outside the release sites. Therefore, the combination of a 10 m monitoring zone, surrounded by a 190 m isolation zone where no wheat plants or related species may be grown and where related species are destroyed prior to flowering, would manage gene flow to other wheat crops and related species (Risk Scenario 3).

197. The applicant has proposed to locate the trial at least 50 m away from the nearest natural waterway, which would reduce the likelihood of plant material being washed away from the sites (Risk Scenario 2). It is a standard licence condition that trial sites be located at least 50 m from waterways to limit the dispersal of viable GM plant material in the event of flooding. A licence condition has also been imposed requiring immediate notification of any extreme weather condition affecting the sites during the release to allow assessment and management of any risks.

198. The applicant has proposed a number of measures to minimise the persistence of any GM wheat plants and seeds in the seed bank at the release sites after harvest of the trial (Risk Scenario 2 and 3). These measures are light tillage and irrigation to promote germination of remaining seed, and monitoring of the trial on a monthly basis for at least two years, and until the trial is free of volunteers for at least 6 months. Volunteer plants that emerge would be destroyed before flowering.

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

200. It is considered by the Regulator that under Australian conditions, a post-harvest monitoring period of at least two years, with monthly inspections, and with no volunteers detected for a minimum of 6 months prior to the end of the time period, would effectively manage survival and persistence of viable wheat seeds in the soil. These measures will minimise the persistence of the GMOs in the environment (Risk Scenario 2). Therefore, a licence condition imposes that after harvest, the trial sites should receive at least 3 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.

201. The applicant proposes that rainfall events of greater than 20 mm in a 24 h period will be deemed to be equivalent to an irrigation event. However a licence condition requires 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 should be tilled to a depth no greater than the depth of sowing. These treatments will ensure 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.

202. In considering potential for spread and persistence of the GMOs, it is important to consider the potential dispersal of grain during sowing and harvesting (mechanical dispersal). This is most likely to result in dispersal of grain into the area immediately around the trial. The licence requires that the planting areas and any other areas where GM material has been dispersed, including during harvest or threshing, must be monitored to manage the possibility of mechanical dispersal of seed from the trial sites and its persistence after the trial. The licence also requires that harvest of GM wheat be conducted separately from other crops. The applicant proposed to conduct harvest by hand or using a dedicated plot harvester. The applicant also proposed that all equipment used in connection with cultivating and harvesting the GMOs such as harvesters, seeders, storage equipment, irrigation piping etc. would be cleaned on site. These measures would minimise human-mediated dispersal of GM plant material (Risk Scenario 2).

203. The applicant does not propose that any of the GM plant material would enter the commercial human food or animal feed supply, and the GM wheat lines have not been assessed for food use by FSANZ. However, non-viable products from GM wheat generated from this trial may be consumed as part of small-scale animal and/or human nutritional studies, under experimental conditions (Risk Scenario 1).

204. It is imposed as a licence condition that nutritional studies involving animals or human volunteers would only be undertaken if approved by an Animal Ethics Committee or a Human Research Ethics Committee, respectively. The Ethics Committees must also be provided with the final risk assessment and risk management plan prepared for application DIR 151 so that they are aware of the Regulator's assessment, including the risk context. The licence includes a condition that material from the GMOs must not be used for feed for animals or food for humans, other than in the proposed animal and human nutritional studies.

205. The applicant proposes to destroy any GMOs not required for experimentation or future planting. The applicant has proposed that milling, crushing and burial be added to the list of approved destruction methods. Crushing and milling are considered effective for destruction, as they render seed non-viable, therefore minimising the risk of germination and/or spread. Deep burial of seed is also considered an effective method of destruction; therefore conditions allowing deep burial, with requirements for monitoring of burial sites, have been included in the licence.

206. Any plant material taken off-site for experimental analysis or future planting would be transported and stored according to the [Regulator's Guidelines for the Transport of GMOs](#). These are standard protocols for the handling of GMOs to minimise exposure of people and other organisms to the GMOs (Risk Scenario 1), dispersal into the environment and gene flow/transfer (Risk Scenario 2 and 3).

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

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

- limit the release to a maximum total area of 1 ha per season per site, at the Boorowa and Ginninderra Experiment Stations, from May 2017 to May 2022
- locate the trial sites at least 50 m away from waterways
- enclose the trial sites with a fence capable of excluding livestock
- surround the planting areas where GMOs are grown with a 10 m monitoring zone, 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 190 m isolation zone, in which no other crops of wheat may be grown, and where growth of related species is controlled
- 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 lines only by hand or by using a dedicated plot harvester
- clean the areas after use
- clean equipment used on the sites after use
- apply measures to promote 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 are detected for a continuous 6 month period
- destroy all GMOs not required for further analysis or future trials
- transport and store GMOs in accordance with the Regulator’s guidelines
- not commence nutritional studies involving animals or human volunteers until endorsed by an Animal Ethics Committee or a Human Research Ethics Committee, respectively
- not allow the GM plant material or products to be used for human food or animal feed, with the exception of the nutritional studies described in the application.

3.2 Other risk management considerations

208. 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

209. 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.

