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

June 2024

Risk Assessment and Risk Management Plan (consultation version) for

DIR 204

Limited and controlled release of wheat genetically modified for increased tolerance to environmental stress

Applicant: Trigall Australia Pty Ltd

This RARMP is open for consultation until 16 July 2024.

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

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

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

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

Summary of the Risk Assessment and Risk Management Plan (Consultation Version) for Licence Application No. DIR 204

Introduction

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

The application

Project Title	Limited and controlled release of wheat genetically modified for increased tolerance to environmental stress
Applicant	Trigall Australia Pty Ltd
Parent organism	Wheat (*Triticum aestivum* L.)
Genetic modifications	
Introduced genes and modified traits	• *HaHB4* gene from *Helianthus annuus* conferring environmental stress tolerance • *bar* gene from *Streptomyces hygroscopicus* as a selectable marker gene conferring glufosinate herbicide tolerance
Genetic modification method	Biolistic transformation
Unique identifier of GMO	IND-ØØ412-7
Trade name of GMO	HB4 wheat
Principal purpose	To gather research and regulatory data for HB4 wheat under Australian growing conditions, including under environmental stress
Previous releases	HB4 wheat is approved for commercial cultivation in Argentina, Brazil and Paraguay
Proposed limits	
Proposed use of GM plants	No use in human food or animal feed proposed
Proposed location/s	Up to 10 sites per year in wheat growing areas of New South Wales, Victoria, Western Australia and South Australia
Proposed release size	Up to 20 hectares per year in total
Proposed period of release	From issue of licence until December 2029

Risk assessment

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both short- and long-term risks are considered.

Credible pathways to potential harm that were considered included exposure of people or animals to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM plants. Potential harms associated with these pathways included adverse health effects in people or animals, and environmental harms due to weediness.

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. The principal reasons for the conclusion of negligible risks are that the proposed limits and controls will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications could lead to harm to people or the environment.

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. Draft licence conditions are detailed in Chapter 4 of the RARMP.

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

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
cm	centimetre
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
ha	hectare
HGT	horizontal gene transfer
IBC	Institutional Biosafety Committee
LC-MS	liquid chromatography–mass spectrometry
m	metre
NLRD	Notifiable Low Risk Dealings
NSW	New South Wales
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin N-acetyltransferase
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SA	South Australia
the Act	*Gene Technology Act 2000*
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and Sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.

5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

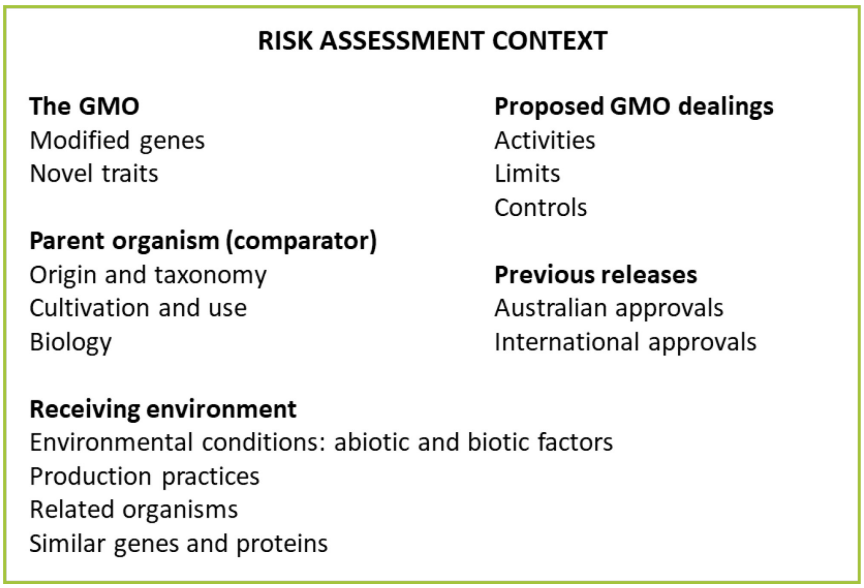

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework.

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

1.1 Interface with other regulatory schemes

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry. These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

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

Section 2 The proposed dealings

9. Trigall Australia Pty Ltd (the applicant, Trigall Australia) proposes to release wheat genetically modified for increased tolerance to environmental stress. The GM wheat has the OECD unique identifier IND-ØØ412-7. Its trade name is HB4 wheat.

10. The purpose of the proposed field trial is to evaluate HB4 wheat under Australian growing conditions, including under environmental stress. The trial will gather research and regulatory data regarding agronomic performance, environmental stress tolerance, nutritional assessment, compositional analysis, molecular analysis, and genetic stability. Some experiments may involve milling GM wheat grains into flour.

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

- conduct experiments with the GMOs
- breed the GMOs
- propagate the GMOs
- grow the GMOs
- use the GMOs in the course of manufacture of a thing that is not a GMO
- import the GMOs
- transport the GMOs
- dispose of the GMOs

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

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

12. The field trial is proposed to take place from issue of the licence until December 2029. This period would cover 5 winter planting seasons and 5 summer planting seasons. The GM wheat would be grown primarily as a winter crop, but occasionally a summer crop cycle may be grown.

13. The GM wheat would be grown on up to 10 trial sites per year, with an area of up to 2 ha per site. The maximum combined planting area would be 20 ha per year.

14. The trial sites would be selected from 135 possible local government areas (LGAs) in New South Wales (NSW), Victoria, Western Australia (WA) and South Australia (SA) (Table 1). The field trials would occur on sites owned by the applicant or on private land in rural areas.

Table 1. LGAs where GM wheat trial sites may be located

NSW	Victoria	WA	SA
Berrigan	Ararat	Albany	Adelaide Plains
Bland	Ballarat	Beverley	Barossa
Blayney	Benalla	Boddington	Light
Cabonne	Buloke	Boyup Brook	Wakefield

NSW	Victoria	WA	SA
Coolamon	Campaspe	Bridgetown-Greenbushes	
Coonamble	Central Goldfields	Brookton	
Cootamundra-Gundagai	Colac Otway	Broomehill-Tambellup	
Cowra	Corangamite	Carnamah	
Dubbo	Gannawarra	Coorow	
Edward River	Glenelg	Corrigin	
Federation	Golden Plains	Cranbrook	
Forbes	Greater Bendigo	Cuballing	
Gilgandra	Greater Geelong	Cunderdin	
Greater Hume	Greater Shepparton	Dalwallinu	
Griffith	Hepburn	Denmark	
Gunnedah	Hindmarsh	Donnybrook-Balingup	
Gwydir	Horsham	Dowerin	
Hay	Indigo	Dumbleyung	
Hilltops	Loddon	Esperance	
Inverell	Macedon Ranges	Gnowangerup	
Junee	Mildura	Goomalling	
Leeton	Mitchell	Greater Geraldton	
Liverpool Plains	Moira	Jerramungup	
Lockhart	Moorabool	Katanning	
Mid-Western	Mount Alexander	Kent	
Moree Plains	Moyne	Kojonup	
Murray River	Northern Grampians	Manjimup	
Murrumbidgee	Pyrenees	Merredin	
Muswellbrook	Southern Grampians	Mingenew	
Narrabri	Strathbogie	Moora	
Narrandera	Swan Hill	Morawa	
Narromine	Wangaratta	Nannup	
Orange	West Wimmera	Narrogin	
Parkes	Wodonga	Northam	
Snowy Valleys	Wyndham	Perenjori	
Tamworth	Yarriambiack	Pingelly	
Temora		Plantagenet	
Upper Hunter		Quairading	
Wagga Wagga		Ravensthorpe	
Walgett		Tammim	
Warren		Three Springs	
Warrumbungle		Toodyay	
Weddin		Victoria Plains	
		Wagin	
		Wandering	
		West Arthur	
		Wickepin	
		Williams	
		Wongan-Ballidu	
		Woodanilling	
		Wyalkatchem	
		York	

15. Only trained and authorised persons would be permitted to deal with the GM wheat.

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

16. 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 each trial site at least 50 m away from the nearest natural waterway
- only permitting authorised persons to access the trial sites
- surrounding each planting area with a 10 m monitoring zone and a 50 m inspection zone that are inspected while the GMOs are flowering to destroy any wheat or sexually compatible plants
- surrounding each inspection zone with a 140 m isolation zone where no wheat or sexually compatible plants are grown
- treating non-GM wheat plants grown on the trial sites as if they are GMOs
- inspecting and cleaning all equipment used on trial sites prior to use for any other purpose
- controlling rodents on trial sites
- cleaning of planting areas post-harvest
- monitoring each post-harvest planting area for volunteers for two years, inspecting every 55 days, and destroying any volunteers found
- tilling and irrigating each planting area during the post-harvest monitoring period
- transporting and storing GMOs in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all GMOs from the trial not required for testing or future planting
- not engaging personnel who have a known allergy to wheat to work with the GM wheat
- not allowing the GMOs or GM products to be used for human food or animal feed.

17. Figure 2 shows the trial site layout proposed by the applicant.

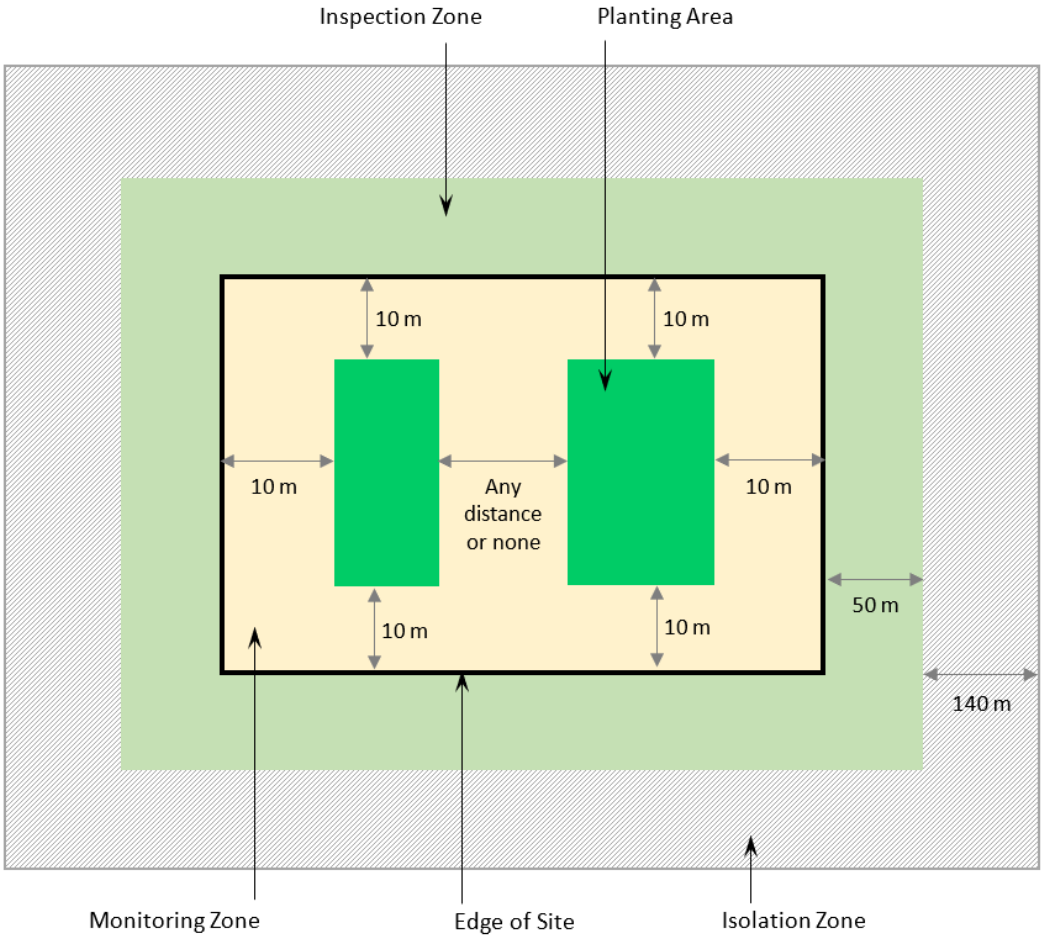

Figure 2. Schematic diagram (not to scale) of proposed trial site layout. Each trial site may include one or multiple planting areas.

