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

Risk Assessment and Risk Management Plan for

DIR 203

Limited and controlled release of cotton genetically modified for herbicide tolerance and insect resistance

Applicant: Monsanto Australia Pty Ltd

May 2024

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 203

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 has been prepared by the Regulator in accordance with 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 concluded that the proposed field trial poses negligible risk 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 203
Applicant	Monsanto Australia Pty Ltd
Project Title	Limited and controlled release of cotton genetically modified for herbicide tolerance and insect resistance[1]
Parent organism	Cotton (*Gossypium hirsutum* L.)
Genetic modifications	
Introduced genes	<u>Introduced genes:</u> • *cp4 epsps*, *dmo* and *bar* conferring herbicide tolerance • *mCry51Aa2*, *Cry1Ac, Cry2ab2* and *Vip3Aa19* conferring insect resistance • Additional genes conferring herbicide tolerance and insect resistance [2] <u>Reporter and selectable marker genes:</u> • *uidA*, *nptII*, *aad* and *aph4*
Genetic modification method	*Agrobacterium*-mediated transformation
Number of lines	Up to 10 lines using single and stacked combinations of the above herbicide tolerance and insect resistance genes
Principal purpose	To evaluate the agronomic performance of the genetically modified cotton under field conditions

[1] The title of the project as supplied by the applicant is "Limited and controlled release of *Gossypium hirsutum* (upland cotton) genetically modified for herbicide tolerance and insect resistance."

[2] Confidential Commercial Information: Some details about gene names and sources in the GM cottons MON 96012 and MON 89151 have been declared as Confidential Commercial Information under section 185 of the Act. This information is available to the prescribed experts and agencies that will be consulted on this application upon request in the course of them performing duties or functions under the Act or under a corresponding State law. CCI is not available to the public.

Previous releases	The application proposes the use of some GM cotton (*G. hirsutum*) varieties previously authorised for release in Australia under the commercial licences DIR 066/2006, DIR 118, DIR 145, DIR 157 and DIR 173, and the limited and controlled licence DIR 147. The application also proposes the use of 2 GM cotton varieties[2] with herbicide tolerance and insect resistance traits, not previously authorised for release in Australia
Proposed limits	
Proposed use of GM plants	No use in human food or animal feed proposed
Proposed locations	Up to 25 trial sites to be selected from 62 possible local government areas in Victoria, New South Wales, Queensland, Western Australia, and the Northern Territory.
Proposed release size	A combined total area of 10 ha in 2024, 50 ha per year in 2025-2027 and 100 ha per year in 2028-2029
Proposed period of release	From September 2024 until September 2029

Risk assessment

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

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

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks. The principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as not using GM plant material in human food or animal feed, will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

Risk management plan

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

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

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
bar	Bialaphos resistance *gene*
Bt	*Bacillus thuringiensis*
CCI	Confidential Commercial Information
CP4 EPSPS	CP4 5-enolpyruvylshikimate-3-phosphate synthase
Cry	Crystal protein
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving Intentional Release
DMO	Dicamba mono-oxygenase protein
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectares
HPPD	4-hydroxyphenylpyruvate dioxygenase
ISAAA	International Service for the Acquisition of Agri-biotech Applications
mCry51Aa2	Modified Cry51Aa2
NLRD	Notifiable Low Risk Dealing
NSW	New South Wales
NT	Northern Territory
OGTR	Office of the Gene Technology Regulator
OECD	Organisation for Economic Co-operation and Development
PC2	Physical Containment Level 2
PPO	Protoporphyrinogen oxidase
Qld	Queensland
RARMP	Risk Assessment and Risk Management Plan
spp.	Species
TGA	Therapeutic Goods Administration
The Act	The *Gene Technology Act 2000*
The Regulations	The Gene Technology Regulations 2001
The Regulator	The Gene Technology Regulator
USA	United States of America
Vic	Victoria
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