210. On the basis of the information submitted by the applicant and records held by the OGTR, the Regulator considers CSIRO suitable to hold a licence. The licence includes a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

211. 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

212. CSIRO is required to submit a contingency plan to the Regulator before planting the GMOs. This plan will detail measures to be undertaken in the event of any unintended presence of the GM wheat outside permitted areas.

213. CSIRO is also required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. This methodology is required before planting the GMOs.

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

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

3.2.4 Reporting requirements

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

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

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

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

3.2.5 Monitoring for compliance

217. 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.

218. 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.

219. 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

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

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

Section 5 Conclusions of the consultation RARMP

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

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

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Appendix A Summary of submissions from prescribed experts, agencies and authorities⁷

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

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

Sub. No.	Summary of issues raised	Comment
1	Notes that the licence will prohibit the use of the GM plant material in human food or animal feed. Does not have any further comments on the licence application at this stage.	Noted
2	Agrees with the overall conclusion of the consultation RARMP that the risk of the proposed limited and controlled release is negligible. In particular, agrees that the risk of genetically modified (GM) plants spreading and persisting as a weed outside the trial site is negligible, but notes the following points would benefit from expanded discussion in the RARMP: <ul style="list-style-type: none"> information on factors reducing the risk of dispersal by birds, such as the seed coat thinness of the cultivars used dispersal by birds of conventional wheat seed environmental factors that limit survival of GM and non-GM wheat, especially drought and disease resistant lines possibility of natural hybridisation between the introduced weedy <i>Triticeae</i> species (<i>Hordeum marinum</i>) and wheat in Australia producing viable offspring. 	Noted Additional text has been added to Chapter 1, Section 4 and Risk scenario 2 of the RARMP, summarising key points from the OGTR’s wheat Biology document. These relate to weediness characteristics of wheat, environmental factors limiting survival and to potential dispersal by birds. Hybridisation between wheat and <i>Hordeum marinum</i> is extremely rare and has not been recorded in Australia. Additional text to this effect has been added to Chapter 1, Section 6.4.
3	Agrees with the conclusion of the consultation RARMP that the risk of the proposed limited and controlled release is negligible.	Noted

⁷ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Appendix B Summary of submissions from the public

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

Issues raised **E:** environment; **EC:** economic issues; **HHS:** human health and safety; **HU:** Herbicide use; **OSA:** outside the scope of the Act.

Other abbreviations the **Act:** *Gene Technology Act 2000*; **APVMA:** Australian Pesticides and Veterinary Medicines Authority; **FSANZ:** Food Standards Australia New Zealand; **GM:** genetically modified; **L:** Licence; **RARMP:** Risk Assessment and Risk Management Plan; the **Regulator:** Gene Technology Regulator; **Sub. No.:** submission number.

Sub. No.	Issue	Summary of issues raised	Comment
1	HHS, HU	The submitter requests that the OGTR reject licence application DIR 151 for the following reasons: <ul style="list-style-type: none"> Asserts that the cocktail of extra herbicides and pesticides required in GM wheat agriculture is harmful to the health of humans, animals and the environment. Questions the safety of the herbicides glyphosate and glufosinate, and their impact on human and animal health and the environment 	Cultivation of GM wheat in the trial will employ practices similar to those used for commercial cultivation of non-GM wheat. The GM wheat lines proposed for release contain genes for disease resistance, drought tolerance, altered oil content or altered grain composition. They do not contain genes for herbicide tolerance, and pesticide usage practices will not differ from those for non-GM wheat cultivation. The APVMA has regulatory responsibility for safety and efficacy of agricultural chemicals, including herbicides, in Australia.
	HHS	<ul style="list-style-type: none"> Claims that there is no safety monitoring of the effect of GM products on health and that studies (cited in the submission) have demonstrated with animals the serious possible health effects from eating GM food. 	No products from this GM wheat trial will enter the commercial human food or animal feed supply. The RARMP concluded that the limited and controlled release of the GM wheat lines included in this application poses negligible risks to the health and safety of people. FSANZ has critically evaluated the studies cited as evidence of adverse effects from GM foods and concluded that these studies provided no grounds to revise its conclusions on the safety of food derived from the previously approved GM crops.
	E	<ul style="list-style-type: none"> Claims that there is no safety monitoring of the effect of GM products in the environment: GM crop cultivation threatens the environment and is linked to loss of genetic diversity and ecosystem collapse. 	Application DIR 151 requests approval for a small field trial of GM wheat plants under limited and controlled conditions. Strict licence conditions have been imposed to restrict the spread and persistence of the GMOs in the environment, including isolation from sexually compatible species, and cleaning and post-harvest monitoring of trial sites. Any GMOs in the field must be destroyed at the conclusion of the trial. Similar conditions have been effective for other GM wheat trials conducted in Australia.
	EC, OSA	<ul style="list-style-type: none"> Claims that taxpayers' funds are used for research that would profit overseas 	The commercial motives of biotechnology companies are outside the scope of responsibility of the Regulator.

Sub. No.	Issue	Summary of issues raised	Comment
		agribusiness companies and biotechnology corporations	