Australian Government
Department of Health
Office of the Gene Technology Regulator

April 2019

Risk Assessment and Risk Management Plan
for

DIR 165

Limited and controlled release of wheat genetically modified for altered iron uptake, transport and bioavailability

Applicant – The University of Melbourne

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 165

Decision

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

The application

Application number	DIR 165
Applicant	The University of Melbourne
Project Title	Limited and controlled release of wheat genetically modified for altered iron uptake, transport and bioavailability[1]
Parent Organism	Bread wheat (*Triticum aestivum*)
Introduced genes and modified traits	Iron-related genes derived from wheat, rice and other plant species: • 57 nicotianamine synthase (NAS) genes involved in iron uptake and transport • Seven genes from a gene family[2] (Class 2) involved in iron bioavailability • Six nicotianamine aminotransferase (NAAT) genes involved in iron uptake • Three deoxymugineic acid synthase (DMAS) genes involved in iron uptake • Six iron-related transcription factor (IRO) genes involved in iron uptake and transport • Six vacuolar iron transporter (VIT) genes involved in iron transport and storage • Six ferritin (Fer) genes involved in iron storage • 55 yellow stripe-like transporter (YSL) genes involved in iron transport Marker genes derived from bacteria: • Two selectable marker genes
Proposed locations	Up to 2 sites in 2019 and 10 sites per year in 2020-2023, to be selected from 131 possible local government areas in Victoria, New South Wales and Western Australia
Proposed release size	Up to 4 ha in 2019 and 20 ha per year in 2020-2023

[1] The original title for the application was: Limited and controlled release of *Triticum aestivum* L genetically modified for improved iron uptake, transport and bioavailability.

[2] The name of this gene family is not provided as it has been declared Confidential Commercial Information (CCI) under Section 185 of the Act. The information is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

Application number	DIR 165
Proposed release dates	April 2019 – December 2023
Primary purpose	To gather research and regulatory data under field conditions

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks.

The risk assessment process considers how the genetic modifications 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), relevant previous approvals and advice received from a wide range of experts, agencies and authorities consulted on the RARMP. Both the short and long term are considered.

Credible pathways to potential harm that were considered included exposure of people or desirable animals to the GM plant material on the trial sites, transfer of the introduced genetic material to non-GM plants outside the trial sites and potential for persistence or dispersal of the GMOs outside the trial sites. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to weediness.

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

Risk management

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

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, 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 GMOs at the end of the trial, and to conduct post-harvest monitoring at the trial sites to ensure GMOs are destroyed.

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Abbreviations

Act	*Gene Technology Act 2000*
Cas9	CRISPR-associated nuclease 9
CCI	Confidential commercial information
cm	centimetres
CRISPR	Clustered regularly interspaced short palindromic repeat
DIR	Dealings involving Intentional Release
DMA	Deoxymugineic acid
DMAS	Deoxymugineic acid synthase
DNA	Deoxyribonucleic acid
Fer	Ferritin
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
ha	hectare
HGT	horizontal gene transfer
hptII	Hygromycin phosphotransferase II
IRO	Iron-related transcription factor
kg	kilograms
LGA	Local government area
m	metres
mg	milligrams
MNP	magnetic nanoparticle
NAAT	Nicotianamine aminotransferase
NAS	Nicotianamine synthase
NHEJ	non-homologous end joining
NLRD	Notifiable Low Risk Dealing
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
RNA	Ribonucleic acid
gRNA	guide RNA
USDA	United States Department of Agriculture
VIT	Vacuolar iron transporter
YSL	Yellow stripe-like transporter

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 describes the risk assessment context for this application.

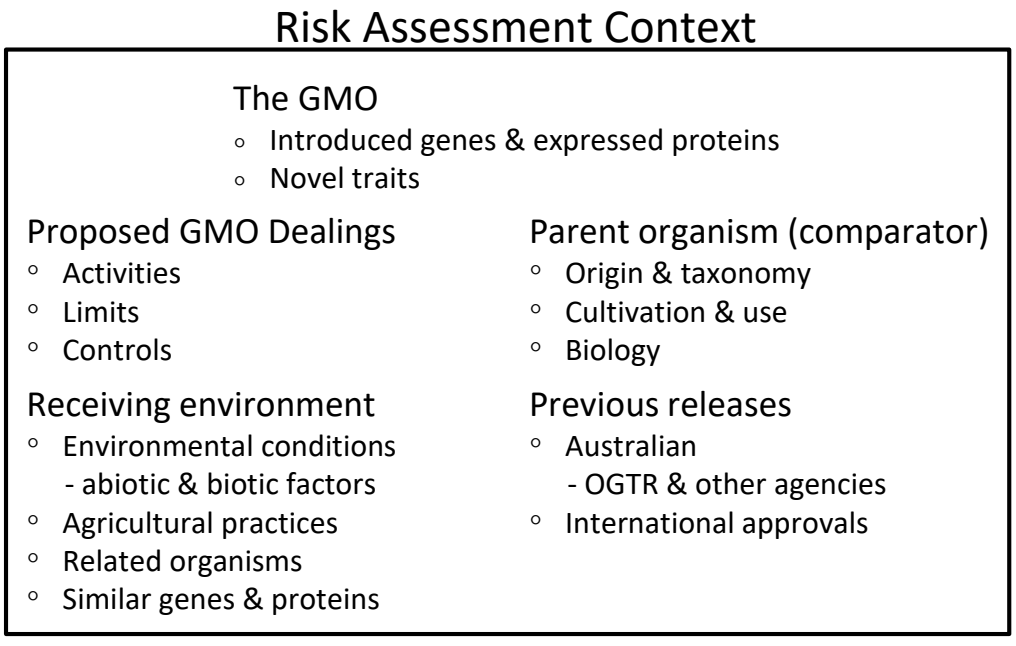

Figure 1. Summary of parameters used to establish the risk assessment context

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.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Two public submissions were received and they are summarised and addressed in Appendix B.

8. 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, the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. 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.

Section 2 The proposed dealings

9. The University of Melbourne proposes to release up to 100 lines of wheat genetically modified for altered iron uptake, transport and bioavailability. The purpose of the release is to gather research and regulatory data under field conditions.

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- growing the GMOs
- using the GMOs in the course of manufacture of a thing that is not a GMO
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

and possession, supply or use of the GMOs for the purposes of, or in the course of, any of the above.

2.1 The proposed limits of the dealings

11. The release is proposed to take place over five growing seasons, from April 2019 to December 2023. In 2019, GM wheat would be grown on up to two trial sites, with an area of up to 2 ha per site, giving a maximum combined planting area of 4 ha. In 2020-2023, GM wheat would be grown on up to ten trial sites per year, with an area of up to 2 ha per site, giving a maximum combined planting area of 20 ha/year.

12. The trial sites would be selected from 131 possible local government areas (LGAs) in New South Wales, Victoria and Western Australia (Table 1).

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

New South Wales	Victoria	Western Australia
Berrigan	Ararat	Albany
Bland	Ballarat	Beverley
Blayney	Benalla	Boddington
Cabonne	Buloke	Boyup Brook
Coolamon	Greater Bendigo	Bridgetown-Greenbushes
Coonamble	Campaspe	Brookton
Cootamundra-Gundagai	Central Goldfields	Broomehill-Tambellup
Cowra	Colac Otway	Carnamah
Dubbo	Corangamite	City of Greater Geraldton
Edward River Council	Gannawarra	Coorow
Forbes	Glenelg	Corrigin
Federation Council	Golden Plains	Cranbrook
Gilgandra	Greater Geelong	Cuballing

New South Wales	Victoria	Western Australia
Greater Hume	Greater Shepparton	Cunderdin
Griffith	Hepburn	Dalwallinu
Gunnedah	Hindmarsh	Denmark
Gwydir	Horsham	Donnybrook-Balingup
Hay	Indigo	Dowerin
Hilltops Council	Loddon	Dumbleyung
Inverell	Macedon Ranges	Esperance
Junee	Mildura	Gnowangerup
Leeton	Mitchell	Goomalling
Liverpool Plains	Moira	Jerramungup
Lockhart	Moorabool	Katanning
Mid-Western Regional Council	Mount Alexander	Kent
Moree Plains	Moyne	Kojonup
Murray River Council	Northern Grampians	Manjimup
Murrumbidgee Council	Pyrenees	Merredin
Muswellbrook	Southern Grampians	Mingenew
Narrabri	Strathbogie	Moora
Narrandera	Swan Hill	Morawa
Narromine	Wangaratta	Narrogin
Orange	West Wimmera	Nannup
Parkes	Wodonga	Northam
Snowy Valleys Council	Wyndham	Perenjori
Tamworth	Yarriambiack	Pingelly
Temora		Plantagenet
Upper Hunter		Quairading
Wagga Wagga		Ravensthorpe
Walgett		Tammin
Warren		Three Springs
Warrumbungle		Toodyay
Weddin		Victoria Plains
		Wagin
		Wandering
		West Arthur
		Wickepin
		Williams
		Wongan-Ballidu
		Woodanilling
		Wyalkatchem
		York

13. 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 in the environment

14. The applicant has proposed a number of controls to restrict the spread and persistence of the GM wheat and the introduced genetic material in the environment. These include:

- locating each trial site at least 50 m away from the nearest natural waterway
- 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 would be grown
- only permitting authorised persons to access the trial sites
- surrounding each trial site with a fence to restrict access by large animals
- treating non-GM wheat plants grown in the trial as if they were GMOs
- inspecting all equipment used on trial sites for GM seeds and cleaning before use for any other purpose

- transporting and storing GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- destroying all plant material from the trial not required for testing or future plantings
- post-harvest monitoring of each trial site monthly for at least 2 years and until the site is free of volunteer plants for at least 6 months, with any wheat volunteers destroyed prior to flowering
- one tillage and three irrigations of each trial site during the post-harvest monitoring period
- not allowing the GMOs or GM products to be used for human food or animal feed.

Section 3 The parent organism

15. The parent organism is *Triticum aestivum* L., which is commonly known as bread wheat or wheat. Wheat is Australia's largest agricultural crop (ABARES, 2018). Wheat cultivation occurs predominantly in the wheat belt from southern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia.

16. Wheat is primarily grown in Australia as a cereal grain for human consumption. Lower quality or damaged wheat crops are used for animal feed or industrial purposes (Blakeney et al., 2009).

17. Detailed information about wheat is contained in the reference document *The Biology of* Triticum aestivum *L. (bread wheat)* (OGTR, 2017), which was produced to inform the risk analysis for licence applications involving GM wheat. Baseline information from this document will be used and referred to throughout the RARMP.

18. Wheat is not native to Australia but is found outside cultivation in all Australian states and territories. The species may not be truly naturalised as it usually grows where cultivated seed is dropped and rarely persists (Henwood and Weiller, 2017). Volunteer wheat grows in agricultural areas in the wheat belt and may be widespread after a wet summer. Farmers often need to control volunteer wheat to reduce survival of wheat pathogens (Holloway, 2017).

19. The weed risk assessment included as Appendix 1 of the wheat biology document (OGTR, 2017) found that wheat possesses few attributes associated with weeds.

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

4.1 Introduction to the GMOs

20. The applicant proposes to release up to 100 lines of wheat genetically modified for altered iron uptake, transport and bioavailability. The introduced or modified iron-related genes are divided into eight classes (Table 2).