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

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

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

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

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

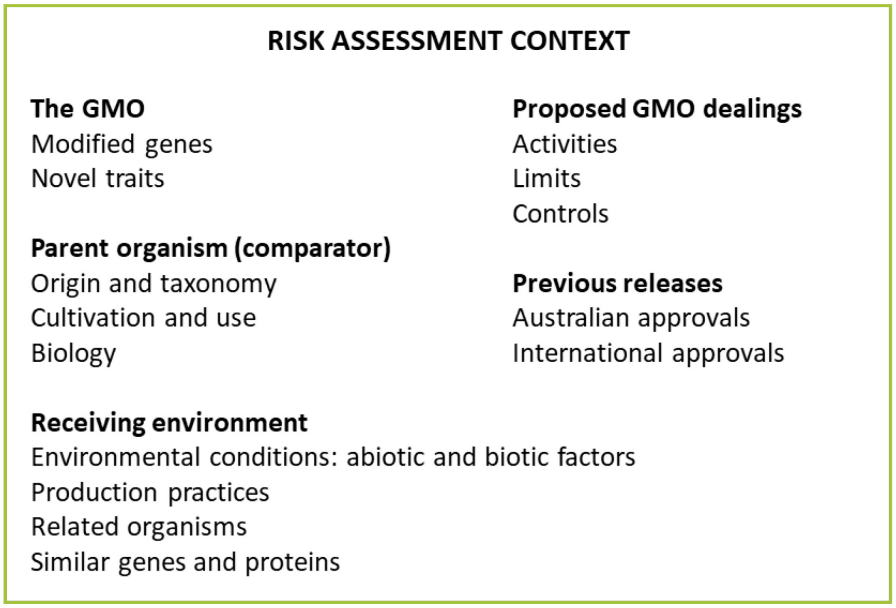

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR, and the Risk Analysis Framework

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils

and the Minister for the Environment - and from 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 their consideration is summarised in Appendix B.

1.1 Interface with other regulatory schemes

7. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA) and the Department of Agriculture, Fisheries and Forestry (DAFF). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

8. APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cottons proposed for release meet the definition of an agricultural chemical product under the Agricultural and Veterinary Chemicals Code Act 1994, due to their production of insecticidal substances and therefore these plants are subject to regulation by the APVMA. The applicant, Monsanto Australia Pty Ltd intends to apply herbicide to the GM cottons during the trial, which is also subject to regulation by the APVMA.

9. The applicant has proposed to import the GM cotton seeds into Australia from North and South America, at different time points throughout the period of the proposed licence. These imports would be subject to permits obtained from DAFF.

Section 2 The proposed dealings

10. The applicant proposes to release up to 10 genetically modified (GM) cotton lines into the environment under limited and controlled conditions. The GM lines have been genetically modified for herbicide tolerance and insect resistance traits, and some lines also contain marker genes.

11. The purpose of the trial is to evaluate the agronomic performance of the GM cotton plants under field conditions.

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

a) conduct experiments with the GMOs;
b) breed the GMOs;
c) propagate the GMOs;
d) use the GMOs in the course of manufacture of a thing that is not the GMOs;
e) grow, raise or culture the GMOs;
f) import the GMOs;
g) transport the GMOs;
h) dispose of the GMOs;

and to possess, supply or use of the GMO for the purposes of, or in the course of, a dealing mentioned above.

13. The GM cottons would not be used for human food or animal feed.

14. Lint derived from the GM cottons is proposed to be used commercially.

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

15. The release is proposed to take place between September 2024 and September 2029, in New South Wales (NSW), Queensland (Qld), Western Australia (WA), Northern Territory (NT) and Victoria (Vic). The proposed maximum number of sites, area per site and combined total area for each year are detailed in Table 1.

Table 1. Proposed duration and maximum number of sites and area per year

Year	Maximum sites	Maximum area (ha) per site	Maximum combined area (ha)
2024	10	3	10
2025	25	10	50
2026	25	10	50
2027	25	10	50
2028	25	25	100
2029	25	25	100

16. The trial sites would be selected from 62 local government areas in NSW, Qld, WA, NT and Vic (Table 2). The trial sites would be located on private land in rural areas. Details of site locations would be provided to the Regulator prior to each planting season.