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

Section 3 The parent organism

19. The parent organism of the GMOs is *Triticum aestivum* L. The terms 'wheat' and 'bread wheat' will be used as general terms to refer to *T. aestivum* throughout this document. The other wheat species grown in Australia is *Triticum turgidum* subsp. *durum* (Desf.) Husn., also known as durum or pasta wheat.

20. Detailed information about the parent organism is contained in the reference document produced to inform the risk analysis process for licence applications involving GM wheat: *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021). This document is available from the Resources page on the OGTR website. Baseline information from this document will be used and referred to throughout the RARMP.

21. Wheat is Australia's largest agricultural crop, with over 12 million hectares of wheat planted in Australia in 2023 (ABARES, 2023). Commercial wheat is cultivated in the 'wheat belt' from south-eastern Queensland through NSW, Victoria, Tasmania, southern SA and southern WA. The major uses of wheat grown in Australia are as either a grain crop for human consumption or a feed grain for livestock (AEGIC, 2020).

22. Wheat is exotic to Australia, but is present outside cultivation in all states and territories of Australia (Atlas of Living Australia website, accessed 4 March 2024). Wheat is not a weed of national significance or a declared weed in any state or territory (Weeds Australia website, accessed 4 March 2024). A weed risk assessment for wheat by the OGTR found that wheat possesses few weedy attributes (OGTR, 2021). Similarly, a weed risk assessment of wheat by the Victorian government placed wheat in the lowest weed risk ranking category (White et al., 2022).

23. Wheat contains a number of allergens. Allergic responses can occur in susceptible individuals due to ingestion of food containing wheat, inhalation of wheat flour, inhalation of wheat pollen, or skin contact with wheat proteins (Stobnicka and Gorny, 2015; Quirce et al., 2016; Venter et al., 2016). Ingestion of food containing wheat can also cause adverse health effects in people with coeliac disease, which is an autoimmune response triggered by gluten in wheat grain, or in people with non-coeliac wheat sensitivity (Rej et al., 2020).

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

4.1 Introduction to the GMOs

24. The GM wheat is known by the trade name HB4 wheat or by the OECD unique event identifier IND-ØØ412-7. It contains two introduced genes, which are described in Table 2.

Table 2. Introduced genes in HB4 wheat

Gene	Source organism	Function
HaHB4	Sunflower (*Helianthus annuus*)	Encodes transcription factor conferring environmental stress tolerance
bar	*Streptomyces hygroscopicus*	Selectable marker gene conferring glufosinate herbicide tolerance

25. The expression of the introduced genes is controlled by introduced regulatory sequences, which are listed in Table 3. As the two introduced genes both have a strong constitutive promoter, the introduced genes are expected to be expressed in all GM wheat plant tissues.

Table 3. Introduced regulatory sequences in HB4 wheat

Genetic element	Source organism	Function
ubi1 (ubiquitin) promoter	Maize (*Zea mays*)	Constitutive promoter
ubi1 5' untranslated exon	Maize (*Z. mays*)	Enhance transgene expression
ubi1 first intron	Maize (*Z. mays*)	Enhance transgene expression
nos terminator	*Agrobacterium tumefaciens*	Terminator

4.2 Method of genetic modification

26. HB4 wheat was generated by biolistic transformation. Information about this method can be found in the document Methods of plant genetic modification, available from the OGTR Risk Assessment References page. The parental wheat variety was co-transformed with plasmids *pIND4-HB4* and *pIND4-Bar.* Plasmid maps of these two plasmids are shown in Figure 3.

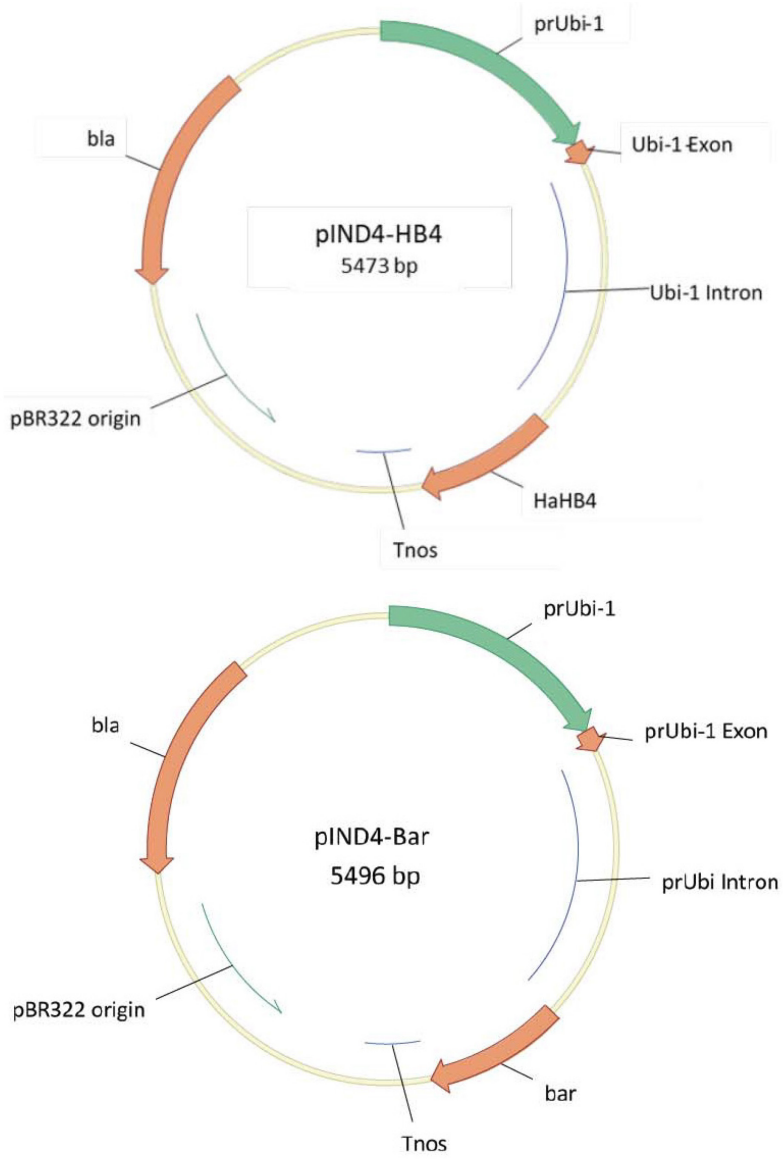

Figure 3. Plasmid maps of plasmids pIND-HB4 and pIND4-Bar. Supplied by applicant.

27. Trigall Genetics (the parent company of Trigall Australia) characterised the genetic modification in HB4 wheat using Southern blotting and genomic sequencing (Trigall Genetics, 2021). The GM wheat was found to contain two complex DNA insertions in a single genetic locus. The insertions included

multiple intact or partial copies of transgenes (Table 4). The insertions have not interrupted any known endogenous wheat genes.

Table 4. Gene copy numbers in HB4 wheat

Gene	Total copies	Intact copies	Intact copies with functional regulatory elements
HaHB4	3	2	1
bar	8	7	3
bla	19	12	0
gus	4	0	0

28. As shown in Table 4, HB4 wheat contains copies of two intentionally introduced gene sequences (*HaHB4* and *bar*) and two unintentionally introduced gene sequences (*bla* and *gus*). The *bla* gene is part of the plasmid backbone of both *pIND4 HB4* and *pIND4-Bar*. It is an *Escherichia coli* gene that encodes the β-lactamase protein conferring ampicillin antibiotic resistance, used as a selectable marker gene for plasmid-carrying bacteria. The *gus* gene apparently originated from a third plasmid that was not intentionally used for transformation but was present during the transformation process. The *gus* gene is an *E. coli* gene encoding the β-glucuronidase enzyme, used as a visual marker to monitor the efficiency of transformation (Trigall Genetics, 2021).

29. The *bla* gene is under the control of a bacterial promoter that would not function in plants. Therefore, the *bla* gene is not expected to be expressed in HB4 wheat.

30. There are no intact copies of the *gus* gene in HB4 wheat, and none of the partial copies have intact promoter sequences. Therefore, the *gus* gene is not expected to be expressed in HB4 wheat.

31. HB4 wheat contains one *HaHB4* gene copy and 3 *bar* gene copies with complete regulatory sequences (Table 4). ELISA and LC-MS techniques confirmed that the *HaHB4* and *bar* genes are expressed in the GM wheat (Trigall Genetics, 2021).

4.3 The introduced genes and encoded proteins

HaHB4

32. The sunflower *HaHB4* gene encodes the transcription factor Hahb-4 (*Helianthus annuus* homeobox-4). The *HaHB4* gene was found to be expressed at very low levels in sunflower plants grown under normal conditions. *HaHB4* expression was strongly induced by drought stress and moderately induced by salt stress, but was not detectably induced by cold stress or heat stress (Gago et al., 2002).

33. GM *Arabidopsis thaliana* plants that constitutively express *HaHB4* were generated. The GM plants had a phenotype of reduced height, smaller leaves and delayed flowering, but no reduction in seed production, in comparison to wild-type plants. Under mild water deficit conditions, wild-type plants wilted, but GM plants expressing *HaHB4* were unaffected. Under severe water deficit conditions, only 9% of wild-type plants survived, but 73% of GM plants survived (Dezar et al., 2005b).

34. Overexpression of the *HaHB4* gene induces a wide range of changes in the Arabidopsis transcriptome, with significant increases or decreases to the level of transcription of 815 genes. The proposed mechanism for drought stress tolerance observed in transgenic plants expressing *HaHB4* is related to inhibition of leaf senescence (Manavella et al., 2006).

35. *HaHB4* expression in sunflower was reported to be induced by mechanical damage or caterpillar feeding activity. GM Arabidopsis and maize plants that constitutively express *HaHB4* demonstrated upregulation of herbivory defence genes and reduced feeding activity by caterpillars in comparison to wild-type plants (Manavella et al., 2008).

36. In the GM wheat, the *HaHB4* gene is expected to confer increased tolerance to environmental stresses, including drought stress, salt stress and biotic stress from invertebrate feeding, to protect crop yield.

bar

37. The *bar* (bialaphos resistance) gene was isolated from *S. hygroscopicus* (Thompson et al., 1987). It encodes a phosphinothricin acetyltransferase (PAT) enzyme that confers tolerance to glufosinate herbicide. PAT acetylates glufosinate, converting it to *N*-acetyl-L-glufosinate which is not toxic to plants (OECD, 2002).

38. The Regulator has previously assessed and approved GM crops containing the *bar* gene for commercial release in Australia, most recently under licence DIR 190.

39. The applicant intends to use the *bar* gene as a selectable marker gene during breeding of the GM wheat. A half dose of glufosinate herbicide will be applied to plants to identify null segregants that do not contain the GM event. The applicant is not proposing to apply glufosinate to the GM wheat in the field trials except for selection during field breeding.

4.4 Potential adverse health effects of the GM wheat

40. In 2022, FSANZ approved the use of HB4 wheat as food. The safety assessment (FSANZ, 2021) considered data regarding:

- potential toxicity or allergenicity of the introduced Hahb-4 protein
- potential toxicity or allergenicity of the introduced PAT protein
- potential toxicity or allergenicity of putative peptides encoded by open reading frames created by the DNA insertions, and
- compositional analysis of whole grain from HB4 wheat.

Based on this information, the FSANZ assessment concluded that no health or safety concerns were identified for HB4 wheat. Food derived from HB4 wheat is considered as safe for human consumption as food derived from non-GM wheat.

41. In a nutritional study, 120 broiler chickens were fed flour from HB4 wheat as 40% of their diet over a six-week period. The chickens fed HB4 wheat gained weight at the same rate as control chickens fed with the non-GM parental wheat. The study also examined general health of the chickens twice per day, as well as monitoring mortality, and did not report any differences between chickens fed the HB4 wheat or the non-GM parental wheat (Miranda et al., 2022).