Table 2: Classes of introduced or modified iron-related genes in the GM wheat

Gene class	Gene family	Altered trait	Type of genetic modification
1	Nicotianamine synthase (NAS)	Iron uptake and transport	Gene introduction
2	CCI[#]	Iron bioavailability	Gene introduction or endogenous gene knockout
3	Nicotianamine aminotransferase (NAAT)	Iron uptake	Gene introduction
4	Deoxymugineic acid synthase (DMAS)	Iron uptake	Gene introduction
5	Iron-related transcription factor (IRO)	Iron uptake and transport	Gene introduction
6	Vacuolar iron transporter (VIT)	Iron transport and storage	Gene introduction or endogenous gene knockout
7	Ferritin (Fer)	Iron storage	Gene introduction
8	Yellow stripe-like transporter (YSL)	Iron transport	Gene introduction

[#]This information has been declared Confidential Commercial Information (CCI) under Section 185 of the Act. The information is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

21. Each GM wheat line will contain up to three introduced genes from Classes 1-8 and up to one introduced selectable marker gene. Some 'knockout' lines would have no introduced genes but would have insertions or deletions in endogenous wheat Class 2 or Class 6 genes making these genetic sequences non-functional. The introduced or modified genes are listed in Table 3.

Table 3: List of introduced or modified genes in the GM wheat

Gene class	Gene name	Source organism	Gene class	Gene name	Source organism
1	*OsNAS1*	*Oryza sativa*	8	*TaYSL6-2-D*	*Triticum aestivum*
	OsNAS2	"		*TaYSL6-3-A*	"
	OsNAS3	"		*TaYSL6-3-B*	"
	54 genes that are CCI[#]	Plant species that are CCI[#]		*TaYSL6-3-D*	"
2	7 genes that are CCI[#]	Plant species that are CCI[#]		*TaYSL6-4-A*	"
3	*TaNAAT1-A**	*Triticum aestivum*		*TaYSL6-4-B*	"
	*TaNAAT1-B**	"		*TaYSL6-4-D*	"
	*TaNAAT1-D**	"		*TaYSL6-5-B*	"
	TaNAAT2-A	"		*TaYSL6-5-D*	"
	TaNAAT2-B	"		*TaYSL8-A*	"
	TaNAAT2-D	"		*TaYSL8-D*	"
4	*TaDMAS-A*	*Triticum aestivum*		*TaYSL9-A*	"
	TaDMAS-B	"		*TaYSL9-D*	"
	TaDMAS-D	"		*TaYSL9-LIKE-A*	"
5	*TaIRO2-A*	*Triticum aestivum*		*TaYSL9-LIKE-B*	"
	TaIRO2-B	"		*TaYSL9-LIKE-D*	"
	TaIRO2-D	"		*TaYSL10-A*	"
	TaIRO2-like-A	"		*TaYSL10-B*	"
	TaIRO2-like-B	"		*TaYSL11-A*	"
	TaIRO2-like-D	"		*TaYSL11-B*	"
6	*TaVIT1-A*	*Triticum aestivum*		*TaYSL11-D*	"
	TaVIT1-B	"		*TaYSL12-A*	"
	TaVIT1-D	"		*TaYSL12-B*	"
	TaVIT2-A	"		*TaYSL12-D*	"
	TaVIT2-B	"		*TaYSL13-B*	"
	TaVIT2-D	"		*TaYSL13-D*	"
7	*TaFer1-A*	*Triticum aestivum*		*TaYSL13-like-A*	"
	TaFer1-B	"		*TaYSL13-like-B*	"
	TaFer1-D	"		*TaYSL13-like-D*	"
	TaFer2-A	"		*TaYSL14-A*	"
	TaFer2-B	"		*TaYSL14-B*	"
	TaFer2-D	"		*TaYSL14-D*	"
8	*TaYSL1-A*	*Triticum aestivum*		*TaYSL15-A*	"
	TaYSL1-B	"		*TaYSL15-B*	"
	TaYSL1-D	"		*TaYSL15-D*	"
	TaYSL2-A	"		*TaYSL15-LIKE-A*	"

Gene class	Gene name	Source organism	Gene class	Gene name	Source organism
8	*TaYSL2-B*	*Triticum aestivum*	8	*TaYSL15-LIKE-B*	*Triticum aestivum*
	TaYSL2-D	"		*TaYSL15-LIKE-D*	"
	TaYSL5-A	"		*TaYSL16*	"
	TaYSL5-B	"		*TaYSL17-A*	"
	TaYSL5-D	"		*TaYSL17-D*	"
	TaYSL6-1-B	"		*TaYSL18*	"
	TaYSL6-1-D	"	Marker	*hptII*	*Escherichia coli*
	TaYSL6-2-A	"		*pat*	*Streptomyces viridochromogenes*
	TaYSL6-2-B	"			

#This information has been declared CCI. The information is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

**Triticum aestivum* is a hexaploid plant with three genomes, known as the A, B and D genomes. Where genes from *T. aestivum* have the same name except for the final letter (A, B or D), these genes are homologs derived from the different wheat genomes.

22. The GM wheat lines with introduced genes also include short regulatory sequences that control expression of the genes (Table 4). The applicant proposes to express marker genes and genes from classes 1, 2 and 5 under the control of constitutive promoters. Genes from classes 3, 4, 6, 7 and 8 would have seed/tissue specific promoters. Further information regarding the activity of the seed/tissue specific promoters is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

Table 4: Introduced regulatory sequences in the GM wheat

Element function	Genetic element	Source organism
Constitutive promoter	*CaMV35S*	Cauliflower mosaic virus
	Ubi1	*Zea mays*
	Actin1	*Oryza sativa*
	Actin2	*Oryza sativa*
	Promoters that are CCI#	*Triticum aestivum*
Seed/tissue specific promoter	Promoters that are CCI#	*Triticum aestivum*
	Promoters that are CCI#	*Hordeum vulgare*
Amplification promoting sequence	*Ubi1 Intron*	*Zea mays*
Termination sequence	*nos*	*Agrobacterium tumefaciens*
	ocs	*Agrobacterium tumefaciens*
	Terminators that are CCI#	*Triticum aestivum*
	Terminators that are CCI#	*Hordeum vulgare*

#This information has been declared CCI. The information is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

23. GM wheat lines may be crossed and progeny grown during the proposed trial. No more than 10 introduced or modified iron-related genes would be stacked in any GM wheat in the proposed trial.

4.2 Methods of genetic modification

24. The applicant proposes to genetically modify (transform) wheat plants in contained facilities under an NLRD authorisation. Transformed wheat lines would be grown for at least two generations in contained glasshouses prior to selecting lines to be used in the field trial.

25. The GM wheat lines with introduced genes would predominantly be produced using *Agrobacterium*-mediated transformation. Some may be produced using the particle bombardment method. Information

about these methods can be found in the document *Methods of plant genetic modification* available from the OGTR Risk Assessment References page.

26. Some of the GM wheat lines with introduced genes may be produced by pollen magnetofection. In this method, the DNA to be introduced is loaded onto positively charged magnetic nanoparticles (MNPs) about 200 nm in size. The MNPs are mixed with plant pollen grains and a magnetic field is applied to transport the MNPs through small apertures in the pollen walls. The DNA load can then integrate into the pollen genome, sometimes in multiple positions. The transformed pollen is used for artificial pollination, generating GM progeny (Zhao et al., 2017).

27. The GM wheat lines with endogenous gene knockout would be generated by CRISPR/Cas9 genome editing. In the proposed CRISPR/Cas9 technique, a plasmid DNA is generated encoding the Cas9 protein and a short guide RNA (gRNA) designed to target a specific endogenous gene. Once wheat is transformed with the plasmid, the expressed Cas9-gRNA complex creates a double-stranded break in the target DNA sequence. Imperfect natural repair of these breaks most often leads to short insertions or deletions (one or a few base pairs) in the target plant DNA sequence, although it can sometimes produce larger deletions (Soyars et al., 2018). The applicant's intent is to make the target genes or associated regulatory sequences non-functional.

28. The applicant proposes to transform wheat with Cas9-gRNA cassettes either by particle bombardment of embryos or by polyethylene glycol-mediated transient transformation of isolated protoplasts (Kim et al., 2018). The genome-edited wheat would be grown in the glasshouse and progeny would be screened to identify segregants that contain the desired gene knockout, but do not contain the Cas9-gRNA cassette. Therefore, the knockout lines selected for the field trial would not contain any introduced plasmid DNA.

29. It is noted that the Regulator is currently reviewing the Gene Technology Regulations, and that part of the scope of the review is to clarify the regulatory status of genome-edited organisms, which is not entirely clear under the current legislation. In this case, the applicant has applied for a licence for dealings with GMOs for all wheat lines included in the application. Therefore, the genome-edited lines that are included in this application will be treated as GMOs.

4.3 The introduced genes, encoded proteins and associated effects

30. Iron is an essential nutrient for human health. Cereal grains contain low levels of iron, and much of this iron is bound to phytate and is not bioavailable in human diets (Vasconcelos et al., 2017). The applicant aims to achieve higher iron content in wheat seeds by introducing genes from classes 1-8 (Table 2) or combinations of these genes into GM wheat lines. The introduced genes have roles in increasing iron uptake from the soil to roots (Classes 1, 3, 4 and 5) , enhancing iron transport from roots to the aboveground plant (Classes 1, 5, 6 and 8), increasing iron storage levels in seeds (Classes 6 and 7) and improving iron bioavailability when seeds are consumed (Class 2). A schematic diagram of iron uptake, transport and storage in wheat is shown in Figure 2.

4.3.1 Class 1 (NAS)

31. The NAS gene family encodes nicotianamine synthase enzymes that catalyse the final step in the production of nicotianamine. Nicotianamine is a molecule made by all higher plants that chelates and transports transition metals including iron and zinc (von Wiren et al., 1999). In grasses, nicotianamine is also a precursor for biosynthesis of phytosiderophores, which are molecules that are secreted from roots to facilitate solubilisation and uptake of iron from the soil (Inoue et al., 2003).

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

copper levels in grain for all GM lines, and increased manganese and magnesium levels for most GM lines, compared to control non-GM wheat (Singh et al., 2017).

33. Several GM crops overexpressing NAS genes have demonstrated tolerance to low iron availability in alkaline soils, which causes leaf chlorosis and poor yield in control non-GM plants (Nozoye, 2018 and references cited therein). In addition, GM tobacco and Arabidopsis overexpressing a NAS gene have shown increased tolerance to high levels of heavy metals, particularly nickel, which cause toxicity to non-GM plants (Kim et al., 2005).

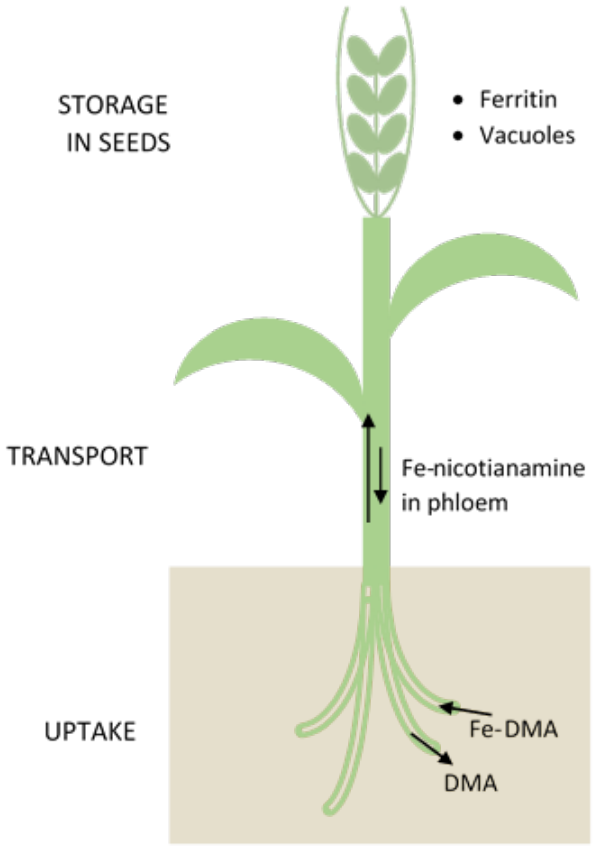

Figure 2. Schematic of iron metabolism in wheat, showing aspects of iron uptake, transport and storage affected by genes included in this application.