Table 2. Local government areas where proposed trial sites may be located

NSW		Qld	WA
Balranald	Hay	Balonne	Ashburton
Berrigan	Inverell	Banana	Broome
Bland	Lachlan Shire Council	Bundaberg Regional	East Pilbara
Bogan	Leeton	Burdekin Shire	Port Hedland
Bourke	Liverpool Plains	Central Highlands	Wyndham-East Kimberley
Brewarinna	Moree Plains	Goondiwindi Regional	**NT**
Carrathool	Murray River	Isaac Regional	Katherine
Central Darling	Murrumbidgee	Lockyer Valley Regional	Roper Gulf
Coolamon	Narrabri	Maranoa Regional	Victoria Daly
Coonamble	Narrandera	Mareeba Shire	**Vic**
Edward River	Narromine	Paroo	Rural City of Mildura
Federation	Parkes	Rockhampton Regional	Shepparton
Forbes	Walgett	South Burnett Regional	Swan Hill
Gilgandra	Wagga Wagga	Southern Downs Regional	
Griffith	Warren	Toowoomba Regional	
Gunnedah	Warrumbungle	Western Downs Regional	
Gwydir	Weddin	Whitsunday Regional	

17. Only trained and authorised staff would be permitted to deal with the GM cotton.

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

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

- transport, storage and disposal of the GM cotton would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- proposed trial sites would be located at least 50 m away from the nearest natural waterway
- non-GM plants used and produced in the trials would be treated as if they were GM
- establishing a 20 metre pollen trap of cotton that may contain conventional cotton or previously authorised commercial cotton varieties or, establishing a cotton exclusion zone of 1.5 km around the trial
- access to the trial sites will be restricted to authorised persons

- following harvest, removal and destruction of all viable or potentially viable material containing the GMOs from trial sites and adjacent areas
- postharvest monitoring of the trial sites and destruction of any volunteers before flowering. With inspections occurring at least once every 35 days, for at least 12 months following harvest, until the trial site has been free of volunteers for at least 6 months
- destruction of any seed that is not required for analysis or planting
- cleaning of equipment prior to use for other purposes and cleaning of the trial site.

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

Section 3 Parent organism – *Gossypium hirsutum* L.

20. The parent organism is cotton (*Gossypium hirsutum* L.), also known as upland cotton. Cotton is exotic to Australia and is grown as an agricultural crop. Cotton is mainly grown in NSW and Qld, but is also grown in Vic, WA and the NT.

21. Cotton is predominately grown as a source of textile and industrial fibre, however cottonseed oil and linters are used in food, and whole white ("fuzzy") cottonseed and cottonseed meal are used in animal feed. A brief description of relevant biological information about the parent organism is provided in this section, for more detailed information please refer to *The Biology of Gossypium hirsutum L. and Gossypium barbadense L.* (OGTR, 2024) which was produced to inform the risk assessment process for licence applications involving GM cotton plants and is available from the OGTR Biology documents page.

22. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (OGTR, 2013). Conventional non-GM cotton is the standard baseline for biological comparison, however it is noted that more than 99% of cotton grown in Australia is GM, with roughly 96% containing both insect resistance and herbicide tolerance traits and the remaining cotton containing herbicide tolerance traits (ISAAA, 2018).

23. Cotton is a domesticated crop that grows best under agricultural conditions. It prefers soils with high fertility and responds well to irrigation. Cotton has been commercially cultivated in Australia since the 1860s (OGTR, 2024). It is a perennial plant that is cultivated as an annual.

24. Areas where cotton can be grown in Australia are mainly limited by water availability, the suitability of the soil, temperature and the length of the growing season. Cotton is grown as a dryland and/or irrigated crop, depending on the rainfall in the production area.

25. In 2022/2023 the cottonseed production area in Australia was estimated at 572,000 hectares (ha); this area is forecast to decrease to 413,000 ha in 2023/2024, in part, due to drier seasonal conditions (ABARES, 2023).

Section 4 The GMO – nature and effect of the genetic modification

26. The applicant proposes the use of 3 herbicide tolerant and 5 insect resistant GM cotton events (Table 3) that may be used as single events and in stacked combinations to generate up to 10 GM cotton lines (Table 4).

4.1 The genetic modifications in the GMOs proposed for release

27. Table 3 lists the introduced genes conferring herbicide tolerance and insect resistance in the GM cotton events. The use of single and stacked combinations of the GM cotton events may result in different combinations of the introduced genes in the proposed GM cotton lines (Table 4), as detailed in paragraphs 28-32 below.

Please note, some information regarding details of introduced genes, proteins, regulatory sequences and their sources for the GM cotton events MON 96012 and MON 89151, and the target 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibiting herbicide in MON 96012, have been declared Confidential Commercial Information (CCI). Under Section 185 of the Act. This information is available to the prescribed experts and agencies that will be consulted on this application upon request in the course of them performing duties or functions under the Act or under a corresponding State law. CCI is not available to the public.