42. As discussed in Section 3, non-GM wheat contains a number of protein allergens. As the Hahb-4 protein is a transcription factor that affects transcription of many genes (see Section 4.3), it is possible that HB4 wheat could have altered levels of some endogenous wheat allergens. A paper studying the effect of *HaHB4* on defence genes found that constitutive expression of *HaHB4* induces increased expression of trypsin inhibitor enzymes in sunflower, maize and Arabidopsis plants, for example a 6-fold increase in transcript levels of trypsin inhibitor 2 in transformed sunflower leaves compared to a non-transgenic control (Manavella et al., 2008). Several proteins from the wheat α-amylase/trypsin inhibitor family have been identified as important allergens in baker's asthma, the allergic response to inhalation of wheat flour (Salcedo et al., 2011). Similarly, constitutive expression of *HaHB4* causes a 5-fold increase in transcript levels of serine protease inhibitor 1 in transformed sunflower leaves (Manavella et al., 2008), and a serine protease inhibitor is a known wheat allergen (Salcedo et al., 2011). It is plausible that HB4 wheat could contain increased levels of wheat allergens from the α-amylase/trypsin inhibitor family and/or the serine protease inhibitor family.

43. The Brazilian risk assessment for HB4 wheat in food or feed analysed data on the differential expression of genes between HB4 wheat grains and wheat grains from the non-GM parental variety. Under normal growing conditions, 258 genes were overexpressed in HB4 wheat grains, and 19 of the

overexpressed genes have been previously identified as potentially allergenic. However, the increased levels of expression of these 19 allergenic genes were still within the range of expression levels found in other wheat genotypes (CTNBio, 2021).

44. No published data was found regarding levels of endogenous wheat allergens in HB4 pollen or vegetative tissue. The *HaHB4* gene is derived from sunflower. In sunflower, water stress strongly induces expression of *HaHB4* in vegetative tissues, but expression is not detectable in reproductive organs (Gago et al., 2002; Dezar et al., 2005a). This suggests that the *HaHB4* gene is evolved to have activity in vegetative tissue rather than reproductive tissue, so is likely to have a greater effect on gene expression levels in vegetative tissue than in reproductive tissue.

45. If HB4 wheat has increased levels of endogenous wheat allergens, people with a wheat allergy could have a stronger reaction to HB4 wheat than to non-GM wheat. In a study of six patients with wheat allergy who had an early asthmatic response to inhalation of wheat flour proteins, the dose-response curves were not linear, but all patients showed a clear trend of increasing dose of inhaled wheat proteins causing increasing impairment of lung function (Salvatori et al., 2008).

46. Gluten, the trigger for autoimmune responses in coeliac disease, accounts for approximately 80% of total protein in wheat grain (Rej et al., 2020). A compositional study found that the total protein content of HB4 wheat grain is not significantly different from the non-GM parental wheat (Ayala et al., 2019). Therefore, the gluten content in HB4 wheat grain could not be different from the non-GM parental wheat by more than a few percent. This is negligible compared to variations in gluten content between non-GM commercial wheat cultivars, which are reported to contain from 5.8 to 12.7 g gluten per 100 g flour (Schuster et al., 2022).

47. The applicant states that HB4 wheat has been extensively field tested in several countries since 2007 and no harmful consequences were observed.

4.5 Characterisation of the GMOs

48. Field trials of HB4 wheat were conducted at 37 field trial sites in Argentina between 2009 and 2017. HB4 wheat had higher average grain yield than the non-GM parental wheat: 16% higher at trial sites with water deficit conditions and 3% higher at trial sites with no water deficit. The difference in grain yield was due to increased grain number per square metre rather than increased grain size. Average water use efficiency of HB4 wheat was calculated as 9% higher than the non-GM parental wheat (Gonzalez et al., 2019).

49. HB4 wheat plants grown in a greenhouse had no significant difference in development times or plant height compared to non-GM parental wheat plants. Anthesis and maturity dates of HB4 wheat were measured at one field trial site, and were not significantly different from non-GM parental wheat (Gonzalez et al., 2019).

50. The applicant states that HB4 wheat has been extensively field tested in several countries. No unintended phenotypic changes were observed in these field trials.

51. A compositional analysis of field-grown HB4 wheat tested grain samples for 43 nutrients and anti-nutrients and forage samples for 10 nutrients. Results indicated that HB4 wheat is compositionally equivalent to non-GM wheat (Ayala et al., 2019).

Section 5 The receiving environment

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

53. Detailed information about non-GM wheat in the Australian environment is presented in the document *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021).

5.1 Relevant abiotic factors

54. The proposed trial sites may be located in any of 135 LGAs in wheat-growing areas of NSW, Victoria, WA and SA. These LGAs have a range of climate types.

55. The key abiotic stresses affecting wheat crops in Australia are drought stress, heat stress and frost (GRDC, 2016a, b).

5.2 Relevant biotic factors

56. In Australian agriculture, a range of invertebrate pests including mites, insects and nematodes feed on wheat plants in the field. Birds, kangaroos, rabbits and mice may also feed on wheat crops (OGTR, 2021).

57. Most of the important pathogens affecting wheat crops are fungal diseases. Some major fungal diseases are transmitted via a 'green bridge' of volunteer cereal plants that survive between growing seasons (GRDC, 2016b).

5.3 Relevant agricultural practices

58. The controls proposed for the field trial are outlined in Section 2.2 of this Chapter. Aside from implementing these controls, it is proposed that the GM wheat would be cultivated using conventional crop management practices for wheat.

59. The GM wheat would be grown as a dryland crop, but drip or pipe irrigation may be used to maintain the crop in challenging weather situations or to control the water regime where necessary. Herbicides and pesticides would be applied according to label instructions to manage the health of the crop. Some GM wheat plants may be tented or bagged to facilitate controlled in-field breeding. The GM wheat would be hand harvested for small areas or harvested with commercial equipment for larger areas.

5.4 Presence of related plants in the receiving environment

60. Bread wheat (*T. aestivum*) is sexually compatible with other bread wheat or durum wheat (*T. turgidum* subsp. *durum*) plants. Bread wheat is commercially cultivated in all of the LGAs where proposed field trial sites may be located, and durum wheat is cultivated in some of these LGAs. Wheat plants would be present as volunteers as well as crops (OGTR, 2021).

61. Triticale, which is a hybrid between wheat and rye, is a minor crop in Australia (Australian grain production, accessed 13 May 2024). There is little evidence available regarding whether triticale can naturally cross with bread wheat in the field. However, a greenhouse study using hand pollination found that about 20% of pollination events between bread wheat and triticale where triticale was the female parent produced viable and self-fertile hybrids (Hills et al., 2007). Therefore, it is possible that GM wheat could cross-pollinate triticale plants.

62. Rye (*Secale cereale*) is also a minor crop in Australia (Australian grain production, accessed 13 May 2024). Very rare natural hybridisation events between bread wheat and rye have been reported in the literature (Hegde and Waines, 2004). However, bread wheat and rye are only crossable where bread wheat is the female parent (Laugerotte et al., 2022). Therefore, it is not possible for GM wheat to cross-pollinate rye plants.

63. No other plant species that naturally cross with bread wheat and produce fertile offspring are present in the Australian environment. Further details are available in the biology document for wheat (OGTR, 2021).

5.5 Presence of similar genes and encoded proteins in the environment

64. The introduced *HaHB4* gene was derived from sunflower. Sunflower is a minor crop grown in Australia for oil production, bird seed and direct human consumption (GRDC, 2017).

65. The introduced *bar* gene was derived from *S. hygroscopicus*, a soil bacterium that was first isolated from Australian soil samples (Jensen, 1931). The *bar* gene is also present in several commercial GM crops grown in Australia.

66. People and animals are routinely exposed to these genes and their encoded proteins in the environment.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

67. In 2022, FSANZ approved use of food derived from HB4 wheat (application A1232).

68. The Regulator has not previously assessed or approved HB4 wheat.

69. The Regulator has previously approved 24 field trial licences for genetically modified wheat, of which 13 licences are for wheat genetically modified for abiotic stress tolerance. Information on these GM wheat licences is available from the OGTR GMO Record. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

70. The Regulator has not approved commercial release of any GM wheat in Australia.

6.2 International approvals

71. HB4 wheat was approved for commercial cultivation in Argentina in 2020, in Brazil in 2023, and in Paraguay in 2023 (ISAAA website; accessed 21 March 2024; BioTrack Product database; accessed 21 March 2024).

72. In Argentina, commercial HB4 wheat was grown on 53,000 ha in 2021-22 and 44,000 ha in 2022-23 (AgbioInvestor, 2024)(USDA Foreign Agricultural Service website; accessed 21 March 2024).

73. HB4 wheat has been approved for food and feed use in Argentina, Brazil, Colombia, Indonesia, Nigeria, Paraguay, South Africa and the United States (ISAAA website; accessed 21 May 2024; USDA Foreign Agricultural Service website; accessed 21 May 2024).

74. HB4 soybean, which is a GM soybean containing the same introduced genes as HB4 wheat, has been approved for commercial cultivation in Argentina, Brazil, Paraguay, the United States and Canada (ISAAA website; accessed 21 March 2024; BioTrack Product database; accessed 21 March 2024).

Chapter 2 Risk assessment

Section 1 Introduction

75. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

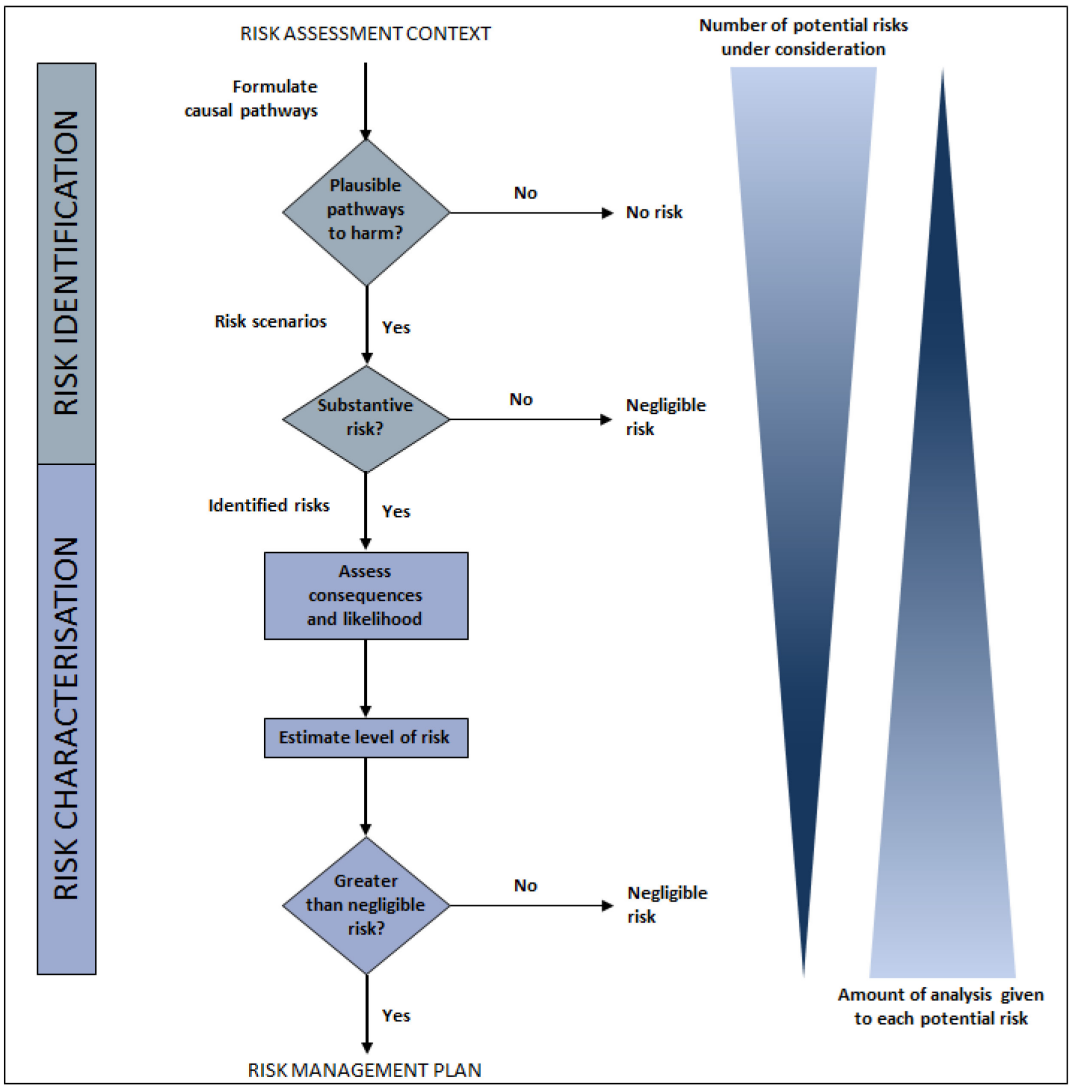

Figure 4. The risk assessment process

76. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

77. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

78. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly

occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 4), i.e. the risk is considered to be no greater than negligible.

79. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

80. A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs are also considered.

Section 2 Risk identification

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

i. the source of potential harm (risk source)

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

iii. potential harm to people or the environment.

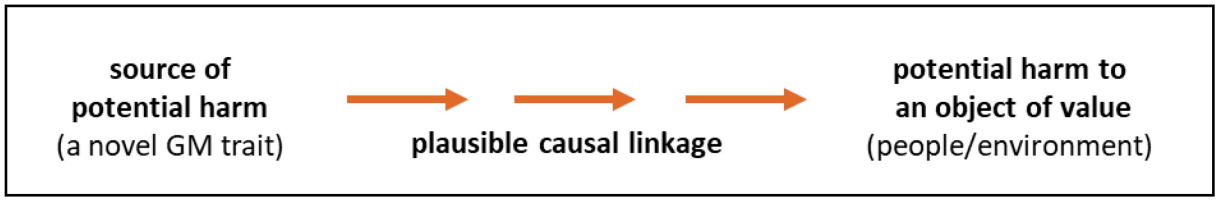

Figure 5. Components of a risk scenario

82. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings,
- the proposed limits including the extent and scale of the proposed dealings,
- the proposed controls to limit the spread and persistence of the GMO, and
- the characteristics of the parent organism(s).

2.1 Risk source

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

84. As discussed in Chapter 1, Section 4, the GM wheat has been modified by introduction of the *HaHB4* gene conferring environmental stress tolerance. This introduced gene will be considered further as a source of potential harm.

85. The GM wheat also contains the introduced *bar* gene that confers glufosinate herbicide tolerance and is used as a selectable marker gene. The *bar* gene has been previously assessed as a herbicide tolerance gene in RARMPs for multiple commercial GM crops (most recently DIR 190), and as a marker gene in RARMPs for multiple GM wheat field trials (most recently DIR 201). These RARMPs are available from the OGTR GMO Record. As the *bar* gene has not been found to pose a substantive risk to either people or the environment in previous assessments, this introduced gene will not be further considered as a source of potential harm.

86. The GM wheat contains gene sequences derived from the marker genes *bla* and *gus*. As discussed in Chapter 1, Section 4.2, these genes are not expected to be expressed as proteins, so would have no effect on the phenotype of the GM wheat. People or other organisms could only be exposed to the DNA sequences, and dietary DNA has no toxicity (Delaney et al., 2018). Therefore, these gene sequences will not be further considered as a source of potential harm.

87. The introduced genes are controlled by introduced regulatory sequences. Regulatory sequences, such as promoters, enhancer sequences and terminators, are naturally present in all plants and the introduced sequences are expected to operate in similar ways to endogenous sequences. The regulatory sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Delaney et al., 2018). Hence, potential for harm from the regulatory elements will not be considered further.

2.2 Causal pathway

88. The following factors are considered when postulating plausible causal pathways to potential harm:

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

89. Although all of these factors are taken into account, some are not included in the risk scenarios because they have been considered in previous RARMPs and are not expected to give rise to substantive risks.

90. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

91. 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

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

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

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

93. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, a plant species may have a different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

94. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 5 and examined in detail in Sections 2.5 - 2.7 (this Chapter).

95. 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 5. Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced *HaHB4* gene	GM wheat grows at the field trial sites ⬇ GM wheat expresses *HaHB4* transcription factor and altered levels of endogenous wheat genes ⬇ Exposure of people who deal with the GM plants, or people in the vicinity of the trial sites, or animals that eat GM plant material	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals	No	• GM plant material would not be used as human food or livestock feed • The small size and short duration of the proposed trial would restrict consumption of GM plant material by wild animals • Proposed limits and controls would minimise exposure of people who may be sensitive to GM plant material • FSANZ has assessed food containing HB4 wheat as safe for human consumption
2	Introduced *HaHB4* gene conferring environmental stress tolerance	GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to non-GM plants outside the trial sites ⬇	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals OR Reduced establishment or	No	• Wheat has low levels of outcrossing • The proposed controls would minimise pollen flow from the GM wheat to non-GM plants outside the trial sites • GM wheat volunteers could be controlled by

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
		Establishment of populations of GM wheat expressing *HaHB4* in the environment	yield of desirable plants OR Increased reservoir for pathogens or pests		most standard weed management measures • FSANZ has assessed food containing HB4 wheat as safe for human consumption
3	Introduced *HaHB4* gene conferring environmental stress tolerance	GM wheat grows at the field trial sites ⬇ Persistence of GM wheat seed at the trial sites or dispersal of GM seed outside the trial sites ⬇ Establishment of populations of GM wheat expressing *HaHB4* in the environment	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased reservoir for pathogens or pests	No	• The proposed controls would minimise persistence of GM wheat at the trial sites • The proposed controls would minimise dispersal of GM wheat outside trial sites • GM wheat volunteers could be controlled by most standard weed management measures • FSANZ has assessed food containing HB4 wheat as safe for human consumption

2.5 Risk scenario 1

Risk Source	Introduced *HaHB4* gene
Causal Pathway	GM wheat grows at the field trial sites ⬇ GM wheat expresses *HaHB4* transcription factor and altered levels of endogenous wheat genes ⬇ Exposure of people who deal with the GM plants, or people in the vicinity of the trial sites, or animals that eat GM plant material
Potential Harm	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals

Risk source

96. The source of potential harm for this postulated risk scenario is the introduced *HaHB4* gene in the GM wheat.

Causal Pathway

97. HB4 wheat would be grown at the field trial sites. The introduced *HaHB4* gene is under the control of a constitutive promoter, so the encoded Hahb-4 protein is likely to be produced in all GM wheat plant tissues. As Hahb-4 is a transcription factor that affects the transcription of many genes (Chapter 1, Section 4.3), any plant tissues containing Hahb-4 are also expected to have altered levels of endogenous wheat proteins.

98. The applicant proposes that HB4 wheat grown in the trial would not be used as human food. Therefore, people would not be exposed to the GM plant material through food.

99. People conducting the dealings could be exposed to GM plant material via inhalation of pollen during flowering, inhalation of wheat dust during harvesting operations, inhalation of flour during milling or experimentation, or skin contact with plant tissues. Due to the small scale of the proposed trial, only a limited number of people would engage in dealings with the GM plant material.

100. Wheat pollen is wind dispersed, and although most pollen falls within 3 m of the source plant, some travels up to 60 m (reviewed in Hegde and Waines, 2004). Therefore, people who are not involved with the trial but who pass within 60 m of a planting area while the GM wheat is flowering could be exposed to low levels of GM pollen via inhalation. However, due to the small scale of the proposed trial and the location of field trial sites in agricultural areas, only a very limited number of people not involved with the trial could be exposed to small amounts of GM pollen.

101. The applicant proposes that HB4 wheat grown in the trial would not be used as animal feed. Therefore, livestock would not be exposed to the GM plant material.

102. Desirable wild animals, including birds and insects, could enter the trial sites and eat GM plant material. However, the small size and short duration of the proposed field trial would restrict the number of animals that could be exposed to the GM wheat.

Potential harm

103. FSANZ has approved use of food derived from HB4 wheat, which is considered as safe for human consumption as food derived from non-GM wheat. The safety assessment (FSANZ, 2021) considered data characterising the introduced Hahb-4 protein and concluded that the Hahb-4 protein is unlikely to be directly toxic or allergenic. A number of other countries have also approved the use of HB4 wheat as food and feed (see Chapter 1, Section 6.2).

104. As Hahb-4 is a transcription factor, the GM wheat is expected to have altered levels of endogenous wheat proteins. Non-GM wheat is not known to produce protein toxins (OGTR, 2021), so altered levels of endogenous proteins in the GM wheat would not cause increased toxicity. However, as discussed in Chapter 1, Section 4.4, non-GM wheat does produce a number of protein allergens, and a study reports that HB4 wheat grain has increased levels of some endogenous allergens, although all within the reported range for non-GM wheat. There is uncertainty regarding whether these increases have biological significance, especially in plant tissues other than grain. If HB4 wheat produces biologically significant increased levels of one or more wheat allergens, people who are sensitive to these wheat allergens could have a stronger reaction to HB4 wheat than to non-GM wheat. The applicant proposes to not engage persons who have a known sensitivity to wheat to conduct dealings with the GM wheat, and this measure would reduce any risks from increased levels of wheat allergens.

105. As discussed in Chapter 1, Section 4.4, the gluten content of the GM wheat is not meaningfully different from non-GM wheat. Therefore, the genetic modifications are not expected to cause harm to people with coeliac disease.

106. A study of GM Arabidopsis and maize plants that constitutively express *HaHB4* demonstrated upregulation of herbivory defence genes, including genes encoding anti-nutrients such as protease inhibitors (Manavella et al., 2008). Therefore, HB4 wheat could have increased levels of anti-nutrients compared to non-GM wheat, which would reduce the nutritional value of the GM plant material. However, a feeding study where chickens were fed flour from HB4 wheat found that HB4 wheat grains had the same nutritional value as non-GM wheat grains (Miranda et al., 2022). Although other tissues of HB4 wheat could still have lower nutritional value than non-GM wheat tissue, this is unlikely to harm desirable wild animals or birds that feed on the GM wheat at trial sites, as there are other food sources in the vicinity of trial sites.

107. As discussed in Chapter 1, Section 4.4, there have been extensive field trials of HB4 wheat outside Australia and no harmful consequences were observed.

Conclusion

108. Risk scenario 1 is not identified as a substantive risk because GM plant material would not be used as human food or livestock feed, the small size and short duration of the proposed trial would restrict consumption of GM plant material by wild animals, and proposed limits and controls would minimise exposure of people who may be sensitive to GM plant material. In addition, FSANZ has assessed food containing HB4 wheat as safe. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.6 Risk scenario 2

Risk Source	Introduced *HaHB4* gene conferring environmental stress tolerance
Causal Pathway	GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to non-GM plants outside the trial sites ⬇ Establishment of populations of GM wheat expressing *HaHB4* in the environment
Potential Harm	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased reservoir for pathogens or pests

Risk source

109. The source of potential harm for this postulated risk scenario is the introduced *HaHB4* gene in the GM wheat, which confers the trait of environmental stress tolerance.

Causal Pathway

110. HB4 wheat would be grown at the field trial sites. When the GM wheat flowers, GM pollen could be carried by wind to sexually compatible plants growing in the vicinity of the trial sites. If these related plants are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM seed.

111. As discussed in Chapter 1, Section 5.4, bread wheat is sexually compatible with other bread wheat or durum wheat plants, and may be able to cross-pollinate triticale plants. Plants of these species could be present in the vicinity of the trial sites either as cultivated crops or as volunteers.

112. Wheat is largely self-pollinating. A study of gene flow in bread wheat found that the average rate of cross pollination from a pollen donor field to recipient plants adjacent to the field was <0.5%. The rate of cross-pollination declined rapidly with distance, and was <0.01% in recipient plants 60 m or more from the donor field (Matus-Cádiz et al., 2004). A second study found that the rate of cross-pollination from a pollen donor field to adjacent recipient plants was <0.5%, and no cross-pollination was detected at distances of 44 m or more (Hanson et al., 2005). Cross-pollination rates in bread wheat vary depending on the recipient cultivar. In a study using 18 different commercial wheat cultivars as recipients, cross-pollination rates from a source field to plants at distances ≤6 m from the pollen source averaged 0.34% for all cultivars combined, but for some recipient cultivars the average rate was <0.1% and for one recipient cultivar the average rate was 1.66% (Gaines et al., 2007).