4.3.2 Class 2

34. The identity of this gene family has been declared CCI. A discussion of the Class 2 genes and their encoded proteins is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.3.3 Class 3 (NAAT) and Class 4 (DMAS)

35. The NAAT gene family encodes nicotianamine aminotransferase enzymes (Takahashi et al., 1999) and the DMAS gene family encodes deoxymugineic acid synthase enzymes (Bashir et al., 2006). NAAT and DMAS enzymes catalyse the two steps in the conversion of nicotianamine (Section 4.3.1) to deoxymugineic acid (DMA). DMA is a phytosiderophore molecule that chelates iron and zinc. Wheat and other grasses respond to iron deficiency, and to a lesser extent zinc deficiency, by secreting DMA from their roots to solubilise soil metal ions and increase their availability for uptake (von Wiren et al., 2000; Tolay et al., 2001).

36. The wheat *TaNAAT1*, *TaNAAT2* and *TaDMAS* genes that are included in this application are highly expressed in wheat root tissues during germination and early seedling growth. Expression was also significantly up-regulated in root tissues in response to iron deficiency (Beasley et al., 2017).

37. GM rice with two introduced barley NAAT genes was grown in alkaline soil with low iron availability. The GM rice demonstrated increased secretion of DMA and improved plant health and yield compared to a non-GM control (Takahashi et al., 2001).

4.3.4 Class 5 (IRO2)

38. The IRO2 gene family encodes an iron-related transcription factor present in grass species. Both *OsIRO2* in rice and *HvIRO2* in barley are strongly up-regulated by iron deficiency (Ogo et al., 2006). *OsIRO2* encodes a transcriptional activator of rice genes involved in iron uptake via DMA chelation (Ogo et al., 2007). GM rice constitutively overexpressing *OsIRO2* had stronger expression of endogenous NAS genes (Section 4.3.1), NAAT and DMAS genes (Section 4.3.3), YSL genes (Section 4.3.7) and others. GM rice overexpressing *OsIRO2* had increased secretion of DMA while a GM rice line where *OsIRO2* was repressed had reduced secretion of DMA (Ogo et al., 2007).

39. GM rice constitutively overexpressing *OsIRO2* was grown in alkaline soil with low iron availability. The GM rice grew better and had higher iron and manganese content in shoots and seeds than a non-GM comparator (Ogo et al., 2011).

4.3.5 Class 6 (VIT)

40. The VIT gene family encodes vacuolar iron transporter proteins (Kim et al., 2006). Vacuoles are storage organelles found in all plants that permit accumulation of nutrients for later use and sequestration of toxins (Krebs et al., 2010). Arabidopsis VIT1 and wheat VIT2 proteins are reported to transport iron and manganese into vacuoles, but not zinc or cadmium (Kim et al., 2006; Connorton et al., 2017). Rice VIT1 and VIT2 proteins are reported to transport iron, manganese and zinc but not cadmium (Zhang et al., 2012).

41. GM rice where endogenous *OsVIT1* or *OsVIT2* genes were knocked out had increased iron and zinc levels in grain and decreased iron and zinc levels in flag leaves compared to non-GM rice, suggesting that VIT activity mediates metal allocation between tissues. Unexpectedly, GM rice with *OsVIT1* or *OsVIT2* genes knocked out also had doubled cadmium content in grain compared to non-GM rice, when grown in soil with high cadmium contamination (Zhang et al., 2012). Some GM wheat or barley lines with an introduced *TaVIT2* gene under the control of an endosperm-specific promoter had increased iron content in grain (Connorton et al., 2017).

42. In the proposed field trial, the applicant may grow GM wheat lines with either knockout of endogenous wheat VIT genes or overexpression of wheat VIT genes under the control of seed/tissue specific promoters.

4.3.6 Class 7 (Fer)

43. Ferritin (Fer) proteins are present in all plants, and assemble into a spherical protein shell that can store several thousand iron atoms. Stored iron is bio-available for cellular needs. Ferritin also protects plants by sequestering excess free iron which causes oxidative stress (reviewed in Briat et al., 2010).

44. GM Arabidopsis plants that did not produce ferritin were reported to have similar iron concentrations in seed to non-GM Arabidopsis, but GM plants were more susceptible to excess iron stress (Ravet et al., 2009). GM banana plants with constitutive overexpression of banana *MusaFer1* had increased iron and zinc content in roots and leaves compared to non-GM banana, and GM plants were more tolerant to both excess iron stress and other oxidative stress (Yadav et al., 2017). GM wheat with constitutive overexpression of wheat *TaFer1* had increased iron content in leaves but not seeds when compared to non-GM wheat. GM wheat and GM Arabidopsis with constitutive overexpression of *TaFer1* had enhanced tolerance to heat stress, drought stress, oxidative stress and excess iron stress compared to controls (Zang et al., 2017).

45. GM rice plants with an introduced soybean ferritin gene driven by an endosperm-specific promoter demonstrated increased levels of iron and zinc in grain compared to non-GM rice (Goto et al., 1999; Vasconcelos et al., 2003). Some GM wheat lines with overexpression of a wheat *TaFer1* gene driven by an

endosperm-specific promoter had increased levels of iron and copper in grain compared to non-GM wheat (Borg et al., 2012).

46. In GM wheat lines for the proposed field trial, the applicant intends to introduce Fer genes under the control of seed/tissue specific promoters.

4.3.7 Class 8 (YSL)

47. Yellow stripe-like (YSL) genes encode a family of proteins that transport chelated iron and other metal ions.

48. Barley *HvYS1* encodes a transporter responsible for primary uptake of iron-phytosiderophore complexes from soil to barley roots. This transporter is highly specific and does not transport phytosiderophore complexes with copper, zinc, nickel, manganese or cobalt, or iron-nicotianamine complexes (Murata et al., 2006). Constitutive overexpression of *HvYS1* in GM rice increases iron content in rice roots, shoots and seed compared to non-GM rice, and also reduces cadmium content, possibly due to down-regulation of less specific transporters (Banakar et al., 2017).

49. Rice *OsYSL2* encodes a metal-nicotianamine transporter responsible for long-distance transport of iron and manganese through the phloem. The rice YSL2 protein does not transport nicotianamine chelating zinc or copper, or phytosiderophore-metal complexes (Koike et al., 2004). In GM rice, silencing or constitutive overexpression of *OsYSL2* caused increased iron levels in roots and reduced iron levels in shoots and seed compared to non-GM rice, indicating disrupted long-distance transport. Overexpression of *OsYSL2* under the control of a phloem and endosperm-specific promoter gave increased iron and manganese levels in GM rice seed (Ishimaru et al., 2010).

50. Rice *OsYSL15* encodes a transporter with roles in both uptake of iron-phytosiderophore complexes from the soil and transport of iron complexes through the phloem. The rice YSL15 protein transports iron chelated by DMA and there are conflicting reports regarding whether it does or does not transport iron chelated by nicotianamine (Inoue et al., 2009; Lee et al., 2009). GM rice with knockdown of *OsYSL15* had growth defects during germination and early growth (Inoue et al., 2009), and had lower iron concentration in shoots and roots than non-GM rice, but had no change in zinc, copper or manganese levels (Lee et al., 2009). GM rice with constitutive overexpression of *OsYSL15* had higher iron concentration in leaves and seeds than non-GM rice, with no effect on zinc, copper or manganese levels, but flowered later and had reduced height compared to controls (Lee et al., 2009).

51. Rice YSL6 is reported to transport manganese-nicotianamine complexes and have a role in detoxifying excess manganese (Sasaki et al., 2011). Rice YSL13 is reported to be involved in iron distribution within rice plants (Zhang et al., 2018). Rice YSL16 is reported to transport copper-nicotianamine complexes but not transport iron, manganese or zinc, and is involved in copper distribution within rice plants (Zheng et al., 2012). Rice YSL18 is a functional iron-DMA transporter but is not involved in direct iron uptake from the soil (Aoyama et al., 2009).

52. In GM wheat lines for the proposed field trial, the applicant intends to introduce YSL genes under the control of seed/tissue specific promoters.

4.3.8 Marker genes

53. The GM wheat plants contain selectable marker genes (Table 3) that confer resistance to an antibiotic or a herbicide. Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. The selectable marker genes are *hptII*, which encodes a hygromycin phosphotransferase enzyme that confers resistance to the antibiotic hygromycin B (Stogios et al., 2011), and *pat*, which encodes a phosphinothricin N-acetyltransferase enzyme that confers tolerance to the herbicide glufosinate (Dröge et al., 1992). The *hptII* gene is derived from *Escherichia coli*, a common gut bacterium that is widespread in human and animal digestive systems and in the environment. The *pat* gene is derived from *Streptomyces viridochromogenes*, a saprophytic, soil-borne bacterium that is not considered to be a pathogen of plants, humans, or other animals (OECD, 1999). More information on marker genes

may be found in the document *Marker genes in GM plants* available from the OGTR Risk Assessment References page.

4.4 Toxicity/allergenicity associated with the introduced genes

54. The applicant has not yet performed any toxicity studies on the GM wheat lines or purified proteins encoded by gene classes 1-8. Gene classes 1-8 do not encode any of the known types of toxic proteins produced by plants (Dang and Van Damme, 2015).

55. The applicant states that each of the introduced gene sequences have been compared to a database of allergens from the Food Allergy Research and Resource Program (FARRP) Allergen Database (Version 18A, February 2018), which contains the amino acid sequences of 2093 peer reviewed known and putative allergenic proteins. None of the alignments met or exceeded the threshold of 35% identity over a protein sequence of 80 or more amino acids. This indicates a lack of immunologically relevant similarities between the products of the genes of interest and known protein allergens.

56. It is noted that IRO2 genes encode transcriptional activators, and the rice IRO2 activator is reported to affect the expression of at least 59 other genes (Ogo et al., 2007). GM wheat lines that constitutively overexpress an IRO2 gene would presumably have increased levels of many wheat proteins. If any of those proteins were endogenous wheat allergens, the GM wheat could have increased levels of allergens.

57. The expression levels of endogenous IRO2 genes in rice are up-regulated by iron deficiency (Ogo et al., 2006). However, GM rice lines that constitutively overexpressed an IRO2 gene had much higher concentrations of IRO2 protein than non-GM rice, under conditions of iron sufficiency or iron deficiency (Ogo et al., 2007). Similarly, a GM wheat line with constitutive overexpression of an IRO2 gene would be expected to have a much higher concentration of IRO2 protein than is ever present in non-GM wheat. Thus, if the IRO2 activator increases the expression of any endogenous wheat allergen, this allergen could be present in GM wheat at levels well above the range seen in non-GM wheat.

58. An intended outcome of the genetic modifications in the proposed field trial is increased iron levels, particularly in GM wheat grains. The iron content of non-GM wheat grains is approximately 30 mg/kg, and in the literature about iron biofortification of GM plants the most successful strategies have achieved increases of about 6-fold in target tissues (Vasconcelos et al., 2017). Iron can be toxic to humans in excess, with ingestion of over 20 mg iron/kg of body weight producing mild toxicity and ingestion of over 40 mg iron/kg of body weight producing moderate or severe toxicity (Balmadrid and Bono, 2009). This suggests that a healthy human would need to ingest over 10% of their body weight of GM wheat grains before symptoms of acute iron toxicity would occur. However, lower levels of iron consumption could be toxic to people with medical conditions such as thalassemia or hereditary haemochromatosis (Barlow-Stewart et al., 2007; Nemeth, 2010).

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

60. A potential unintended effect in GM wheat lines in the proposed field trial is accumulation of metals other than iron. A number of heavy metals are highly toxic to humans and animals, including arsenic, lead, mercury and cadmium (Flora et al., 2008; Jaishankar et al., 2014; Clemens and Ma, 2016). In particular, potential accumulation of cadmium should be considered because: (a) more than 80% of human cadmium exposure is from consumption of cereals and vegetables; (b) many populations around the world already have cadmium intake above recommended levels, so moderate increases in cadmium exposure could have toxic effects; and (c) in terms of chemical characteristics, cadmium mimics iron and zinc, so may be taken up by biological pathways that are used for biofortification (Khan et al., 2014; Clemens and Ma, 2016).