Table 3. Details of the GMOs

Event	Previous release(s)	Introduced genes	Traits
MON 96012	None in Australia, small scale contained trials in the USA[a]	CCI*	Glyphosate, glufosinate, CCI* (HPPD inhibiting), dicamba, and PPO-inhibiting herbicide tolerance
MON 15947	None. Is a segregant of commercial line MON 15985 released under DIR 066/2006 DIR 124 and DIR 145	*Cry2Ab2*	Lepidopteran-resistance
MON 89151	None in Australia, small scale contained trials in the USA	CCI*	Lepidopteran-resistance
MON 88702	Limited and controlled release under DIR 147	*mCry51Aa2*	Hemipteran and thysanopteran-resistance
MON 15985	Commercial release under DIR 066/2006, DIR 124 and DIR 145	*Cry1Ac, Cry2Ab2*	Lepidopteran-resistance
COT102	Commercial release under DIR 124, DIR 145 and DIR 157	*Vip3Aa19*	Lepidopteran-resistance
MON 88913	Commercial release under DIR 066/2006, DIR 124 and DIR 145	*cp4 epsps*[a]	Glyphosate tolerance
MON 88701	Commercial release under DIR 145	*dmo, bar*[a]	Glufosinate and dicamba tolerance

[a] Bialaphos resistance (*bar*), *cp4 5-enolpyruvylshikimate-3-phosphate synthase* (*cp4 epsps*), *dicamba mono-oxygenase* (*dmo*), protoporphyrinogen oxidase (PPO), United States of America (USA).

*This information has been declared Confidential Commercial Information (CCI) under Section 185 of the Act.

Table 4. Proposed GM cotton lines with labelled possibilities for events to be included in single and stacked combinations

LINES	MON 96012	MON 15947	MON 89151	MON 88702	MON 15985	COT102	MON 88913	MON 88701
1	1 line							
2		1 line						
3			1 line					
4				1 line				
5-8	Up to 4 lines of any combination							
9-10				Up to 2 lines of any combination				

28. Details of genes in proposed lines 1, 3 and 5-8 (Table 4) are declared CCI.

29. Proposed line 2, MON 15947 (Table 4) contains the *Cry2Ab2* gene derived from *Bacillus thuringiensis* that encodes the Cry2Ab2 protein conferring lepidopteran resistance. Event MON 15947 is a segregant of the commercial GM cotton line MON 15985 which was authorised for release under DIR 066/2006 (Table 3). Event MON 15985 contains inserts for *Cry1Ac* and *Cry2Ab2*. Event MON 15947 only contains the *Cry2Ab2* insert (Table 3), which is reported to have segregated independently from *Cry1Ac* (USAID, 2007).

30. Proposed line 4, MON 88702 (Table 4) contains a modified *Cry51Aa2* gene (*mCry51Aa2*) derived from *B. thuringiensis* that encodes the mCry51Aa2 protein conferring hemipteran and thysanopteran resistance. Event MON 88702 has previously been authorised for a limited and controlled release under DIR 147 (Table 3).

31. Proposed lines 5-8 may be generated using stacked combinations of MON 96012, MON 15947, MON 89151 and MON 88702 (Table 4). These lines may contain any combination(s) of the introduced genes; *Cry2Ab2 (*MON 15947), *mCry51Aa2* (MON 88702) (Table 3) and CCI genes in MON 96012 and MON 89151.

32. Proposed lines 9-10 may be generated using stacked combinations of MON 88702 with the commercial GM cotton events MON 15985, COT102, MON 88913 and MON 88701 (Table 4), which have been authorised for release under DIR 066/2006, DIR 124, DIR 145 and DIR 157. These lines may contain any combination(s) of the introduced genes *mCry51Aa2* (MON 88702)*, Cry1Ac, Cry2Ab2* (MON 15985)*, vip3Aa19* (COT102)*, cp4 epsps* (MON 88913), *dmo* and *bar* (MON 88701). These crosses have previously been authorised for a limited and controlled release under DIR 147 (Table 3) and the introduced genes have previously been assessed to pose negligible risks to human health and the environment under the above listed licences.

Method of genetic modification

33. Events MON 96012, MON 88913, MON 88701, MON 89151, MON 88702, MON 15985 and COT102 were generated using *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genetic modifications into plants. More information can be found in the document *Methods of Plant Genetic Modification* which is available from the Resources page on the OGTR website. Event MON 15947 was a selectively bred progeny of commercial event MON 15985 generated via conventional breeding.