Interspecific cross-pollination from bread wheat to durum wheat occurs at lower levels than intraspecific cross-pollination between bread wheat plants (Matus-Cádiz et al., 2004).

113. The *HaHB4* gene is derived from sunflower. In sunflower, water stress strongly induces expression of *HaHB4* in vegetative tissues, but expression is not detectable in reproductive organs (Gago et al., 2002; Dezar et al., 2005a). This indicates that *HaHB4* has not evolved to have activity in reproductive tissue in its source plant, although it could have incidental activity in reproductive tissue when controlled by a constitutive promoter. The applicant conducted extensive field trials of HB4 wheat, and reported that the genetic modification did not alter any reproductive aspects, including pollen viability, of the HB4 wheat compared to the non-GM parent.

114. The applicant proposes to surround each GM wheat planting area with a monitoring zone, inspection zone and isolation zone, as described in Chapter 1, Section 2.2. GM wheat plants flowering during the field trial would be separated from any sexually compatible non-GM crops by a distance of at least 200 m, and would be separated from any sexually compatible non-GM volunteer plants by a distance of at least 60 m. In addition, GM wheat planting areas would be monitored after harvest and any wheat volunteers would be destroyed before flowering. The suitability of the proposed controls to manage pollen flow is discussed in detail in Chapter 3, Section 3.1.3. These controls are expected to minimise pollen flow from the GM wheat to sexually compatible non-GM plants outside the trial sites.

115. In the unlikely event of pollen flow from the GM wheat to sexually compatible non-GM crops or volunteer plants in the environment despite the proposed controls, the recipient plants would still be predominantly self-pollinated. A small proportion of seeds produced by the non-GM crops or volunteer plants could be hybrid GM seeds that contain the *HaHB4* gene. The hybrid GM seeds could be eaten by people or by desirable animals, or could grow into GM wheat plants in the environment.

116. Wheat is a domesticated plant that does not compete well with other vegetation (OGTR, 2021), as reflected by its low weed risk rating (Chapter 1, Section 3). The introduced *HaHB4* gene in GM wheat is expected to confer increased tolerance to drought stress, salt stress and biotic stress from invertebrate feeding, but it would not reverse the traits acquired during wheat domestication, that have contributed to its non-weedy habit. Therefore, even if GM wheat plants entered the environment as volunteers, they would not be expected to spread widely or establish populations with long term persistence.

Potential harm

117. If the GM wheat entered the Australian environment, the postulated potential harms are increased toxicity or allergenicity to people, increased toxicity to desirable animals, reduced establishment or yield of desirable plants, or an increased reservoir for pathogens or pests.

118. As discussed in Risk Scenario 1, FSANZ has approved use of HB4 wheat as food, and HB4 wheat is not expected to be toxic to people or animals.

119. As discussed in Risk Scenario 1, it is uncertain whether HB4 wheat has increased levels of any endogenous wheat allergens. However, as discussed in the causal pathway above, GM wheat could only plausibly be a small proportion of any wheat crop or volunteer wheat population in the environment. Even if the GM wheat contained increased levels of endogenous wheat allergens, this would have a negligible effect on total allergen levels in flour milled from a predominantly non-GM wheat crop. Similarly, low-level presence of the GM wheat would have a negligible effect on total allergen levels in airborne pollen produced by a predominantly non-GM wheat crop or volunteer wheat population.

120. The introduced *HaHB4* gene is expected to confer increased tolerance to environmental stresses, especially drought stress (Chapter 1, Section 4.3). Therefore, GM wheat volunteers could have increased persistence in the environment compared to non-GM wheat volunteers. For example, many Australian wheat growing areas have winter-dominated rainfall, and grow wheat as a winter crop. In these agricultural areas, GM wheat volunteers could survive better over the dry summer than non-GM wheat volunteers. Persistent GM wheat volunteers could provide a reservoir for wheat pests

or pathogens, such as fungal diseases, that could attack subsequent wheat crops (Chapter 1, Section 5.2). Persistent GM wheat volunteers could also become weeds that reduce the establishment or yield of subsequent non-wheat crops.

121. However, farmers typically manage crop plant volunteers in the same way that they manage other weeds. GM wheat volunteers could be controlled by most standard weed management measures, such as cultivation, heavy grazing or the use of appropriate herbicides. GM wheat volunteers are unlikely to be controlled by glufosinate herbicides, due to the introduced *bar* gene conferring glufosinate tolerance. Aside from glufosinate, there are a wide range of other herbicides (from at least four different mode of action groups) that are registered for control of volunteer wheat, volunteer cereals, and/or grass weeds during summer fallow (APVMA website, accessed 30 April 2024).

Conclusion

122. Risk scenario 2 is not identified as a substantive risk because wheat has low levels of outcrossing, the proposed field trial controls would minimise pollen flow from the GM wheat to non-GM plants outside the trial sites, and any GM wheat volunteers could be controlled by most standard weed management measures. In addition, FSANZ has assessed food containing HB4 wheat as safe. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.7 Risk scenario 3

Risk Source	Introduced *HaHB4* gene conferring environmental stress tolerance
Causal Pathway	GM wheat grows at the field trial sites ⬇ Persistence of GM wheat seed at the trial sites or dispersal of GM seed outside the trial sites ⬇ Establishment of populations of GM wheat expressing *HaHB4* in the environment
Potential Harm	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals OR Reduced establishment or yield of desirable plants OR Increased reservoir for pathogens or pests

Risk source

123. The source of potential harm for this postulated risk scenario is the introduced *HaHB4* gene in the GM wheat, which confers the trait of environmental stress tolerance.

Causal Pathway

124. HB4 wheat would be grown at the field trial sites and would produce seed. If a GM wheat seedbank persisted at the trial sites after completion of the trial, or if GM seed dispersed outside the trial sites, volunteer GM wheat expressing *HaHB4* could grow in the environment.

125. As discussed in Risk Scenario 2, the *HaHB4* gene is derived from sunflower. In sunflower, water stress strongly induces expression of *HaHB4* in vegetative tissues, but expression is not detectable in reproductive organs or seed (Gago et al., 2002; Dezar et al., 2005a). This indicates that *HaHB4* has not evolved to have activity in seed in its source plant, although it could have incidental activity in seed when controlled by a constitutive promoter. Following extensive field trials of HB4 wheat, the

applicant reported that there were no unintended changes to phenotype, including seed germination and dormancy characteristics, of HB4 wheat compared to the non-GM parental wheat.

Persistence of GM wheat on the trial sites

126. The applicant proposes to clean the trial sites after harvest. This is expected to destroy live GM wheat plants on the trial sites, but would not be expected to destroy all GM wheat seeds. For example, if the trial sites are cleaned by tillage, GM seeds lost during harvest would remain on the trial sites under the soil surface.

127. Wheat has low seed dormancy. A small field trial of wheat persistence in Europe found that 87% of wheat volunteers emerged in the first month post-harvest, 11% in the second month, 1% in the third month, and no volunteer emergence was observed in the following three months (Kalinina et al., 2015). A large field trial over nine years in Australia found that emergence of volunteer wheat was greatly reduced by two months after harvest, but viable wheat seed could persist for at least six months post-harvest during dry seasons in no-tillage plots (Wicks et al., 2000). In previous Australian field trials of GM wheat licensed by the Regulator, most wheat volunteers emerged within a few months after harvest, but sometimes small numbers of wheat volunteers emerged under favourable germination conditions up to 20 months after harvest.

128. The applicant proposes post-harvest monitoring of each trial site for two years with tillage and irrigation. Any wheat volunteers found would be destroyed prior to flowering. The suitability of the proposed controls to manage persistence of GMOs is further discussed in Chapter 3, Section 3.1.4. These control measures are expected to minimise persistence of viable GM wheat seeds on the trial sites.

Dispersal of GM wheat outside the trial sites

129. Dispersal of GM wheat seeds outside the trial sites could potentially occur through the activity of people or animals or through transport by wind or water.

130. Human activity is the most important dispersal pathway for non-GM wheat seed (OGTR, 2021). Important mechanisms for inadvertent seed dispersal by people include dispersal via equipment such as harvesters, and grain loss during transport. The applicant has proposed controls for the field trial including only permitting authorised persons to access the trial sites, inspecting and cleaning all equipment used on trial sites prior to use for any other purpose, and transporting and storing GMOs in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. In addition, the GM wheat would not be used as human food, so there would be no opportunity for grain dispersal during transport or processing for food use. These control measures would minimise dispersal of GM wheat seed outside the trial sites by human activity.

131. Dispersal of non-GM wheat seed by animals is discussed in *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021). Pathways known to contribute to dispersal of wheat seed are consumption and excretion of whole seeds by cattle and sheep, transport of seeds in the wool of sheep, transport of seeds by rodents for hoarding, or consumption and excretion of whole seeds by birds.

132. The applicant proposes that the GM wheat will not be used as animal feed. Therefore, cattle and sheep would not be permitted to enter the trial sites, and would not disperse GM wheat seed. The applicant proposes to control rodents on trial sites. In addition, planting areas would be surrounded by a 10 m monitoring zone kept free of vegetation or mown, which would deter rodents from transporting seeds through the area. The applicant does not propose specific controls to restrict dispersal of GM wheat by birds. However, <0.3% of wheat seeds consumed by birds are reported to remain viable after excretion (Woodgate et al., 2011), and only a small proportion of any excreted seed would be deposited in places conducive to germination. The potential for dispersal of GM wheat seeds by animals or birds would be further reduced by the small scale of the proposed field trial, and

by cleaning the field trial sites after harvest. Therefore, the proposed control measures would minimise dispersal of GM wheat seed outside the trial sites by animal activity.

133. Wheat seeds are not usually dispersed by wind as wheat has non-shattering seed heads, seeds are heavy and they lack specialised structures to aid windborne dispersal (OGTR, 2021). It is possible that GM wheat seeds could be dispersed by high winds if a severe storm occurred while mature seed was present on plants or the soil surface. Wheat seeds on the soil surface could also be transported by water during heavy runoff or flooding. The applicant has proposed that all field trial sites would be located at least 50 m from any natural waterway, which would minimise the potential for seed dispersal through flooding.

Potential harm

134. If the GM wheat entered the Australian environment, the postulated potential harms are increased toxicity or allergenicity to people, increased toxicity to desirable animals, reduced establishment or yield of desirable plants, or an increased reservoir for pathogens or pests. These potential harms are all discussed in Risk Scenario 2.

135. As discussed in Risk Scenario 1, FSANZ has approved use of HB4 wheat as food, and HB4 wheat is not expected to be toxic to people or animals.

136. As discussed in Risk Scenario 2, GM wheat volunteers could be controlled by most standard weed management measures.

Conclusion

137. Risk scenario 3 is not identified as a substantive risk because the proposed field trial controls would minimise persistence of GM wheat at the trial sites and dispersal of GM wheat outside trial sites. In addition, GM wheat volunteers could be controlled by most standard weed management measures and FSANZ has assessed food containing HB4 wheat as safe. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

139. 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.

140. 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.

141. For DIR 204, uncertainty is noted particularly in relation to potential for increased levels of endogenous wheat allergens in the GM wheat, especially in plant tissues other than the grain.

142. Additional data, including information to address this uncertainty, 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.

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

Section 4 Risk evaluation

144. 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.

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

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

146. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 5 and include:

- GM plant material would not be used as human food or animal feed
- limits on the size and duration of the proposed release
- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM wheat plants and their genetic material
- wheat has low levels of outcrossing
- GM wheat volunteers could be controlled by most standard weed management measures
- FSANZ has assessed food containing HB4 wheat as safe.

147. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM wheat into the environment are considered to be negligible. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no additional controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment [1].

[1] 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.

Chapter 3 Risk management plan

Section 1 Background

148. 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.

149. 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.

150. All licences are subject to 3 conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors and Section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder must also be reported to the Regulator.

151. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

152. 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 (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

153. 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, draft licence conditions have been proposed to limit the release to the proposed size, locations and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

3.1 Limits and controls on the release

154. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by the applicant. Many of these are discussed in the three risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 Consideration of limits proposed by the applicant

155. The applicant proposes to conduct the field trial between the date of issue of the licence and December 2029. This would limit the duration of the trial to five years and a few months. The GM wheat would be grown at a maximum of ten trial sites per year, with a planting area of up to 2 ha per site. The

small size and short duration of the trial would restrict the exposure of people and desirable animals to the GMOs (Risk scenario 1).