61. The applicant has analysed the grain mineral contents of GM wheat lines with introduced NAS genes grown in glasshouse experiments, and has stated that there are no differences in cadmium, lead or arsenic levels between the GM lines and non-GM wheat. GM rice overexpressing rice *OsNAS2* and soybean ferritin genes had grain cadmium, lead and arsenic levels below detection limits when grown in normal soil, and

when grown in cadmium-contaminated soil there was no difference between grain cadmium levels in the GM rice lines and non-GM controls (Trijatmiko et al., 2016). The YSL family of transporters are known to transport a range of metals including iron, manganese and copper (Section 4.3.7). Many members of the YSL family have not been tested for metal specificity, so proteins from this family could potentially transport cadmium or other toxic heavy metals. GM wheat lines with knockout of VIT genes may have increased cadmium levels, as seen for rice VIT knockout lines (Section 4.3.5). Therefore, some of the GM wheat lines proposed for release may accumulate higher levels of cadmium or other toxic heavy metals than non-GM wheat.

62. There is no evidence that the *hptII* or *pat* genes or the proteins they encode are toxic or allergenic (see document *Marker genes in GM plants* available from the OGTR Risk Assessment References page). GM foods containing the *hptII* and *pat* genes have been assessed and approved for sale in Australia (FSANZ website, accessed 4 December 2018).

4.5 Characterisation of the GMOs

63. GM wheat lines containing *OsNAS2*, which may be included in the proposed field trial, were previously grown in a field trial under licence DIR 128 issued to the University of Adelaide. The *OsNAS2* lines grown in the DIR 128 field trial had significantly increased iron content in grains compared to a non-GM control. Some *OsNAS2* lines also produced a higher number of tillers, leading to 20-30% increases in shoot biomass and grain yield. Other agronomic characteristics of the *OsNAS2* lines were similar to the non-GM parent wheat. Further information regarding the field trial results for *OsNAS2* lines is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

64. The applicant states that GM wheat lines would be grown in contained glasshouses and undergo functional characterisation to identify any unintended phenotypes. Only GM wheat with expected phenotypes would be released in the field trial.

Section 5 The receiving environment

65. 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).

66. Detailed information about non-GM wheat in the Australian environment is presented in the document *The biology of* Triticum aestivum *L. (bread wheat)* (OGTR, 2017).

5.1 Relevant abiotic factors

67. The proposed trial sites may be located in any of 131 LGAs, which have a range of climates and soil types. In most of the grain belt of Australia, water availability is the main limiting factor for wheat production. Wheat plants are also susceptible to damage from frost or from heat stress, particularly during flowering. Wheat requires a number of soil nutrients including nitrogen, phosphorus, potassium, and zinc, and it is common for agricultural soils to lack some combination of the nutrients needed for optimal yield (GRDC, 2015, 2016a, b).

5.2 Relevant biotic factors

68. Wheat crops in Australia can be severely affected by diseases, which are predominantly caused by either fungal pathogens or nematodes. Arthropod pests are not normally a major problem in wheat cultivation, but sometimes build up to an extent that control is warranted (GRDC, 2015, 2016a, b).

69. Vertebrate pests of wheat crops include mice, birds, kangaroos and rabbits. Mice and birds often eat seeds, while kangaroos and rabbits prefer to graze on young green plants (OGTR, 2017).

5.3 Relevant agricultural practices

70. 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 agronomic practices for wheat. The GM wheat would be grown as a dryland crop, but drip or pipe irrigation may be used if necessary due to challenging weather situations. Herbicides or pesticides would be applied according to label instructions, in the same way that these chemicals are used in non-GM wheat crops.

71. Some GM wheat plants may be tented to facilitate controlled breeding. Some harvested GM seed may be milled into flour in order to test flour quality traits.

72. After harvest, the trial sites may be replanted to the GM wheat, left fallow, or planted with a rotation crop if approved.

5.4 Presence of related plants in the receiving environment

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

74. *Triticum aestivum* can spontaneously hybridise with a number of closely related species from the *Triticum-Aegilops* genera complex (Zaharieva and Monneveux, 2006). The only other *Triticum* species present in Australia is *T. turgidum* (durum wheat), which is cultivated for pasta production (Atlas of Living Australia, accessed 15 November 2018). No *Aegilops* species (goatgrasses) are cultivated or naturalised in Australia (Weeds in Australia, accessed 15 November 2018).

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

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

5.5 Presence of similar genes and encoded proteins in the environment

77. The introduced genes in classes 1-2 were isolated from rice, which is a common food crop that is grown in the Australian environment, and from other plant species. The identities of the donor plant species, other than rice, have been declared CCI. A discussion of the distribution and use of these plant species in Australia is found in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

78. The introduced genes in classes 3-8 were isolated from wheat, which is a staple food crop and widespread in the Australian environment. Therefore, people are routinely exposed to these genes and their encoded proteins.

79. The *hptII* gene is derived from a common bacterium that is present in human and animal digestive systems, and the *pat* gene is derived from a common soil bacterium. Both humans 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

6.1.1 Approvals by the Regulator

80. The Regulator has issued 21 licences for field trials of GM wheat in Australia. Further information about these field trials is available in the document *Genetically modified (GM) wheat trials* available from the Fact sheets page on the OGTR website.

81. GM wheat lines containing two of the introduced genes, *OsNAS2* and *hptII*, were approved for limited and controlled release under licences DIR 128 and DIR 152. GM wheat lines containing the *pat* gene were approved for limited and controlled release under licence DIR 142. No other genes from the current application were included in previous licences.

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

6.1.2 Approvals by other government agencies

83. The Regulator is responsible for assessing and managing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products.

84. The applicant has not sought approvals of the GMOs from other regulatory agencies.

85. The applicant proposes that some breeding and development of the GMOs may occur overseas, and seed would be returned to Australia. This would require an import permit from the Department of Agriculture and Water Resources.

6.2 International approvals

86. Field trials of GM wheat have been approved in a number of countries including the United States, Canada and several European countries. These approvals are for a range of modified traits, including herbicide tolerance, disease resistance, improved yield and abiotic stress tolerance (USDA APHIS Biotechnology Permits, CFIA Approved Confined Research Trials, EU GM Register; accessed 15 November 2018).

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

88. GM wheat has not been approved for commercial cultivation in any country.

Chapter 2 Risk assessment

Section 1 Introduction

89. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by, or as the result of, gene technology (Figure 2). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

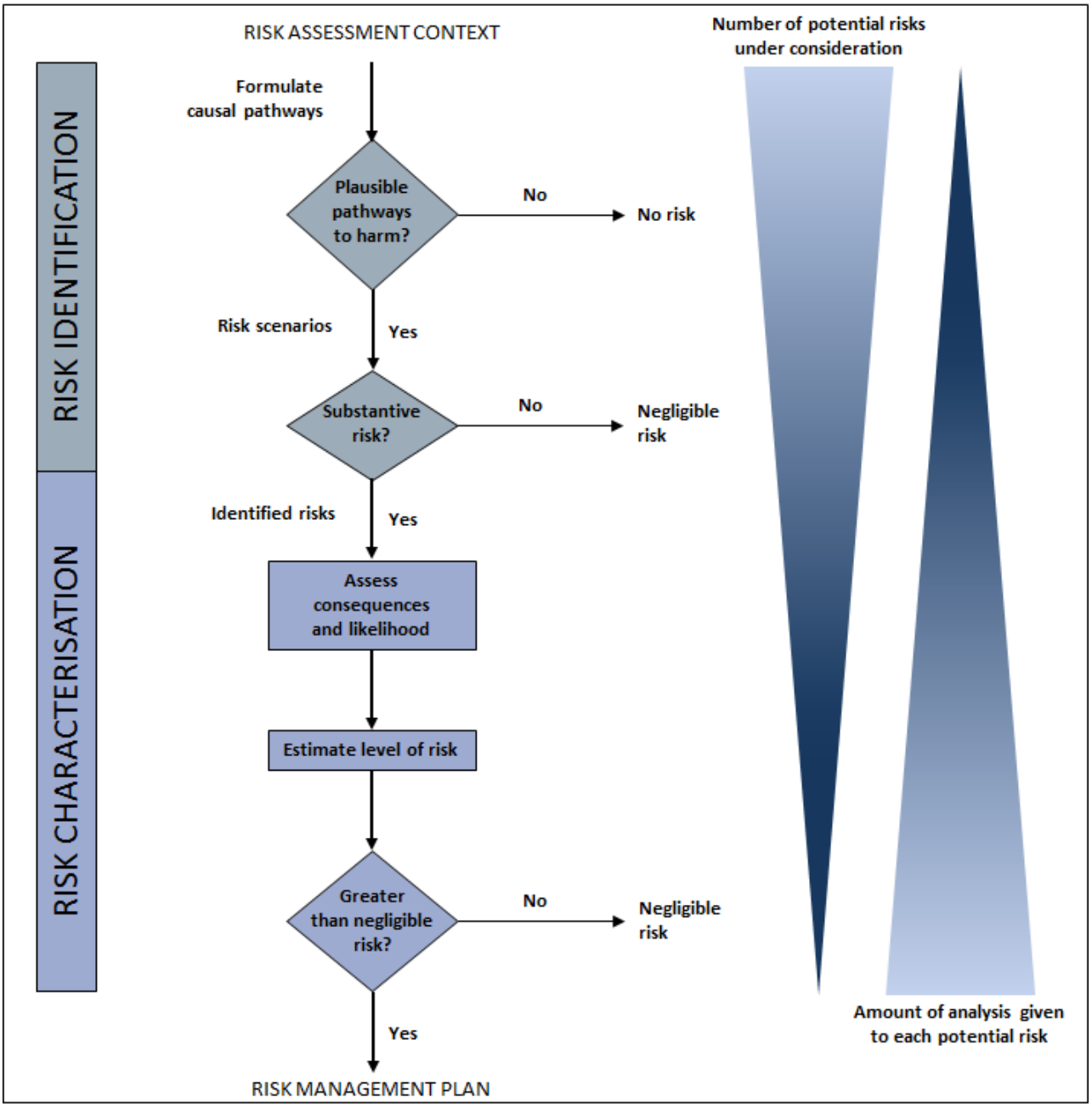

Figure 1 The risk assessment process

90. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants (Keese et al., 2014).

91. 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 for how this exposure to the GMO could give rise to harm for people or the environment. These are risk scenarios.

92. Postulated 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 long or short term, do not advance in the risk assessment process (Figure 2). These scenarios are considered to pose negligible risk.

93. 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.

Section 2 Risk Identification

94. Postulated risk scenarios are comprised of three components (Figure 3):

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.

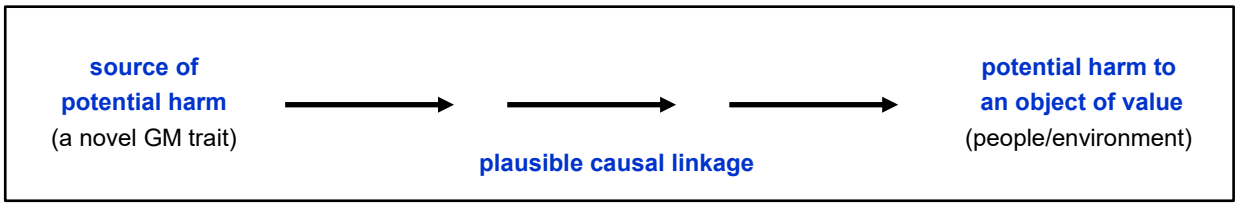

Figure 3. Risk scenario

95. 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

96. 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.

2.1.1 The introduced genetic elements

97. As discussed in Chapter 1 (Table 2 and Table 3), the GM wheat lines have been modified by the introduction or knockout of genes from eight different classes, conferring altered iron uptake, transport and bioavailability. These introduced or knocked out genes are considered further as potential sources of risk.