Introduced regulatory elements

34. Gene constructs used to generate MON 88702, MON 15985, COT102, MON 88913 and MON 88701 contain genetic regulatory sequences that control gene expression. These regulatory

sequences are derived from plants and bacteria that are known pathogens. By themselves, these regulatory sequences do not cause disease or toxicity. For details, please refer to DIR 147, DIR 066/2006, DIR 157, DIR 118 and DIR 173 where these regulatory sequences were assessed as posing no risk to human health and the environment.

35. Details of regulatory sequences used to control gene expression in MON 96012 and MON 89151, and reported effects on protein expression, are declared CCI.

Introduced selectable marker genes

36. The GM cotton events MON 15947, MON 15985 and COT102 contain antibiotic resistance selectable markers and/or reporter genes derived from *Escherichia coli* (*E. coli*) (Table 5). The selectable markers may be present in the proposed GM cotton lines 5-8 if the cross involves MON 15947, and proposed GM cotton lines 9-10 if the cross involves MON 15985 and/or COT102.

Table 5. Selectable markers in GM cotton events

EVENT	GENE	SOURCE	FUNCTION
MON 15947	*uidA*	*E. coli*	selectable marker – reporter
MON 15985	*nptII*	*E. coli*	selectable marker – antibiotic resistance
	aad	*E. coli*	selectable marker – antibiotic resistance
	uidA	*E. coli*	selectable marker – reporter
COT102	*aph4*	*E. coli*	selectable marker – antibiotic resistance

37. During initial development, MON 96012 and MON 89151 contained a *spectinomycin* antibiotic resistance selectable marker. This marker was removed during subsequent development of the events. Further details of this selectable marker are declared CCI.

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

38. As the commercial GM cotton events MON 15985, COT102, MON 88913 and MON 88701 have been extensively assessed in the past, this section of the RARMP will focus on evaluating the toxicity/allergenicity of proteins associated with the introduced genes of MON 96012, MON 89151, MON 15947 and MON 88702.

39. Please note that details of toxicity/allergenicity of proteins associated with the introduced genes in MON 96012 and MON 89151 are declared CCI.

Insecticidal Cry proteins

40. Crystal (Cry) proteins which are also known as delta-endotoxins, confer resistance against lepidopteran, hemipteran and thysanopteran insects. Cry proteins are derived from *B. thuringiensis*, a common soil-borne bacteria, that is widely used as an organic pesticide in agriculture, as it produces protein toxins that are specific to certain insects. In humans, there have been reports of allergic reactions to *B. thuringiensis* microbial products in topical insecticidal sprays. Evaluations by the USEPA concluded that reactions to *Bacillus thuringiensis* (*Bt*) sprays have been due to non-Cry proteins produced during fermentation or to other ingredients added to the insecticidal formulations (EPA, 2001).

41. Cry proteins are expressed by *B. thuringiensis* during sporulation as inactive crystalline protoxins. They become activated when the crystalline inclusions are ingested, solubilised in an alkaline midgut environment to inactive protoxins, and cleaved by proteases in the insect midgut to produce an active toxin. This active toxin then binds to specific receptors on the brush border membrane of the midgut epithelium, leading to formation of membrane pores (Yu et al., 1997; Bravo et al., 2007). The formation of pores causing cell lysis, resulting in impaired digestion and insect death (Schnepf et al., 1998; OECD, 2007; Soberón et al., 2009). Many Bt toxins are expressed in their inactive protoxin form, limiting their toxicity to target insects that have alkaline midgut environments, specific proteases, and specific receptors for the toxins to bind to when activated. Some Cry proteins

expressed by *B. thuringiensis,* including Cry2 proteins, do not undergo the protease-mediated C-terminal cleavage step as they appear to be naturally truncated (Gill et al., 1992)

42. Lepidopteran, hemipteran and thysanopteran insects, the target species of Cry proteins, exhibit alkaline midgut environments. This is in contrast to non-target organisms such as birds and mammals which exhibit acidic midgut environments that result in the degradation of Cry proteins, preventing their activation. Non-target organisms such as birds and mammals also do not express receptors for the *B. thuringiensis* Cry proteins and are therefore not adversely affected (Schnepf et al., 1998; OECD, 2007).