156. The applicant proposes that only authorised persons would enter the trial sites. Standard licence conditions included in the draft licence state that only people authorised by the licence holder are covered by the licence and permitted to deal with the GMOs. In addition, the licence holder must inform all people dealing with the GMOs of relevant licence conditions. These measures would ensure that the field trial is conducted in accordance with the specified limits and controls (important for all risk scenarios).

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

157. The applicant proposes not allowing the GMOs or GM products to be used for human food or animal feed. A draft licence condition states that GM plant material must not be used as food for humans or feed for animals. This condition would restrict the exposure of people and desirable animals to the GMOs (Risk scenario 1), and would also minimise dispersal of the GMOs by livestock or during transport or processing for human food use (Risk scenario 3).

158. The applicant proposes not engaging personnel who have a known allergy to wheat to work with the GM wheat. As discussed in Risk scenario 1, HB4 wheat has increased levels of some endogenous allergens, and there is uncertainty regarding whether these increases have biological significance, especially in plant tissues other than grain. If HB4 wheat produces biologically significant increased levels of certain wheat allergens, people who are sensitive to these wheat allergens could have a stronger reaction to HB4 wheat than to comparable non-GM wheat. Due to the small scale of the proposed trial, only a limited number of people would work with the GM wheat, but it is possible that one or more of the potential workers would have a known wheat allergy. A licence condition (above) does not permit the GM wheat to be used as food, so people working with the GM wheat would not be exposed to wheat allergens via consumption. People working with the GM wheat could be exposed to wheat allergens through inhalation or through skin contact. Respiratory wheat allergies are both more frequent and more severe than skin-mediated wheat allergies (Stobnicka and Gorny, 2015), which typically only cause mild localised skin symptoms. Therefore, it is considered appropriate to protect people with wheat allergy from inhaling GM wheat material, but unnecessary to protect them from skin contact with GM wheat material. A draft licence condition states that the licence holder must not permit a person to conduct any dealing which may expose the person to inhalation of GM plant material unless the licence holder has determined that the person does not have a known allergy to wheat. The licence holder could comply with the licence condition either by excluding any workers with a known wheat allergy from dealings at times when there may be airborne GM plant material, or by ensuring that any workers with a known wheat allergy use masks or other protective equipment that prevent them from inhaling airborne GM plant material.

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

159. Figure 2 in Chapter 1 shows a schematic diagram of the field trial setup proposed by the applicant. Each GM wheat planting area would be surrounded by a 10 m monitoring zone and a 50 m inspection zone. The monitoring and inspection zones would be inspected while the GMOs are flowering to destroy any wheat or sexually compatible plants. The inspection zone would be surrounded by a 140 m isolation zone where no wheat or sexually compatible plants would be deliberately grown. In two published studies of pollen-mediated gene flow from wheat at field trial scale, involving seven individual trials using 0.25 ha pollen donor plots, average gene flow rates were 0.02% to wheat plants 40 m from the pollen donor plots and 0.004% to wheat plants 100 m from the pollen donor plots (Matus-Cádiz et al., 2004; Loureiro et al., 2012). One hybrid seed was found in 230,000 wheat seeds collected at distances of 180 m to 300 m from the pollen donor plots (Matus-Cádiz et al., 2004). Therefore, the combination of a 10 m monitoring zone and a 50 m inspection zone (60 m isolation distance) is expected to minimise pollen flow from the GM wheat to scattered non-GM volunteer plants, and the combination of a 10 m monitoring zone, 50 m inspection zone and 140 m isolation zone (200 m isolation distance) is expected to minimise pollen flow from the GM wheat to non-GM crops outside the trial site (Risk scenario 2). The proposed field trial setup is included in the draft licence.

160. The applicant did not specify the proposed frequency of inspections of the monitoring and inspection zones to destroy any wheat or sexually compatible plants. The draft licence requires inspections at least every 14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering.

161. The applicant proposes that more than one planting area could be established at each trial site. Under the conditions in the draft licence, where more than one planting area is established at a field trial site, all planting areas must be inside a 10 m monitoring zone surrounding the whole trial site (see Figure 1 in licence). Any land between planting areas is also considered part of the monitoring zone.

3.1.4 Consideration of proposed controls regarding persistence of the GMOs

162. After harvest of each trial site, the applicant proposes cleaning of planting areas and destroying all GMOs from the trial not required for testing or future planting. Draft licence conditions require that trial sites must be cleaned (which would destroy any surviving GM plants) within 14 days after harvest, and that harvested GM seed not required for experimentation or future planting must be destroyed as soon as practicable. In addition, to deal with the case of failed crops that are not harvested, draft licence conditions require that GMOs must be harvested or destroyed within 8 months after planting, and that if all GMOs in a planting area have been destroyed, the GMOs are considered harvested and the area is considered cleaned.

163. The applicant has only proposed post-harvest cleaning and inspections for planting areas, but these measures would also be needed on some other areas of the trial sites to ensure that all GMOs from the trial are destroyed. A draft licence condition specifies that the areas requiring post-harvest cleaning and inspections are the planting area, monitoring zone, areas where equipment has been cleaned, and any other areas where GMOs are known to have dispersed. The monitoring zone is considered to require post-harvest cleaning and inspections due to potential seed dispersal during harvest, as the applicant is proposing to use commercial harvesters that must drive into and turn on the monitoring zone. In previous GM wheat trial sites of a similar size to those proposed under DIR 204, and using commercial harvesting equipment, emergence of hundreds or thousands of post-harvest wheat volunteers has been reported in the monitoring zone during post cleaning inspections.

164. The applicant has proposed that GMOs would be destroyed by herbicide application, root cutting and shredding/mulching, uprooting, light tillage to a depth of no more than 5 cm, burning/incineration, autoclaving, seed grinding, milling, or seed burial to a depth of at least 1 m. These methods are considered effective for rendering wheat plants and/or seed non-viable, and have been included in the draft licence. To ensure the effectiveness of destruction by seed burial, a licence condition specifies how this must be carried out, including a requirement that seeds must be sufficiently irrigated at time of burial to encourage decomposition.

165. The applicant has proposed that non-GM wheat plants grown on the trial sites, such as non-GM wheat varieties grown in the planting area as comparators or for breeding purposes, would be treated as if they are GMOs. Non-GM wheat in the trial site may be cross-pollinated by the GM wheat, resulting in hybrid seeds. It is therefore appropriate to require non-GM wheat to be destroyed in the same manner as GM wheat, to manage persistence of the GMOs, and this measure is included in the draft licence.

166. After harvest, the applicant proposes to inspect the trial sites for a period of two years and destroy any volunteer wheat plants found. As discussed in Risk Scenario 3, viable wheat seeds in a seedbank are expected to germinate within two years. To demonstrate that the seedbank is free of GM wheat, a draft licence condition requires inspections for a period of at least two years and until no wheat volunteers have been detected in the area for at least the final six months of inspections.

167. The proposed frequency of post-harvest inspections is every 55 days. However, the applicant also states that wheat volunteers found during inspections would be destroyed prior to flowering, which would prevent pollen flow to non-GM plants outside the trial site (Risk scenario 2) as well as seed set and

persistence (Risk scenario 3). Wheat typically requires 1275 degree-days[2] to grow from emergence to flowering (Bowden et al., 2008), which in hot weather (average daily temperature 26°C), would be about 49 days. Considering variations in flowering time between cultivars and between individual plants, trial sites should be inspected at least every 35 days to ensure that wheat volunteers are detected before flowering. This is required by the draft licence.

168. During the post-harvest monitoring period for each trial site, the applicant proposes one light tillage to a depth of no more than 5 cm and 3 irrigations or equivalent natural rainfall events to promote seedbank germination. An Australian field trial found that wheat seed banks were most persistent during dry seasons in no-tillage plots (Wicks et al., 2000). Shallow tillage after harvest, combined with irrigation, will promote germination, however, deep tillage can encourage prolonged dormancy in seeds (Ogg and Parker, 2000). Therefore, the draft licence requires a shallow post-harvest tillage along with at least 3 post-harvest watering events, at intervals of at least 28 days, with the last required watering event occurring during the final volunteer-free period.

169. The measures described above are expected to minimise the persistence of the GM wheat on the field trial sites (Risk scenario 3).

3.1.5 Consideration of proposed controls to limit dispersal of the GMOs

170. The applicant proposes to inspect and clean all equipment used on trial sites prior to use for any other purpose. The applicant also proposes to transport and store GMOs in accordance with the Regulator's current Guidelines for the Transport, Storage and Disposal of GMOs. These measures would help to minimise dispersal of GMOs outside the trial sites by human activity (Risk Scenario 3) and are included in the draft licence.

171. The application indicates that the GM wheat may be harvested and threshed either by hand or using commercial equipment. The draft licence requires that the GM wheat must be harvested and threshed separately from any other crop and that threshing must take place on a planting area or monitoring zone or in a facility approved by the Regulator. These conditions would help to minimise dispersal of GMOs outside the trial sites by human activity.

172. The applicant proposes to control rodents on trial sites. This could be done by use of traps and/or baits in the planting area and surrounding areas. The applicant also proposes maintaining the 10 m monitoring zone either vegetation-free or with vegetation kept mown at a maximum height of 10 cm, to avoid attracting or harbouring rodents. As discussed in Risk Scenario 3, these measures would minimise dispersal of GM wheat seeds by rodents. The draft licence requires these measures to be taken from planting of GM wheat seeds in a planting area until the planting area is cleaned. Cleaning of a planting area, as defined in the licence, includes removal of most of the GM seeds from the soil surface where they could be readily accessed by rodents or dispersed by other means.

173. The applicant proposes to locate each planting area at least 50 m away from the nearest natural waterway. This measure would restrict the potential for GM wheat seeds to be dispersed by flooding (Risk Scenario 3), and is included in the draft licence. In addition, draft licence conditions require that planting areas must not be located in flood-prone areas, and that the licence holder must immediately notify the Regulator of any extreme weather event affecting a trial site, to allow assessment and management of any risks.

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

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

- limit the duration of the release to the period between issue of the licence and December 2029

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

- limit the size of the release to a maximum of 10 sites per year, with each site having a maximum planting area of 2 ha
- not allow the GM plant material to be used for human food or animal feed
- not permit persons with an allergy to wheat to conduct dealings that may expose them to inhalation of GM plant material
- surround each planting area with a 10 m monitoring zone, maintained in a manner that does not attract or harbour rodents, that is inspected while the GMOs are flowering to destroy any wheat or sexually compatible plants
- surround the monitoring zone with a 50 m inspection zone that is inspected while the GMOs are flowering to destroy any wheat or sexually compatible plants
- surround the inspection zone with a 140 m isolation zone where no wheat or sexually compatible plants may be grown
- destroy all harvested GM seed not required for further analysis or future planting
- clean the planting areas after harvest and clean other areas where seed has been dispersed
- apply measures to promote the germination of any wheat seeds that may be present in the soil after harvest, including watering and shallow tillage
- monitor each trial site for at least 24 months after harvest and destroy any wheat plants that may grow, until no volunteers have been detected for a continuous 6 months period
- clean all equipment used on the trial sites
- transport and store the GMOs in accordance with the Regulator's guidelines
- implement measures to control rodents within the planting areas
- locate trial sites at least 50 m from any natural waterways.

3.2 Other risk management considerations

175. 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

176. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

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

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

178. In addition, the applicant organisation must have access to an Institutional Biosafety Committee (IBC) and be an accredited organisation under the Act.

3.2.2 Contingency plan

179. If a licence were issued, Trigall Australia would be required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM wheat outside permitted areas.

180. Before planting the GMOs, Trigall Australia would also be required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

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

3.2.4 Reporting requirements

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

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

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

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

3.2.5 Monitoring for compliance

184. 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.

185. 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.

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

Section 4 Issues to be addressed for future releases

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

188. This includes additional data regarding levels of endogenous wheat allergens in the GM wheat in comparison to non-GM wheat, especially in plant tissues other than the grain.