98. The GM wheat lines also contain the *hptII* or *pat* genes that were used as selectable marker genes. These genes and their products have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas. Further information about these genes can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. As these genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

99. The introduced genes are controlled by introduced regulatory sequences. These are derived from maize, rice, wheat, barley, a bacterium (*Agrobacterium tumefaciens*) and a plant virus (Cauliflower mosaic virus). Regulatory sequences are naturally present in 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 (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

2.1.2 Unintended effects

100. The genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the proteins encoded by the introduced genes, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding (Ladics et al., 2015; Schnell et al., 2015). Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015; Anderson et al., 2016). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, unintended effects resulting from the process of gene introduction will not be further considered for this application.

101. The genetic modifications involving knockout of genes by CRISPR/Cas9 have the potential to cause two classes of unintended effects. The first class of unintended effects are significant genomic deletions or rearrangements at the intended site of gene editing (Hahn and Nekrasov, 2018), leading to altered expression of endogenous genes. The applicant will use CRISPR/Cas to generate double-stranded breaks in DNA sequences that will be randomly repaired by non-homologous end joining (NHEJ). The conventional plant breeding technique of mutagenesis also generates double-strand breaks repaired by NHEJ, and can also produce significant genomic deletions or rearrangements (Shirley et al., 1992). As discussed in the previous paragraph, conventional breeding using mutagenesis has a long history of safe use. The second class of unintended effects is off-target gene editing, leading to inadvertent knockout of additional genes with sequences that nearly match the intended site of gene editing. Off-target CRISPR/Cas9 gene editing is rare in plants (Hahn and Nekrasov, 2018; Soyars et al., 2018). It is also noted that all DNA breaks generated by conventional mutagenesis are untargeted. Therefore, unintended effects arising from genome editing will not be further assessed for this application.

2.2 Causal pathway

102. The following factors are taken into account when postulating plausible causal pathways to potential harm:

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

103. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs.

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

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

2.3 Potential harm

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

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

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

2.4 Postulated risk scenarios

108. Five risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 5 and examined in detail in Sections 2.4.1 – 2.4.6.

109. In the context of the activities proposed by the applicant and considering both the short and long term, none of the five 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 or knocked out genes conferring altered iron uptake, transport and bioavailability	GM wheat grows at the field trial sites ⬇ GM wheat composition is different from non-GM wheat ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites	Increased toxicity or allergenicity to people	No	• GM plant material would not be used as human food • Proposed limits on the release would minimise the exposure of people to GM plant material
2	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability	GM wheat grows at the field trial sites ⬇ GM wheat composition is different from non-GM wheat ⬇ Exposure of animals that eat GM plant material	Toxicity to desirable animals	No	• GM plant material would not be used as livestock feed • Fences would exclude large animals from the trial sites • The small size and short duration of the proposed trial would restrict the exposure of desirable animals to GM plant material
3	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability	GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to related food or feed crops outside the trial sites ⬇ Exposure of people or animals that eat the hybrid GM seed	Increased toxicity or allergenicity to people OR Toxicity to desirable animals	No	• Wheat has low levels of outcrossing • The proposed controls would minimise pollen flow from the GM wheat to crops outside the trial sites
4	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability	GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to related plants outside the trial sites ⬇ GM hybrid seed grows into volunteer plants ⬇ Spread and persistence of GM wheat in the environment	Increased toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment and yield of desirable plants OR Reduced quality of biotic environment	No	• The proposed controls would minimise pollen flow from the GM wheat to related plants outside the trial sites • Wheat has limited ability to survive outside cultivation • GM wheat volunteers could be controlled by standard weed management measures

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
5	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability	GM wheat grows at the field trial sites ⬇ Persistence or dispersal of GM seed outside the trial limits ⬇ GM seed grows into volunteer plants ⬇ Establishment of GM wheat populations in the environment	Increased toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment and yield of desirable plants OR Reduced quality of biotic environment	No	• The proposed controls would minimise persistence of the GM wheat on the trial sites • The proposed controls would minimise dispersal of the GM wheat outside trial sites • Wheat has limited ability to survive outside cultivation • GM wheat volunteers could be controlled by standard weed management measures

2.4.1 Risk scenario 1

Risk Source	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability
Causal Pathway	⬇ GM wheat grows at the field trial sites ⬇ GM wheat composition is different from non-GM wheat ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites ⬇
Potential Harm	Increased toxicity or allergenicity to people

Risk source

110. The source of potential harm for this postulated risk scenario is the introduced or knocked out genes conferring altered iron uptake, transport and bioavailability in the GM wheat lines.

Causal pathway

111. GM wheat would be planted at the trial sites. Due to the genetic modifications, the GM wheat lines would have different composition, in terms of protein levels and/or metal content, to non-GM wheat. The GM wheat would not be used as human food, so people would not be exposed to plant material from the trial through ingestion. People may be exposed to GM plant material through inhalation of pollen when the GMOs flower, through direct skin contact with GM plant material, or possibly through inhalation of flour, as the applicant proposes to mill some GM seed into flour.

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

113. Wheat pollen is wind dispersed, and although usually more than 90% of wheat pollen falls within 3 m of the source plant, some pollen 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 trial site while the GM wheat is flowering could be exposed to low levels of GM pollen through inhalation. As the applicant proposes a maximum of ten trial sites per year, the maximum duration of planting under the field trial is 5 years, and the sites would be located in agricultural areas, only a limited number of people not involved with the trial could be exposed to low levels of GM pollen.

Potential harm

114. All of the introduced genes, or homologs, are present in crop plants that are commonly consumed by people. As discussed in Chapter 1, Section 4.4, there is no evidence suggesting that the proteins encoded by the introduced genes are themselves toxic or allergenic. Also, the intended increased levels of iron in the GM wheat are not expected to be toxic.

115. As discussed in Chapter 1, Section 4.4, some of the GM wheat lines could have higher levels of cadmium or other toxic heavy metals than non-GM wheat. As this is an early stage trial, no data is available regarding the potential for accumulation of cadmium or other toxic heavy metals in these GM wheat lines. Absorption of cadmium through the skin is negligible (Agency for Toxic Substances and Disease Registry, accessed 12 Dec 2018). Absorption of cadmium through inhalation of plant material is known to occur, as smokers on average accumulate twice the cadmium burden of non-smokers, due to high cadmium levels in tobacco leaves. However, even heavy smokers receive only about 10% of the FAO/WHO Provisional Tolerable Weekly Intake for cadmium from smoking (EFSA, 2009). Inadvertent inhalation of GM pollen or flour by people involved in the proposed trial would be at lower levels than deliberate inhalation of plant material by smokers. Also, people involved in the proposed trial could inhale pollen or flour during at most a few weeks in the year, while smokers typically smoke every day. It is implausible that people involved in

the proposed trial could be exposed to levels of cadmium or other toxic heavy metals that pose any health concern from the GM wheat.

116. Non-GM wheat can produce allergic responses in susceptible individuals via inhalation of pollen or inhalation of flour (Pahr et al., 2012). Common symptoms of respiratory allergy to wheat include rhinitis, conjunctivitis and asthma (Houba et al., 1998). As discussed in Chapter 1, Section 4.4, some GM wheat lines include a transcriptional activator that would increase levels of many wheat proteins, and there is uncertainty regarding whether these proteins include allergen/s. If the levels of wheat allergen/s are increased, this could increase the severity of an allergic reaction in a person with a respiratory allergy to wheat who is exposed to the GM wheat pollen or flour. The applicant proposes that respiratory allergy to wheat will be discussed during licence training and that no people who are known to be allergic to wheat will be allowed to work with the GM wheat.

Conclusion

117. Risk scenario 1 is not identified as a substantive risk because the GM plant material would not be used as human food and proposed limits and controls would minimise the exposure of people to GM plant material. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk Source	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability
Causal Pathway	⬇ GM wheat grows at the field trial sites ⬇ GM wheat composition is different from non-GM wheat ⬇ Exposure of animals that eat GM plant material ⬇
Potential Harm	Toxicity to desirable animals

Risk source

118. The source of potential harm for this postulated risk scenario is the introduced or knocked out genes conferring altered iron uptake, transport and bioavailability in the GM wheat lines.

Causal pathway

119. GM wheat would be planted at the trial sites. Due to the genetic modifications, the GM wheat lines would have different composition, in terms of protein levels and/or metal content, to non-GM wheat. Animals, including mammals, birds and invertebrates, in the vicinity of the trial sites could eat the GM plant material.

120. The GM wheat would not be used as animal feed. The applicant proposes to surround each trial site with a fence that would exclude large animals. Thus, livestock and large native animals would not be exposed to the GM plant material.

121. Desirable animals such as small native mammals or birds could enter the trial sites and feed on the GM wheat. The small size and short duration of the proposed field trial (Chapter 1, Section 2.1) would restrict the numbers of native animals or birds that could be exposed to the GM plant material.

122. Wheat is not bee-pollinated and bees are not attracted to wheat plants (USDA, 2017).

Potential harm

123. All of the introduced genes, or homologs, are present in crop plants that are commonly consumed by animals. As discussed in Chapter 1, Section 4.4, there is no evidence suggesting that the proteins encoded by the introduced genes are toxic, or that the intended increased levels of iron in the GM wheat are toxic.

124. As discussed in Chapter 1, Section 4.4, some of the GM wheat lines could have higher levels of cadmium or other toxic heavy metals than non-GM wheat grown under the same conditions. The only quantitative study suggesting that genetic modifications present in the GM wheat could lead to increased cadmium content found that GM rice with knockout of VIT genes had seed cadmium levels 1.7-2.1 fold higher than non-GM rice (Zhang et al., 2012). It is possible that some introduced genes in the GM wheat could also increase cadmium accumulation; for instance, many of the Class 8 YSL genes are metal ion transporters that have not been tested for specificity. As the applicant is proposing to cross GM wheat lines, potentially a GM wheat stack containing both knockout of VIT genes and an introduced gene contributing to cadmium accumulation could have cadmium levels well over 2-fold higher than non-GM wheat.

125. The average level of cadmium in non-GM wheat forage is 0.19 mg/kg (EFSA, 2004), and the average cadmium level in non-GM wheat grain is about 0.08 mg/kg (Adams et al., 2004). For most domestic animals, gross symptoms of toxicity commence when feed contains cadmium levels of approximately 5 mg/kg (EFSA, 2004). It is highly unlikely that GM wheat forage grown under normal conditions could contain cadmium levels over 25-fold higher than non-GM wheat to reach known toxic levels of 5 mg cadmium/kg. As wheat grain contains lower levels of cadmium than foliage, it is even less likely that GM wheat grain could be toxic to seed-eating animals or birds.

126. However, the level of cadmium accumulation in wheat depends on soil cadmium concentration. Non-GM wheat grown on agricultural soil that was highly contaminated with cadmium was reported to have grain cadmium levels up to 8-fold higher than average wheat grain cadmium levels (Adams et al., 2004). Therefore, if a trial site was located on soils with high cadmium contamination, foliage of some types of GM wheat could potentially be mildly toxic if grazed.

127. In addition, there is uncertainty regarding potential harm because sensitivity to cadmium toxicity varies between animal species (EFSA, 2004). Sensitivity to cadmium also varies between developmental stages, for instance, lower dosages of cadmium cause kidney damage to ducklings than to adult ducks (Furness, 1996). If any desirable animal species or developmental stage that might feed on the GM wheat is particularly sensitive to cadmium or other toxic heavy metals, GM wheat with higher levels of cadmium or other heavy metals than non-GM wheat could potentially have toxic effects on this sensitive species.