Cry2ab2 in MON 15947

43. The Cry2ab2 protein conferring lepidopteran resistance is derived from *B. thuringiensis*. MON 15947 is a segregate of MON 15985 which contains the *Cry1Ac* and *Cry2ab2* gene. MON 15947 only contains the *Cry2ab2* gene, which is reported to have segregated independently from *Cry1Ac*. In MON 15985, the *Cry1Ac* and *Cry2ab2* genes are reported to be inserted at different positions on the plant genome, with data collected over multiple generations of crossing and backcrossing identifying no significant variation from expected segregation ratios, confirming that the *Cry1Ac* and *Cry2ab2* genes are maintained as single dominant Mendelian traits (EPA, 2018). These findings are consistent with the reported independent segregation of *Cry2ab2* from *Cry1Ac* in MON 15985.

44. The Cry2ab2 protein has been extensively assessed by regulatory bodies worldwide and in the peer reviewed scientific literature. In Australia, MON 15985 expressing the Cry2ab2 protein has previously been authorised for commercial release under DIR 066/2006, DIR 124 and DIR 145, in which it was concluded that there is no toxicity or allergenicity associated with the *Cry2ab2*-encoded Cry2ab2 protein. GM cotton expressing *Cry2ab2* has also been assessed as safe for human consumption by FSANZ (FSANZ, 2002).

uidA in MON 15947

45. Event MON 15947 also contains the reporter gene beta-glucuronidase (*uidA*) derived from *E. coli* (Table 5). *uidA* and its 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. As this gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application. More detail on marker genes can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website.

mCry51Aa2 in MON 88702

46. The mCry51Aa2 protein conferring hemipteran and thysanopteran resistance is encoded by the modified *Cry51Aa2* (*mCry51Aa2*) gene, derived from *B. thuringiensis.* Compared to the native sequence, the amino acid sequence of the modified protein mCry51Aa2 has 9 changes, namely 8 amino acid substitutions and one deletion of 3 amino acids which results in a sequence similarity of 96.4%. Like other Cry proteins, mCry51Aa2 was shown to be produced as a protoxin and is reported to have the same mode of action (Jerga et al., 2016).

47. The OGTR has previously authorised the GM cotton MON 88702 expressing *mCry51Aa2* for a limited and controlled release under DIR 147. Data presented and assessed for MON 88702 in DIR 147 suggested that there is no toxicity or allergenicity associated with the *mCry51Aa2*-encoded Cry protein. Event MON 88702 has also been assessed as safe for human consumption by FSANZ (FSANZ, 2018).

4.3 Characterisation of the GMOs

48. The applicant has stated that new events MON 89151 and MON 96012 are in early-stage investigations and that the proposed field trials will assess the efficacy of the GM cotton for insect

resistance and herbicide tolerance traits. Should the early investigations be successful, the applicant plans to collect data from the field trials for future regulatory submissions to the APVMA and OGTR for possible commercial release.

49. The applicant has reported that the introduced genetic modifications in the GM cottons are not known to affect seed numbers or play a role in seed dormancy and are not known to confer any other phenotypic changes that would affect the ability of the GM cottons to persist amongst existing plants. They have reported the MON 96012 and MON 89151 GM cotton plants grown in glasshouses to have exhibited normal cotton plant phenotypes.

50. Plant characteristics relating to the persistence of insecticidal events MON 15947, MON 89151, MON 88702, MON 15985 and COT102, are not expected to be altered. The herbicide tolerant events MON 88913, MON 88701 and MON 96012 may survive better than non-GM cotton in agricultural environments where volunteer cotton is managed with the application of their target herbicide(s). In which instance, alternative herbicides or mechanical measures may be used to control these volunteers. Please see Chapter 2 for more details of management of GM cotton volunteers.

51. Details of the spatiotemporal expression of the proteins associated with the introduced genes in MON 89151 and MON 96012 are declared CCI.

52. Unprocessed cotton contains the natural toxicant gossypol, and cyclopropenoid fatty acids which are anti-nutrients (OGTR, 2024). The applicant has not provided any information as to whether the genetic modifications in the proposed GM lines would alter gossypol and cyclopropenoid fatty acid content. However, GM cotton with similar genetic modifications to those which are proposed for use in this application (MON 88702, MON 88913 and COT102) have been reported to exhibit comparable gossypol and cyclopropenoid fatty acids levels to that in conventional or null segregant cotton (FSANZ, 2004, 2005, 2018). Event MON 88701 however, was reported to exhibit marginally higher levels of gossypol and cyclopropenoid fatty acids than the non-GM cotton line Coker 130. As mean values remained within levels observed in commercial cotton varieties, FSANZ concluded that these increases were not meaningful from a nutritional perspective (FSANZ, 2013).