Section 5 Conclusions of the consultation RARMP

189. The risk assessment 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. These negligible risks do not require specific risk treatment measures.

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

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

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

   (b) words importing a gender include every other gender;

   (c) words in the singular number include the plural and words in the plural number include the singular;

   (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

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

   (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

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

2. In this licence:

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

‘Clean’ means, as the case requires:

   (a) in relation to Equipment or a facility, remove and/or Destroy the GMOs, if any; or

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

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

      ii. remove Wheat seeds from the soil surface to the reasonable satisfaction of the Regulator.

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

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

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

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

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

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

   (a) uprooting;

   (b) root cutting and shredding/mulching;

   (c) Tillage, but only in accordance with condition 39;

(d) treatment with herbicide;

(e) burning/incineration;

(f) autoclaving;

(g) milling/hammer milling;

(h) crushing or grinding of seed;

(i) burial, but only in accordance with condition 40;

(j) a method approved in writing by the Regulator.

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

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

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

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

'GM' means genetically modified.

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

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

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

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

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

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

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

(a) whether the information or opinion is true or not; and

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

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

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

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

'Regulator' means the Gene Technology Regulator.

'Related Species' means durum wheat or triticale plants.

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

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

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

Note: Tillage must be in accordance with condition 39.

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

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

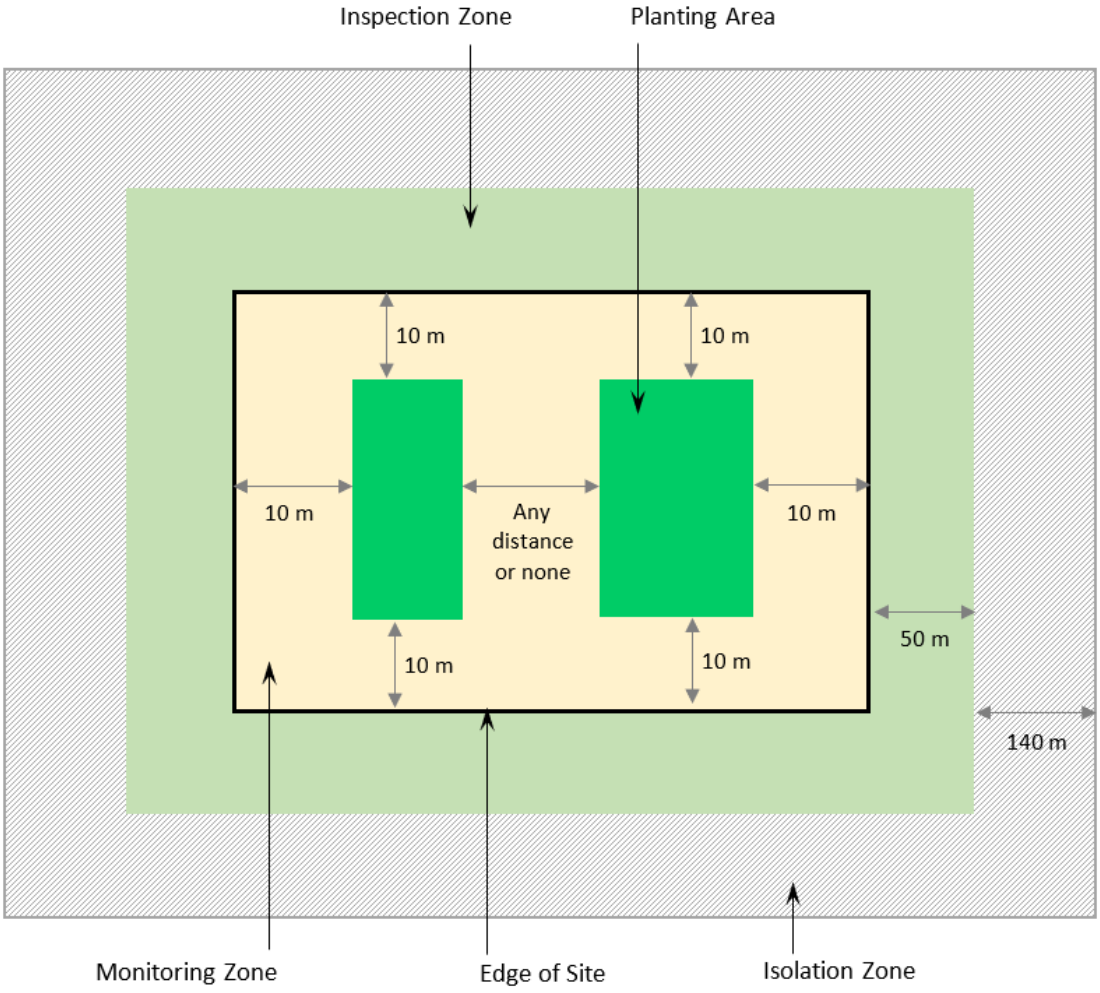

Figure 1. Diagram (not to scale) showing the relationship between Planting Area, Monitoring Zone, Site, Inspection Zone and Isolation Zone.

Section 2 General conditions and obligations

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

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

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

5. The licence holder is Trigall Australia Pty Ltd.

6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by this licence.
7. The GMOs with which dealings are authorised by this licence are those listed at **Attachment A**.
8. The dealings authorised by the licence are to:
   (a) conduct experiments with the GMOs;
   (b) breed the GMOs;
   (c) propagate the GMOs;
   (d) use the GMOs in the course of manufacture of a thing that is not the GMOs;
   (e) grow the GMOs;
   (f) import the GMOs;
   (g) transport the GMOs;
   (h) dispose of the GMOs;

   and the possession, supply or use of the GMOs in the course of any of these dealings.
9. This licence does not apply to dealings with the GMOs conducted as a Notifiable Low Risk Dealing (NLRD) or pursuant to another authorisation under the Act.

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

General obligations of the licence holder

10. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.
11. The licence holder must be able to access and control all Planting Areas, Monitoring Zones, Inspection Zones, Isolation Zones and approved facilities to the extent necessary to comply with this licence.

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

12. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:
    (a) the particular condition, including any variations of it;
    (b) the cancellation or suspension of the licence;
    (c) the surrender of the licence.
13. The licence holder must not permit a person covered by this licence to conduct any dealing with the GMOs unless:
    (a) the person has been informed of any applicable licence conditions, including any variation of them; and
    (b) the licence holder has obtained from the person a signed and dated statement that the person:
        i. has been informed by the licence holder of the licence conditions including any variation of them; and
        ii. has understood and agreed to be bound by the licence conditions, or variation.
14. The licence holder must inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator.

General obligations of persons covered by the licence

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

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

Section 3 Limits and control measures

3.1 Limits on the release

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

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

Area and duration

Period	Maximum number of Sites per calendar year	Maximum combined area of Planting Areas in each Site
From issue of licence until December 2029	10	2 ha

Local government areas in which Sites may be located

New South Wales	Victoria	Western Australia	South Australia
Berrigan	Ararat	Albany	Adelaide Plains
Bland	Ballarat	Beverley	Barossa
Blayney	Benalla	Boddington	Light
Cabonne	Buloke	Boyup Brook	Wakefield
Coolamon	Campaspe	Bridgetown-Greenbushes	
Coonamble	Central Goldfields	Brookton	
Cootamundra-Gundagai	Colac Otway	Broomehill-Tambellup	
Cowra	Corangamite	Carnamah	
Dubbo	Gannawarra	Coorow	
Edward River	Glenelg	Corrigin	
Federation	Golden Plains	Cranbrook	
Forbes	Greater Bendigo	Cuballing	
Gilgandra	Greater Geelong	Cunderdin	
Greater Hume	Greater Shepparton	Dalwallinu	
Griffith	Hepburn	Denmark	
Gunnedah	Hindmarsh	Donnybrook-Balingup	
Gwydir	Horsham	Dowerin	
Hay	Indigo	Dumbleyung	
Hilltops	Loddon	Esperance	
Inverell	Macedon Ranges	Gnowangerup	
Junee	Mildura	Goomalling	
Leeton	Mitchell	Greater Geraldton	
Liverpool Plains	Moira	Jerramungup	
Lockhart	Moorabool	Katanning	
Mid-Western	Mount Alexander	Kent	
Moree Plains	Moyne	Kojonup	

New South Wales	Victoria	Western Australia	South Australia
Murray River	Northern Grampians	Manjimup	
Murrumbidgee	Pyrenees	Merredin	
Muswellbrook	Southern Grampians	Mingenew	
Narrabri	Strathbogie	Moora	
Narrandera	Swan Hill	Morawa	
Narromine	Wangaratta	Nannup	
Orange	West Wimmera	Narrogin	
Parkes	Wodonga	Northam	
Snowy Valleys	Wyndham	Perenjori	
Tamworth	Yarriambiack	Pingelly	
Temora		Plantagenet	
Upper Hunter		Quairading	
Wagga Wagga		Ravensthorpe	
Walgett		Tammim	
Warren		Three Springs	
Warrumbungle		Toodyay	
Weddin		Victoria Plains	
		Wagin	
		Wandering	
		West Arthur	
		Wickepin	
		Williams	
		Wongan-Ballidu	
		Woodanilling	
		Wyalkatchem	
		York	

3.2 Control measures

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

Conditions to restrict exposure

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

20. The licence holder must not permit a person covered by this licence to conduct any dealing which may expose the person to inhalation of Plant Material unless the person has provided a statement that the person does not have a known allergy to Wheat.

Note: The licence holder should document compliance with this condition, as per Condition 13.

Conditions to restrict pollen flow

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

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

23. The Monitoring Zone and Inspection Zone must be maintained in a manner appropriate to allow the identification of Volunteers and Related Species while inspections of these areas are required by condition 26.

Note: Condition 49(d) requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone and Inspection Zone.

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

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

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

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

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

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

Conditions to restrict seed dispersal

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

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

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

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

30. Measures must be implemented to control rodents within each Planting Area from at least 7 days prior to planting the GMOs, while the GMOs are being grown and until the Planting Area is Cleaned.

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

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

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

Conditions relating to harvesting

32. All GMOs planted within a Planting Area must be harvested or Destroyed within 8 months after the first planting of any GMO within that Planting Area.

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

    (a) the GMOs are taken to have been harvested; and

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

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

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

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

Conditions to restrict persistence of GMOs on trial sites

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

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

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

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

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

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

38. While post-Cleaning inspection requirements apply to an area:
    (a) the area must be maintained in a manner appropriate to allow identification of Volunteers; and
    (b) no plants may intentionally be grown in the area unless:
        i. the area is planted as a new Planting Area in accordance with condition 16; or
        ii. the plants are agreed to in writing by the Regulator; and
    (c) the area must not be used for grazing livestock; and
    (d) prior to an application for Sign off, the area must receive at least three watering events as described in **Attachment B**, at intervals of at least 28 days, with the final required watering event occurring within the six months prior to submission of the Sign off application; and
    (e) within the six months prior to submission of the Sign off application, and before the final required watering event, the area must be Tilled.

Tillage

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

Destruction by burial

40. If Destruction of GMOs occurs by burial:

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

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

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

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

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

Processing or experimentation with the GMOs

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

(a) a Planting Area before Cleaning; or

(b) a Monitoring Zone before Cleaning; or

(c) a facility approved in writing by the Regulator.

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

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

Transport or storage of the GMOs

43. Transport or storage of the GMOs must:

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

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

(c) comply with all other conditions of this licence.

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

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

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

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

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

Contingency plan

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

Section 4 Sign off

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

(a) post-Cleaning inspection activities have been conducted for at least 24 months on the area; and

(b) conditions have been conducive for germination and detection of Volunteers; and

(c) no Volunteers have been detected in the area during the six months prior to the Sign off request.