128. It is noted that some plants naturally accumulate higher levels of cadmium than wheat. For example, a study of non-GM wheat and canola grown on soils with different levels of cadmium found that when wheat and canola plants were grown under identical conditions, the cadmium concentration in canola grain was approximately 3-fold higher than in wheat grain (Brennan and Bolland, 2004). Canola is a common rotation crop in the Australian wheat belt, where the GM wheat field trial is proposed to take place. Therefore, it is unlikely that the GM wheat would contain higher levels of cadmium than other food sources available to animals and birds in the wheat belt.

Conclusion

129. Risk scenario 2 is not identified as a substantive risk because the GM plant material would not be used as animal feed, fences would exclude large animals from feeding in the trial sites, and the small size and short duration of the proposed trial would restrict the exposure of other desirable animals to GM plant material. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk Source	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability
Causal Pathway	⬇ GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to related food or feed crops outside the trial sites ⬇ Exposure of people or animals that eat the hybrid GM seed ⬇

Potential Harm	Increased toxicity or allergenicity to people OR Toxicity to desirable animals

Risk source

130. The source of potential harm for this postulated risk scenario is the introduced or knocked out genes conferring altered iron uptake, transport and bioavailability in the GM wheat lines.

Causal pathway

131. GM wheat would be planted at the trial sites. When the GM wheat flowers, GM pollen could be carried by wind to sexually compatible crops growing in the vicinity of the trial sites. If these related crops are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM seed. The hybrid seed could be used for human food or animal feed.

132. It should be noted that vertical gene flow per se is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome.

133. As discussed in Chapter 1, Section 5.4, bread wheat is sexually compatible with bread or durum wheat crops. Crossing of bread wheat with rye or triticale crops is possible but rare. All of these species are considered as potential recipient crops for pollen flow in this risk scenario.

134. 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 nearby plants 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).

135. The applicant proposes that each planting area for GMOs would be surrounded by monitoring, inspection and isolation zones totalling 200 m. No crops of wheat or sexually compatible plants would be grown in any of these zones. Based on the cross-pollination studies above, a 200 m separation distance is expected to minimise pollen flow from the GM wheat to related food or feed crops.

136. Wheat pollen grains contain two sperm cells, and in a pollination event these two sperm simultaneously fertilise an embryo and an endosperm (Sabelli and Larkins, 2009). If a GM pollen grain pollinates a flower on a non-GM plant, the introduced genes will be present in the embryo and endosperm of the resultant hybrid GM seed, but will not be present in any other part of the maternal plant. Some of the classes of introduced genes would have little effect if they were expressed only in hybrid GM seed, as their encoded proteins function in other parts of the plant (e.g. enzymes involved in root uptake of metal ions).

Potential harm

137. As discussed in Chapter 1, Section 4.4, some of the GM wheat lines could have higher levels of cadmium or other toxic heavy metals than non-GM wheat. If humans or livestock ate wheat products with significantly increased levels of cadmium or other heavy metals, this could lead to toxicity. The maximum permitted level of cadmium in wheat grain for human consumption in Australia is 0.1 mg/kg (Food Standards Australia New Zealand, 2017).

138. However, as discussed above, the proposed 200 m separation distance would minimise pollen flow from the GM wheat to any non-GM wheat or related crops, so the proportion of hybrid GM seeds in any non-GM crop would range between zero and extremely low. In addition, as discussed above, some of the

introduced genes with roles in metal uptake would have little effect if only present in hybrid seeds. Thus, hybrid GM seeds would not be expected to cause any measurable increase in the levels of cadmium or other toxic heavy metals in non-GM food or feed crops.

139. It is estimated that 0.2 – 1% of children have a food allergy to non-GM wheat, although most children outgrow the allergy by the age of 12 (reviewed in Cianferoni, 2016). As discussed in Chapter 1, Section 4.4, some GM wheat lines include a transcriptional activator that would increase levels of many wheat proteins, which could include allergen/s. If a person with a wheat food allergy ate wheat with increased levels of allergens, this could increase the severity of an allergic reaction. However, as discussed above, the proportion of hybrid GM seeds in any non-GM crop grown in the vicinity of the GM wheat would be negligible, so the non-GM crop would not be expected to have any measurable increase in levels of allergens. In addition, people with known wheat allergies generally avoid eating wheat products.

Conclusion

140. Risk scenario 3 is not identified as a substantive risk because wheat has low levels of outcrossing and the proposed controls would minimise pollen flow from the GM wheat to crops outside the trial sites. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk Source	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability
Causal Pathway	⬇ GM wheat grows at the field trial sites ⬇ Pollen flow from the GM wheat to related plants outside the trial sites ⬇ GM hybrid seed grows into volunteer plants ⬇ Spread and persistence of GM wheat in the environment ⬇
Potential Harm	Increased toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment and yield of desirable plants OR Reduced quality of biotic environment

Risk source

141. The source of potential harm for this postulated risk scenario is the introduced or knocked out genes conferring altered iron uptake, transport and bioavailability in the GM wheat lines.

Causal pathway

142. GM wheat would be planted at the 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 and fertilise these non-GM plants to produce hybrid GM seed. If the maternal plants are volunteers, the hybrid GM seed could fall to the ground and grow into volunteer GM plants. If the maternal plants are part of a crop, the hybrid GM seed could be lost during harvest or transport and grow into volunteer GM plants. Volunteer GM wheat could potentially spread and persist in the environment. People and desirable animals could then be exposed to the GM wheat.

143. As discussed in Chapter 1, Section 5.4, bread wheat is sexually compatible with bread wheat or durum wheat plants. Crossing of bread wheat with rye or triticale is rare, and hybrids produced are usually sterile. Therefore, it is highly unlikely that hybrids between GM wheat and rye/triticale could spread and

persist in the environment. Thus, this risk scenario will only consider crossing between GM wheat and bread or durum wheat plants.

144. As discussed in Risk Scenario 3, the proposed monitoring, inspection and isolation zones provide a 200 m separation distance between the GM wheat and any sexually compatible crops, and are expected to minimise pollen flow to related crops.

145. The applicant proposes to inspect the 10 m monitoring zone and 50 m inspection zone for wheat or related species, and destroy any volunteer plants found, but does not propose to inspect the isolation zone for individual wheat plants. Therefore, volunteer wheat plants could be present in the isolation zone, starting approximately 60 m from GMOs on the trial sites. Populations of volunteer wheat in the isolation zone would typically be low, but could be larger if, for instance, wheat was grown there in the previous season, there was good rainfall, and volunteers have not been controlled by weed management. A study found that the rate of cross-pollination from a donor wheat field to recipient plants 60 m or greater from the donor field was <0.01% (Matus-Cádiz et al., 2004). Although this rate could be up to an order of magnitude higher for some recipient wheat cultivars (Gaines et al., 2007), the proportion of GM hybrid seeds produced on non-GM volunteer plants near the trial sites would still be very low.

146. Even if hybrid GM wheat seeds were produced in the environment, wheat is a domesticated plant that has limited ability to survive outside cultivation. For instance, during domestication wheat lost its natural seed dispersal mechanism of seed shattering and lost seed dormancy traits that allow seeds to delay germination until environmental conditions are favourable (reviewed in OGTR, 2017). The introduced or knocked out genes are not expected to alter seed dormancy, seed shattering or other seed dispersal traits. Therefore, it is highly unlikely that populations of GM wheat could spread and persist in the environment.

Potential harm

147. Potential harms that could arise from populations of volunteer GM wheat in the environment include toxicity or allergenicity to people, toxicity to desirable animals, reduced establishment and/or yield of desirable plants, or reduced quality of the biotic environment.

148. People do not harvest and eat volunteer wheat, so people would not be exposed to GM volunteer wheat by ingestion. People could inhale pollen from GM volunteer wheat populations. As discussed in Risk Scenario 1, it is implausible that inhalation of the GM pollen could be toxic to people. GM wheat pollen could have higher levels of allergens than non-GM wheat pollen. However, as the proposed trial sites are located in Australia's wheat belt, pollen produced by small populations of volunteer GM wheat could only be a tiny proportion of the airborne wheat pollen produced by large scale wheat cultivation, so would have little effect on airborne wheat allergen concentrations.

149. Desirable animals, including birds, could eat GM volunteer wheat, particularly if it spread to livestock pastures. As discussed in Risk Scenario 2, it is highly unlikely that the GM wheat would be toxic to domesticated animals, but there is uncertainty regarding whether the GM wheat could be toxic to some other animal species. However, small populations of GM wheat volunteers could probably only be a minor part of any animal's diet, which minimises the potential for toxicity to desirable animals.

150. Establishment of populations of GM volunteer wheat could potentially reduce the establishment and/or yield of desirable plants such as agricultural crops or native vegetation. This is unlikely because wheat is a poor competitor with other vegetation (OGTR, 2017). As discussed in Chapter 1, Section 4.4, several of the classes of introduced genes may confer tolerance to low iron availability, which could make the GM wheat more competitive than non-GM wheat when growing on soils where iron deficiency inhibits plant growth. As further discussed in Chapter 1, Section 4.4, introduced NAS genes may confer increased tolerance to high levels of metals, which could make some GM wheat lines more competitive than non-GM wheat when growing on soils polluted with heavy metals. Constitutive overexpression of Fer genes has been reported to enhance tolerance to heat stress, drought stress and oxidative stress (Zang et al., 2017), which could increase plant competitiveness in difficult growing conditions. There is uncertainty regarding

whether introduced Fer genes would confer these abiotic stress tolerances when expressed under the control of a seed/tissue specific promoter, as proposed by the applicant.

151. Further information regarding the potential for GM wheat containing Class 2 genes to compete with other vegetation is included in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

152. The ability of volunteer GM wheat to compete with desirable plants is restricted because the genetic modifications are not expected to change the susceptibility of the GM wheat to conventional weed management. Thus, GM wheat volunteers could be controlled by standard weed management measures, such as cultivation or the use of appropriate herbicides, if required.

153. The final potential harm that is considered is whether populations of GM volunteer wheat could reduce the quality of the biotic environment. GM wheat volunteers, like non-GM wheat volunteers, could host wheat pathogens, which could spread to cultivated wheat. If, as discussed above, GM wheat has increased competitiveness compared to non-GM wheat, GM volunteers could potentially have higher population density than non-GM volunteers and host a larger reservoir of pathogens. In addition, some types of GM volunteer wheat would secrete large quantities of DMA into the soil. As all grasses can take up DMA-iron complexes, this could potentially increase iron availability for grassy weeds, and improve the growth of these weeds on iron deficient soil. However, some other grasses naturally secrete higher levels of phytosiderophores than non-GM wheat (Bashir et al., 2006), so it is unclear that the GM wheat would secrete levels of phytosiderophores outside the normal range for grasses.

Conclusion

154. Risk scenario 4 is not identified as a substantive risk because the proposed controls would minimise pollen flow from the GM wheat to related plants outside the trial sites, wheat has limited ability to survive outside cultivation, and GM wheat volunteers could be controlled by standard weed management measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.5 Risk scenario 5

Risk Source	Introduced or knocked out genes conferring altered iron uptake, transport and bioavailability
Causal Pathway	⬇ GM wheat grows at the field trial sites ⬇ Persistence or dispersal of GM seed outside the trial limits ⬇ GM seed grows into volunteer plants ⬇ Establishment of GM wheat populations in the environment ⬇
Potential Harm	Increased toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment and yield of desirable plants OR Reduced quality of biotic environment

Risk source

155. The source of potential harm for this postulated risk scenario is the introduced or knocked out genes conferring altered iron uptake, transport and bioavailability in the GM wheat lines.

Causal pathway

156. GM wheat would be grown at the trial sites. If GMOs persisted at the trial sites after completion of the trial, or if GM seed dispersed outside the trial sites, volunteer GM wheat could potentially establish populations in the environment.

Persistence of GMOs on trial sites

157. The applicant proposes to clean the trial sites after harvest. This is expected to minimise persistence of live GM wheat plants on the trial sites, but would not be expected to remove or destroy all GM seeds.

158. White-grained wheat cultivars, which are the types usually grown in Australia, have little seed dormancy. Freshly harvested seed samples from 16 white-grained cultivars, when placed in conditions of sufficient moisture, were 50% germinated within 2-8 days (Mares and Mrva, 2001). 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).

159. The applicant proposes post-harvest monitoring of each trial site for at least two years with tillage and irrigation. Any wheat volunteers found would be destroyed prior to flowering. Based on the seed dormancy studies above, and experience from previous wheat field trial licences issued by the Regulator, these control measures are expected to minimise persistence of viable GM wheat seeds on the trial sites.

Dispersal of GM seed outside trial sites

160. Dispersal of GMOs outside the limits of the trial sites could potentially occur through the activity of people or animals or by wind or water.

161. Human activity is the most important dispersal pathway for non-GM wheat seed (OGTR, 2017). The applicant has proposed a number of controls related to dispersal of GM wheat by people. Only authorised persons would be permitted to access the trial sites. All equipment used on trial sites would be inspected for GM seeds and cleaned before use for any other purpose. All GM plant material would be transported and stored in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These control measures are expected to minimise dispersal of GM wheat outside the trial sites by human activity.

162. Animals can potentially spread plant seed by movement of seeds adhering to fur, feathers or feet, consumption and excretion of whole seeds, or by removing and hoarding seed (Chambers and MacMahon, 1994). Wheat seeds do not possess adaptations for adhesion to the exterior of animals, such as hooks, barbs or sticky surfaces.

163. The applicant proposes to fence the trial sites, which is expected to exclude large herbivores. However, small herbivores might ingest and excrete viable seeds. As white-grained wheat cultivars have large seeds with a thin seed coat, they are expected to be readily broken down and digested by mammals (OGTR, 2017). A bird feeding study found that approximately 0.25% of wheat seeds ingested by corellas were excreted as viable seeds, and 0.1% of wheat seeds ingested by galahs were excreted as viable seeds (Woodgate et al., 2011). Under field conditions, probably only a small proportion of viable seeds excreted by birds would be deposited in places conducive to germination.

164. Seeds may be removed and hoarded by birds, ants or rodents (Chambers and MacMahon, 1994). Although most hoarded seeds are later consumed, a small proportion may remain. A literature survey has identified only three Australian bird species reported to hoard seeds, all crows or ravens (de Kort and Clayton, 2006) (Queensland Government Department of Environment, accessed 19 December 2018). Ants may transport seeds to nest sites over distances that are typically between tens of centimetres and a few metres (Gómez and Espadaler, 1998), so would probably not transport seeds outside the proposed 10 m monitoring zone. Mice can collect and carry seed over distances estimated as up to 50 m (Andersson and

deVicente, 2010). The applicant proposes that the monitoring zone surrounding each GM wheat planting area will be maintained in a manner that facilitates detection of wheat and related species. If the monitoring zone is kept either free of vegetation or planted with vegetation mown to a height of less than 10 cm, as was required for previous wheat field trial licences issued by the Regulator, this would deter rodents from entering the monitoring zone. Therefore, this control measure would deter rodents from transporting GM seeds to areas outside the trial sites.

165. 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, 2017). 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.

Establishment of GM volunteer populations in the environment

166. As discussed in Risk Scenario 4, wheat is a domesticated plant that has limited ability to survive outside cultivation. Therefore, even if GM wheat seeds grew into volunteer plants outside the limits of the trial, it is highly unlikely that populations of GM wheat could spread and persist in the environment.

Potential harm

167. The potential harms from this risk scenario are the same as the potential harms from Risk Scenario 4, which also considered the consequences of establishment of GM wheat populations in the environment.

168. As discussed in Risk Scenario 4, GM wheat volunteers could be controlled by standard weed management measures, if required.

Conclusion

169. Risk scenario 5 is not identified as a substantive risk because the proposed controls would minimise persistence of the GM wheat on the trial sites, the proposed controls would minimise dispersal of GM seed outside trial sites, wheat has limited ability to survive outside cultivation, and GM wheat volunteers could be controlled by standard weed management measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

170. 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.

171. 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.

172. 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.

173. For DIR 165, uncertainty is noted particularly in relation to:

- potential for increased toxicity of the GM wheat to people or animals
- potential for increased allergenicity of the GM wheat to people
- potential for the genetic modifications to increase plant competitiveness and survival, particularly relating to increased tolerance to abiotic stresses

174. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.

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

Section 4 Risk evaluation

176. 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.

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

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

178. Five 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:

- no GM plant material would enter 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 and its genetic material
- GM wheat has limited ability to survive outside cultivation
- GM wheat volunteers could be controlled by standard weed management measures

179. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM wheat plants into the environment are considered negligible. The *Risk Analysis Framework* (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. Hence, no controls are required to treat these negligible risks. The Regulator concludes that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

180. 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.

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

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

183. 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

184. The assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM wheat. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed containment measures (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

185. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release to the proposed size, 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 full in the licence.

3.1 Licence conditions to limit and control the release

186. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by the University of Melbourne. Many of these are discussed in the five 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 University of Melbourne

187. The applicant proposes that the duration of the field trial would be limited to five years, between April 2019 and December 2023. In 2019, GM wheat would be grown on a maximum of two trial sites, with an area of up to 2 ha per site. In 2020-2023, GM wheat would be grown on a maximum of ten trial sites per year, with an area of up to 2 ha per site. The small size and short duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk Scenarios 1 and 2).

188. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions included in the licence state that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions. In addition, the applicant proposes that no person with a known respiratory allergy to wheat would be permitted to deal with the GMOs. As discussed in Chapter 1, Section 4.4, only introduced Class 5 genes are considered to pose a potential allergenicity risk. Therefore, a licence condition states that the licence holder must not permit a person with a known respiratory allergy to wheat to conduct any dealing under the licence which would expose that person to inhalation of plant material from GM wheat overexpressing a Class 5 gene. These measures would limit the exposure of people to potential harm from the GM wheat (Risk Scenario 1).

3.1.2 Consideration of proposed controls to manage exposure to the GMOs

189. The applicant proposes not allowing the GMOs or GM products to be used for human food or animal feed. A licence condition states that GM plant material must not be used as food for humans or feed for animals. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenarios 1 and 2).

3.1.3 Consideration of proposed controls to manage pollen flow from the GMOs

190. The applicant proposes surrounding each GM wheat planting area with a 10 m monitoring zone and a 50 m inspection zone. Both of these areas 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. These isolation distances were considered in Risk Scenarios 3 and 4 and in previous RARMPs (e.g. DIR 152) and are expected to minimise pollen flow from the GMOs to non-GM plants outside the trial sites, so are included in the licence.

191. The applicant proposes that the monitoring and inspection zones would be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until 28 days after all GMOs in the planting area have finished flowering. The applicant suggests that the beginning of flowering be defined as a plant reaching Zadoks score 60 and the end of flowering be Zadoks score 69 or higher (Zadoks et al., 1974). However, wheat is an asynchronous plant (Zadoks et al., 1974), meaning that the main stem could finish flowering (Zadoks score 69 or higher) while some tillers are still flowering, so defining flowering using the Zadoks scale could be unclear and the licence does not use this definition. The applicant proposes continuing inspections for 28 days after all GMOs have finished flowering. It is desirable to have one inspection after the completion of flowering of the GMOs, in case any plants were missed in the previous inspection, but no further inspections are necessary. Therefore, a licence condition requires the monitoring and inspection zones to be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering.

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

3.1.4 Consideration of proposed controls to manage persistence of the GMOs

193. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or future plantings. In order to manage persistence of GMOs, it is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed. Licence conditions require that each trial site must be cleaned (which would destroy any surviving GM plants) within 35 days after harvest, and that harvested GM seed not required to conduct experiments or for future planting must be destroyed as soon as practicable. In addition, to deal with the case of failed crops that are not harvested, licence conditions require that GMOs must be harvested or destroyed within eight months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been cleaned.

194. The applicant proposes that GM wheat would be destroyed using one or more of the following methods: grinding, milling, herbicide application, root cutting and shredding/mulching, uprooting, burning/incineration, tilling, autoclaving or burial to a depth of at least 1 m. All of these methods are considered effective in destroying one or more life stages of the GM wheat, so are included in the 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.

195. The applicant proposes to treat non-GM wheat plants grown in the trial as if they were GMOs. Non-GM wheat grown at the trial site may be cross-pollinated by GM wheat and bear hybrid seeds. It is therefore appropriate to require non-GM wheat to be destroyed after harvest in the same manner as GM wheat, to manage persistence of the GMOs, and this measure is included in the licence.

196. After harvest, the applicant proposes to monitor each trial site monthly for at least 2 years and until the site is free of volunteer plants for at least 6 months. Any wheat volunteers found would be destroyed prior to flowering, to prevent pollen flow to non-GM plants outside the trial site. Wheat typically requires 1275 degree-days to grow from emergence to flowering (Bowden et al., 2008), which in hot weather (average daily temperature 26°C), would be about 49 days. Allowing for variation between cultivars and between individual plants, monitoring the trial sites at least every 35 days would be sufficient to detect volunteers before flowering. The total monitoring period of at least two years, with at least the last six months volunteer-free, was discussed in Risk Scenario 5 and is expected to minimise persistence of GM wheat at the trial sites, so is included in a licence condition.

197. The applicant does not specify which areas of the trial sites would be subject to post-harvest inspections. Harvest seed loss would occur on the planting area, and also potentially on land surrounding the planting area where the harvester drives during harvesting. The applicant states that commercial harvesters may be used to harvest trial sites. One commercial combine harvester used in Australia has a turning circle diameter of 14 m (New Holland Agriculture, 2014), indicating that if this harvester were used to harvest a planting area, during turns it would drive at least 7 m into the 10 m monitoring zone. Therefore, a licence condition states that the planting areas and associated monitoring zones require post-harvest inspections.

198. The applicant proposes to inspect areas used for equipment clean down on a monthly basis for the presence of volunteers. A licence condition requires that any area used to clean equipment used in connection with the GMOs, and any area where GMOs have dispersed in the course of dealings under this licence, must be cleaned as soon as practicable, and then monitored in the same way as the planting areas after cleaning. The applicant proposes to inspect burial sites at least every 70 days for the presence of volunteers or disturbance, and if any disturbance is observed, take appropriate remedial action including notification to the Regulator. As volunteers are not expected to emerge on burial sites under normal circumstances, the suggested inspection frequency over the period of a year is considered sufficient and is included in a draft licence condition. If seed is dispersed during burial, this area would require cleaning as an area in which the GMOs have been dispersed in the course of dealings under the licence, and post-cleaning conditions would apply.

199. The applicant proposes at least one tillage (to a depth of no more than 5 cm) and three irrigations / natural rainfall events for each trial site during the post-harvest monitoring period. 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, followed by irrigation, will germinate much of the wheat seed lying on the soil surface (Ogg and Parker, 2000). However, deep cultivation could place wheat seeds in an environment where they are unable to germinate, enforcing dormancy. The licence includes conditions requiring that the post-harvest trial sites receive at least three irrigations and one tillage, with the last required irrigation occurring after tillage and at a time that would promote germination of volunteers within the final volunteer-free period. The licence specifies that tillage must be to a depth no greater than 5 cm, and that natural rainfall events may be taken as irrigation only with the agreement of the Regulator. These measures are expected to promote germination of the GM wheat seedbank and manage persistence of GM seed on the trial sites (Risk Scenario 5).

3.1.5 Consideration of proposed controls to manage dispersal of the GMOs

200. The applicant proposes to inspect all equipment and clothing used on trial sites for GM seeds and to clean the equipment before use for any other purpose. The applicant also proposes to transport and store GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These measures are expected to minimise dispersal of GMOs outside the trial sites by human activity (Risk Scenario 5) and are included in the licence.

201. The application does not discuss how the GM wheat would be threshed. As required for previous wheat field trial licences issued by the Regulator, a licence condition states that GM wheat must be threshed separately from any other crop, and threshing must take place on the planting areas, monitoring zones or in a facility approved by the Regulator.