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

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

Section 5 Reporting and documentation

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

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

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

Notice	Content of notice	Timeframe
a. Changes to contact details	Changes to any of the contact details of the project supervisor that were notified in the licence application or subsequently	As soon as practicable
b. Ongoing suitability to hold a licence	i. any relevant conviction of the licence holder; or ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and	As soon as practicable after any of these events occur
	iv. any information related to the licence holder's ongoing suitability to hold a licence, that is requested by the Regulator	Within the timeframe stipulated by the Regulator
c. People covered by the licence	i. names of all organisations and persons, or functions or positions of the persons, who will be covered by the licence, with a description of their responsibilities; and *Note: Examples of functions or positions are 'project supervisor', 'site manager', 'farm labourer' etc.* ii. detail of how the persons covered by the licence will be informed of licence conditions	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
d. Testing methodology	A written methodology to reliably detect the GM Wheat described in this licence.	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
e. Contingency plan	A Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)

f. Training records	Copies of the signed and dated statements referred to in condition 13 if requested by the Regulator	Within the timeframe stipulated by the Regulator
g. Additional information required by the Act	i. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or ii. any contraventions of the licence by a person covered by the licence; or iii. any unintended effects of the dealings authorised by the licence *Note: The Act requires, for the purposes of the condition 47(g), that:* • *the licence holder will be taken to have become aware of additional information of a kind mentioned in Condition 47(g) if he or she was reckless as to whether such information existed; and* • *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in Condition 47(g), if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed* *Note: Contraventions of the licence may occur through the action or inaction of a person.*	Without delay after becoming aware of any new information *Note: An example of notification without delay is contact made within a day of a contravention of the licence via the OGTR free call phone number 1800 181 030. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location, if required*
h. Further details regarding additional information	Any further details requested by the Regulator in relation to information provided under condition 47(g)	Within the timeframe stipulated by the Regulator

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

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

Notice	Content of notice	Timeframe
a. Intention to plant	i. Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the Site (e.g. Google Maps) and any other descriptions ii. Detail of how the licence holder will access and control the Planting Area and the associated Monitoring Zone, Inspection Zone and Isolation Zone, in accordance with condition 11 *Note: this should include a description of any contracts, agreements, or other enforceable arrangements.* iii. Date on which the GMOs will be planted iv. Period when the GMOs are expected to Flower v. Period when harvesting is expected to commence vi. How all areas requiring post-Cleaning inspections are intended to be used until Sign off, including proposed post-harvest crops (if any) vii. Details of how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers	At least 7 days prior to each planting (to be updated as soon as practicable if the notified details change)

Notice	Content of notice	Timeframe
	viii. History of how the Site has been used for the previous two years	
b. Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition	Within 7 days of any planting
c. Extreme Weather	Any Extreme Weather event that is expected to affect or has already affected an area where the GMOs are or may be present. *Note: The Contingency Plan must be implemented if the GMOs are detected outside areas requiring Cleaning (Condition 45).*	As soon as practicable
d. Harvest	Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting
e. Cleaning	i. Date(s) on which required Cleaning was performed on any areas of land ii. Method(s) of Cleaning	Within 7 days of completion of Cleaning
f. Destruction by burial	Date of burial, location of burial including GPS co-ordinates, and details of measures used to ensure that the burial site will not be disturbed for the period required by Condition 40.	Within 7 days of burial of any GMOs
g. Inspection activities	Information recorded in a Logbook as per the inspection requirements (Conditions 26, 37 and 49).	Within 35 days of inspection

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

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

(a) date of the inspections; and

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

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

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

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

(e) details of the developmental stage of the GMOs while they are being grown; and

(f) details of any post-Cleaning rainfall events including measurements at or near the area, or any irrigation events; and

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

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

(i) details of rodent control methods used and any evidence of rodent activity, while rodent control methods are required.

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

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

ATTACHMENT A

DIR No: 204

Full Title: Limited and controlled release of wheat genetically modified for increased tolerance to environmental stress

Organisation Details

Postal address: Trigall Australia Pty Ltd

1/52 Barnes St

Tamworth NSW 2340

IBC Details

IBC Name: PTM Solutions Australia Institutional Biosafety Committee

GMO Description

GMOs covered by this licence

Genetically modified wheat known by the OECD unique identifier IND-ØØ412-7, which expresses the introduced genes listed below.

Parent Organism

Common Name: Wheat

Scientific Name: *Triticum aestivum* L.

Modified traits

Category: Abiotic stress tolerance

Selectable marker – herbicide tolerance

Description: The GM wheat line expresses one introduced gene conferring environmental stress tolerance and one introduced selectable marker gene (Table 1, below).

Purpose of the dealings with the GMO

The purpose of the release is to gather research and regulatory data for the GM wheat under Australian growing conditions. The GM wheat grown in this field trial is not permitted to be used for human food or animal feed.

Table 1. Introduced genes expressed in the GM wheat

Gene	Source organism	Function
HaHB4	Sunflower (*Helianthus annuus*)	Encodes transcription factor conferring environmental stress tolerance
bar	*Streptomyces hygroscopicus*	Selectable marker gene conferring glufosinate herbicide tolerance

ATTACHMENT B

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

Examples of acceptable watering events are:

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

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

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

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

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

References

ABARES (2023). Australian Crop Report: December 2023. (Canberra, Australia: Australian Bureau of Agricultural and Resource Economics and Sciences).

AEGIC (2020). Wheat 2030: Anticipated trends in global consumption. (Australian Export Grains Innovation Centre).

AgbioInvestor (2024). Global GM crop area review.

Ayala, F., Fedrigo, G.V., Burachik, M., and Miranda, P.V. (2019). Compositional equivalence of event IND-OO412-7 to non-transgenic wheat. Transgenic Res *28*, 165-176.

Bowden, P., Edwards, J., Ferguson, N., McNee, T., Manning, B., Roberts, K., Schipp, A.*, et al.* (2008). Wheat growth and development. (NSW DPI).

CTNBio (2021). Parecer Técnico: 7795/2021. (Brazil: Comissão Técnica Nacional de Biossegurança).

Delaney, B., Goodman, R.E., and Ladics, G.S. (2018). Food and feed safety of genetically engineered food crops. Toxicological Sciences *162*, 361-371.

Dezar, C.A., Fedrigo, G.V., and Chan, R.L. (2005a). The promoter of the sunflower HD-Zip protein gene *Hahb4* directs tissue-specific expression and is inducible by water stress, high salt concentrations and ABA. Plant Science *169*, 447-456.

Dezar, C.A., Gago, G.M., Gonzalez, D.H., and Chan, R.L. (2005b). *Hahb-4*, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. Transgenic Research *14*, 429-440.

FSANZ (2021). Safety assessment - Application A1232. (Food Standards Australia New Zealand).

Gago, G.M., Almoguera, C., Jordano, J., Gonzalez, D.H., and Chan, R.L. (2002). *Hahb-4*, a homeobox-leucine zipper gene potentially involved in abscisic acid-dependent responses to water stress in sunflower. Plant, Cell & Environment *25*, 633-640.

Gaines, T.A., Byrne, P.F., Westra, P., Nissen, S.J., Henry, W.B., Shaner, D.L., and Chapman, P.L. (2007). An empirically derived model of field-scale gene flow in winter wheat. Crop Science *47*, 2308-2316.

Gonzalez, F.G., Capella, M., Ribichich, K.F., Curin, F., Giacomelli, J.I., Ayala, F., Watson, G.*, et al.* (2019). Field-grown transgenic wheat expressing the sunflower gene HaHB4 significantly outyields the wild type. Journal of Experimental Botany *70*, 1669-1681.

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

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

GRDC (2017). GrowNotes Sunflowers. (Grains Research and Development Corporation).

Hanson, B.D., Mallory-Smith, C.A., Shafii, B., Thill, C.D., and Zemetra, R.S. (2005). Pollen-mediated gene flow from blue aleurone wheat to other wheat cultivars. Crop Science *45*, 1610-1617.

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

Hills, M.J., Hall, L.M., Messenger, D.F., Graf, R.J., Beres, B.L., and Eudes, F. (2007). Evaluation of crossability between triticale (X *Triticosecale* Wittmack) and common wheat, durum wheat and rye. Environmental Biosafety Research *6*, 249-257.

Jensen, H.L. (1931). Contributions to our knowledge of the Actinomycetales. II. The definition and subdivision of the genus Actinomyces, with a preliminary account of Australian soil Actinomycetes. Proceedings of The Linnean Society of New South Wales *56*, 345-370.

Kalinina, O., Zeller, S.L., and Schmid, B. (2015). Persistence of seeds, seedlings and plants, performance of transgenic wheat in weed communities in the field and effects on fallow weed diversity. Perspectives in Plant Ecology, Evolution and Systematics *17*, 421-433.

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

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

Laugerotte, J., Baumann, U., and Sourdille, P. (2022). Genetic control of compatibility in crosses between wheat and its wild or cultivated relatives. Plant Biotechnology Journal *20*, 812-832.

Loureiro, I., Escorial, M.C., Gonzalez, A., and Chueca, M.C. (2012). Pollen-mediated gene flow in wheat (*Triticum aestivum* L.) in a semiarid field environment in Spain. Transgenic Research *21*, 1329-1339.

Manavella, P.A., Arce, A.L., Dezar, C.A., Bitton, F., Renou, J.P., Crespi, M., and Chan, R.L. (2006). Cross-talk between ethylene and drought signalling pathways is mediated by the sunflower *Hahb-4* transcription factor. The Plant Journal *48*, 125-137.

Manavella, P.A., Dezar, C.A., Bonaventure, G., Baldwin, I.T., and Chan, R.L. (2008). *HAHB4*, a sunflower HD-Zip protein, integrates signals from the jasmonic acid and ethylene pathways during wounding and biotic stress responses. The Plant Journal *56*, 376-388.

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

Miranda, P.V., Iglesias, B.F., Charriere, M.V., and Burachik, M. (2022). Drought tolerant wheat IND-OO412-7 is nutritionally equivalent to its non-transgenic comparator. GM Crops & Food *13*, 119-125.

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

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

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

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

Philips, J.G., Martin-Avila, E., and Robold, A.V. (2022). Horizontal gene transfer from genetically modified plants - Regulatory considerations. Frontiers in Bioengineering and Biotechnology *10*.

Quirce, S., Boyano-Martinez, T., and Diaz-Perales, A. (2016). Clinical presentation, allergens, and management of wheat allergy. Expert Review of Clinical Immunology *12*, 563-572.

Rej, A., Aziz, I., and Sanders, D.S. (2020). Coeliac disease and noncoeliac wheat or gluten sensitivity. Journal of Internal Medicine *288*, 537-549.

Salcedo, G., Quirce, S., and Diaz-Perales, A. (2011). Wheat allergens associated with baker's asthma. Journal of Investigational Allergology and Clinical Immunology *21*, 81-92.

Salvatori, N., Reccardini, F., Convento, M., Purinan, A., Colle, R., De Carli, S., Garzoni, M.*, et al.* (2008). Asthma induced by inhalation of flour in adults with food allergy to wheat. Clinical & Experimental Allergy *38*, 1349-1356.

Schuster, C., Huen, J., and Scherf, K.A. (2022). Comprehensive study on gluten composition and baking quality of winter wheat. Cereal Chemistry *100*, 142-155.

Stobnicka, A., and Gorny, R.L. (2015). Exposure to flour dust in the occupational environment. International Journal of Occupational Safety and Ergonomics *21*, 241-249.

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

Trigall Genetics (2021). Application to FSANZ for the inclusion of wheat event IND-ØØ412-7 with increased tolerance to environment stresses in Standard 1.5.2 Food Produced Using Gene Technology. (Food Standards Australia New Zealand).

Venter, C., Maslin, K., Arshad, S.H., Patil, V., Grundy, J., Glasbey, G., Twiselton, R.*, et al.* (2016). Very low prevalence of IgE mediated wheat allergy and high levels of cross-sensitisation between grass and wheat in a UK birth cohort. Clinical and Translational Allergy *6*, 22.

Virtue, J.G. (2008). SA weed risk management guide. (Adelaide: Government of South Australia: Department of Water, Land and Biodiversity Conservation).

White, M., Cheal, D., Carr, G.W., Adair, R., Blood, K., Muir, A., and Meagher, D. (2022). Advisory list of environmental weeds in Victoria 2022. (Heidelberg, Victoria: Department of Environment, Land, Water and Planning).

Wicks, G.A., Felton, W.L., Murison, R.D., and Martin, R.J. (2000). Changes in fallow weed species in continuous wheat in northern New South Wales 1981-1990. Australian Journal of Experimental Agriculture *40*, 831-842.

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