202. The applicant proposes to surround each trial site with a fence to restrict access by large animals. This measure is expected to restrict dispersal of GMOs by large herbivores (Risk Scenario 5) and also to restrict exposure of livestock and large native animals to potential toxicity from ingestion of the GMOs (Risk Scenario 2). In addition, the presence of a fence would reduce the likelihood of people who are not involved in the trial inadvertently entering the trial site, so could help reduce human exposure to potential allergenicity from inhalation of the GM pollen (Risk Scenario 1) and help restrict human dispersal of GMOs (Risk Scenario 5). The licence includes a condition requiring each planting area and surrounding monitoring zone to be inside a fence.

203. The applicant has not proposed any rodent control measures. As discussed in Risk Scenario 5, it is possible that rodents could remove and cache GM wheat seeds from the trial sites. A licence condition states that the monitoring zone must be maintained in a manner that does not attract or harbour rodents, for instance kept either free of vegetation or planted with vegetation mown to a height of less than 10 cm, as required for previous wheat field trial licences issued by the Regulator. This is expected to deter rodents from transporting seed through the monitoring zone. An additional licence condition requires implementation of measures to control rodents within each planting area. Both of the licence conditions above apply while the GMOs are being grown and 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.

204. The applicant proposes that all trial sites would be located at least 50 m from any natural waterway. This measure is expected to minimise the potential for seed dispersal through flooding, and is included in the licence. An additional licence condition requires immediate notification of any extreme weather condition affecting trial sites to the Regulator, to allow assessment and management of any possible dispersal of GMOs.

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

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

- limit the duration of the release to a maximum of five years, between April 2019 and December 2023

- limit the size of the release to a maximum of two sites in 2019 and ten sites per year in 2020-2023, with each site having a maximum area of 2 ha
- not allow the GM plant material to be used for human food or animal feed
- surround each planting area with a monitoring zone of at least 10 m, 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
- clean each planting area after harvest and clean any area in which seed has been dispersed
- treat non-GM wheat plants grown in the trial as if they were GMOs
- destroy all harvested GM seed not required for further analysis or future planting
- apply measures to promote the germination of any wheat seeds that may be present in the soil after harvest, including irrigation and shallow tillage
- monitor each trial site for at least 24 months after harvest and until the site is free of volunteer plants for at least 6 months, with any wheat volunteers destroyed prior to flowering
- clean all equipment used on the trial sites
- transport and store the GMOs in accordance with the Regulator's guidelines
- surround each trial site with a fence
- 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

206. 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

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

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

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

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

3.2.2 Contingency plan

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

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

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

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

3.2.4 Reporting requirements

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

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

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

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

3.2.5 Monitoring for compliance

215. 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.

216. 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.

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

Section 4 Issues to be addressed for future releases

218. 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. This includes:

- compositional characterisation of the GM wheat lines, particularly with respect to potential for increased toxicity
- biomolecular characterisation of the GM wheat lines, particularly with respect to potential for increased allergenicity
- additional phenotypic characterisation of the GM wheat lines, particularly with respect to increased tolerance to abiotic stresses leading to potential for increased weediness.

Section 5 Conclusions of the RARMP

219. The RARMP concludes that this limited and controlled release of GM wheat poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

220. Licence conditions have been 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.

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

Advice received by the Regulator from prescribed experts, agencies and authorities[3] on the consultation RARMP is summarised below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Comment
1	Opposes the release of genetically modified wheat within the Shire. States that Council has adopted a policy that does not support the growing, storage or transport of genetically modified crops within the Shire, for marketing reasons. Notes that there is no current legislative power enabling the Council to enforce this policy. Requests that the Shire's submission be forwarded to the applicant.	The submission was forwarded to the applicant. The Regulator is required to assess GMO applications in accordance with the Gene Technology Act 2000, the object of which is to protect the health and safety of people and the environment. Marketing and trade issues are outside the scope of assessments conducted by the Regulator. These issues are the responsibility of the State and Territory governments and industry.
2	No concerns raised.	Noted.
3	Agrees with the overall conclusions of the RARMP that the risks to the environment are negligible.	Noted.
	The RARMP would benefit from further discussion of the toxicity of GM wheat seed to desirable animals and birds. It is possible that birds, invertebrates and small mammals will be exposed to the GM wheat seed over the five year trial. There are no controls that restrict exposure of birds or small animals. It is agreed that levels of cadmium in the GM wheat seed are unlikely to reach levels toxic to animals or birds. There is some uncertainty regarding the level of cadmium that may be present in the GM wheat seed. According to the RARMP, levels of cadmium in non-GM wheat are 0.2 mg/kg. However, other studies report differences in cadmium levels in wheat cultivars (Jafarnejadi et al., 2011; Corguinha et al., 2015). The genetic modification may increase cadmium levels 2-fold in wheat grain as seen in rice grain genetically modified with similar genes (Zhang et al., 2012). Different species and developmental stages have different sensitivities to cadmium. For example, ducklings given 20 mg/kg dietary cadmium had kidney damage whereas studies of adult ducks showed no kidney damage (Furness, 1996). Drakes fed 200 mg/kg	Risk Scenario 2 in Chapter 2 of the RARMP discusses potential toxicity of the GM wheat to desirable animals. The text was expanded to include information about the varying levels of cadmium in wheat grown in different soils, and that some developmental stages of animals can have higher sensitivity to cadmium. Text was also added to compare the potential cadmium levels of the GM wheat to cadmium concentrations in other food sources available to birds and animals in the environment surrounding the trial sites.

[3] Prescribed experts, agencies and authorities include the Gene Technology Technical Advisory Committee, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	of cadmium had severe lesions on their testes and reduced egg production. In contrast, at 20 mg/kg there were moderate gonadal alterations in 20% of the test animals. Exposure of laying quail to cadmium administered by injection at 0.1, 0.3, 1, 3 and 10 mg/kg body weight demonstrated decreased egg production at 1 and 3 mg/kg and decreased eggshell thickness and lowered fertility rate at 0.3 mg/kg (Rahman et al., 2007).	
	The RARMP would benefit from further discussion of dispersal of GM wheat seed outside the trial sites. There are no controls to prevent dispersal by birds or small animals other than rodents. There is uncertainty regarding endozoochory of wheat seeds especially in bird species. The biology document states white wheat cultivars have thin seed coats and are large so likely to be easily broken down in the digestive system of animals (OGTR, 2017). Galahs and corellas are recognised as the primary bird pests of wheat and are in highest abundance in the wheat belt (Tracey et al., 2007). Both bird species can excrete viable wheat seeds, albeit at very low levels. The RARMP would benefit from additional discussion of factors that impact on dispersal by endozoochory such as the seed coat thickness of the cultivars used in this trial, abundance of galahs and corellas in the wheat belt, or germination rates of excreted seed in the wild versus under ideal laboratory conditions.	Risk Scenario 5 in Chapter 2 of the RARMP discusses potential dispersal of wheat seeds by consumption and excretion. The text was expanded to state that white wheat cultivars, as grown in Australia, are readily digested by mammals and that only a small proportion of viable excreted seeds would germinate in the environment.
4	Agrees with the conclusion of the RARMP.	Noted.
	The Regulator should consider whether testing of gene edited plants is required prior to field release.	Appendix A to the licence describes the GMOs covered by the licence. A note under the description of gene edited lines clarifies that only GM wheat segregants free from the CRISPR/Cas9 machinery used for gene editing are authorised for release under this licence. GM wheat lines containing the *cas9* gene or CRISPR genetic sequence are not covered by the licence.
	The Regulator should consider including discussion of stacking in the RARMP.	Clarification was sought from the applicant regarding the potential extent of stacking of GM wheat lines. The applicant indicated that a maximum of ten introduced or edited iron-related genes would be stacked in crosses between GM wheat lines. This limit has been added to the descriptions of the GMOs in Chapter 1, Section 4.1 of the RARMP and Appendix A of the licence. Text discussing the effects of stacking has been added to Risk Scenario 2 in Chapter 2 of the RARMP and to the CCI Attachment to the RARMP.

Submission	Summary of issues raised	Comment
	The Regulator should consider whether the scale of the release represents additional risks for people or the environment.	As discussed in Chapters 2 and 3 of the RARMP, the licence conditions are considered effective in managing any risks to people or the environment. The OGTR further investigated the capacity of the applicant to manage large numbers of trial sites in compliance with licence conditions. The OGTR is satisfied that the University of Melbourne has suitable expertise and processes in place to comply with licence conditions, and will not plant a larger number of sites than they have resources to manage. The applicant was also consulted to determine whether there had been any changes to their field trial plans since the submission of the application. The applicant revised the requested number of trial sites for the first year of the field trial down from ten sites to two sites. This reduces the total scale of the release by 16%. The revised scale has been incorporated into the finalised RARMP and Condition 24 of the licence.
5	Notes that the licence will prohibit the use of the GM plant material in human food or animal feed. Does not have any further comments.	Noted.
6	There is an unknown potential that these GMO lines may accumulate higher levels of cadmium than occurs in normal wheat. The documents detail appropriate risk management plans for this issue (as well as normal risks associated with GMO plants). Overall, while acknowledging there is a degree of uncertainty about cadmium accumulation, accept that appropriate risk management plans are in place.	Noted.
	The proposed controls seem okay for the identified risks. The uptake of other heavy metals was identified, and seems to be dealt with properly.	Noted.
7	Some of the GM wheat lines proposed for release may accumulate higher levels of cadmium or other toxic heavy metals than non-GM wheat. Therefore, any accidental release of these GM wheat lines to the environment could be problematic due to their toxicity. It is presumed that the OGTR's draft licence conditions are adequately stringent for containment of these genes.	As discussed in section 3.1 of the RARMP, the proposed licence conditions are expected to minimise pollen flow from the GMOs to non-GM plants outside the trial sites, to minimise persistence of GMOs on the trial sites, and to minimise dispersal of GMOs outside the trial sites. It is noted that the licence conditions for DIR 165 are similar to licence conditions imposed for previous GM wheat field trial licences. To date, 16 GM wheat field trials have been grown in Australia under licences issued by the Regulator and there has been no escape of GM

Submission	Summary of issues raised	Comment
		wheat into the environment.
	Overall, supported the OGTR's conclusion that DIR 165 poses negligible risk of harm to human health and safety and the environment.	Noted.

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Appendix B Summary of submissions from the public on the consultation RARMP

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

Submission	Summary of issues raised	Comment
1	What is 'negligible risk'? These sound like cop out words used to avoid any prosecution if things go wrong. Can anyone put their name to categorically guaranteeing that this proposed release would pose negligible risk to human health and safety or to the environment?	The Regulator's approach to risk analysis is outlined in the *Risk Analysis Framework*, a key guidance document produced by the OGTR and accessible on the OGTR website. As defined in this document, a 'negligible risk' is one of no discernible concern, where there is no present need to invoke mitigating action. This is consistent with the more general meaning of the term: very little risk, or a risk so small that it may be neglected or disregarded. Licence DIR 165 was issued by the Gene Technology Regulator. According to the gene technology legislation, the Regulator must not issue a licence unless satisfied that any risks posed by the proposed activities can be managed in such a way as to protect the health and safety of people and the environment.
2	My comments fall on deaf ears. Genetic modification is so bad for us. It is all about power, greed and money for few at the expense of humanity. When will they realize they have to stop destroying the world?	All submissions received in relation to a RARMP are read and considered by the Regulator prior to her decision on whether or not to issue a licence. Summaries of each submission, and responses to the issues raised, are also published as an attachment to the finalised RARMP. In making a decision regarding a licence application, the Regulator is required to assess whether the particular GMOs and activities included in the licence application pose risks to human health and safety or the environment and whether those risks are able to be managed. In the case of licence application DIR 165, the RARMP concludes that the proposed field trial poses negligible risks to human health and safety or the environment.