Section 5 The receiving environment

5.1.1 Relevant abiotic factors

53. The abiotic factors relevant to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of Gossypium Hirsutum L. and Gossypium barbadense L.*(cotton) (OGTR, 2024). To summarise, factors restricting where cotton can be grown in Australia are water availability (through rainfall or irrigation), soil suitability and temperature. Cotton seedlings may be killed by frost, growth and development of cotton plants below 12°C is minimal, and a long, hot growing season is crucial for achieving good yields.

5.1.2 Relevant biotic factors

54. The major insect pests of cotton are lepidopteran species. In Australia, the most damaging lepidopteran pests are cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*). However, beet armyworm (*Spodoptera exigua*), cluster caterpillar (*Spodoptera litura*) and pink bollworm (*Pectinophora gossyipiella*) can also affect cotton production (OGTR, 2024). These lepidopteran pests are now managed through the widespread adoption of GM cotton varieties with *Bt* toxin genes that specifically target these insect pests.

55. Many cotton growing areas across Australia also have important non-lepidopteran insect pests. These include cotton aphids (*Aphis gossypii*), green mirids (*Creontiades dilutus*), brown mirids (*C. pacificus*), two-spotted spider mites (*Tetranychus urticae*), silverleaf whitefly (*Bemisia tabaci*), thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*), green vegetable bugs (*Nezara viridula*), solenopsis mealybugs (*Phenacoccus solenopsis*) and Reniform nematode (*Rotylenchulus reniformis*) (CRDC and CottonInfo, 2017).

56. Australian cotton is affected by a number of soil-borne and foliar fungal diseases, along with oomycete, bacterial and viral diseases. Fungal pathogens cause the major diseases Verticillium wilt (*Verticillium dahliae*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*; FOV). Common seedling diseases of cotton are black root rot (*Thielaviopsis basicola*) and damping off (caused by *Rhizoctonia solani*, *Pythium* spp. and *Phytophthora* spp.). Leaves may be affected by Alternaria leaf spot (*Alternaria* spp.) and cotton bunchy top virus spread by aphids. Boll rots are caused by different pathogens, including fungi, bacteria and oomycetes (CRDC and CottonInfo, 2017).

57. Cotton is susceptible to competition from weeds. Problematic weeds range from large plants such as Noogoora burr (*Xanthium occidentale*), Bathurst burr (*Xanthium spinosum*), thornapples (*Datura* spp.) and sesbania (*Sesbania canabina*), to vines such as cowvine and bellvine (*Ipomoea* spp.), yellow vine or spine-less caltrop (*Tribulus* spp.), to grasses such as nut grass (*Cyperus rotundus*) (CRDC, 2013). Some weed species are alternate hosts for diseases of cotton, e.g. many weeds are hosts for *V. dahliae* (CRDC and CottonInfo, 2017).

5.1.3 Relevant agricultural practices

58. It is anticipated that the agronomic practices for cultivation of the GM cotton will not differ significantly from industry best practices used in Australia. All cotton plants would be grown following standard cotton agricultural management practices and would receive applications of water, fertilisers, herbicides and insecticides similar to current commercially grown non-GM and GM cotton crops. Herbicide tolerance and insect resistance traits in the GM cotton will inform practices with respect to weed management within the crop and pesticide application. Cultivation practices for cotton are discussed in more detail in *The Biology of Gossypium hirsutum L.and Gossypium barbadense L.(cotton)* (OGTR, 2024). The management of GM cotton volunteers with the use of herbicides is discussed in Chapter 2.

59. The GM cotton is proposed to be grown at field trial sites as either an irrigated or a dryland crop, with channel and drip irrigation used as necessary. In small areas, seed may be hand-planted or planted with a small plot cone-seeder, and in larger areas, seed may be planted with commercial equipment. Harvesting of cotton bolls may occur by hand or with commercial equipment. The proposed GM cottons may also be grown in glasshouse conditions approved under a Notifiable Low Risk Dealing (NLRD).

60. Disposal of GM cottons and other plants on trial sites is proposed to occur by either destructive analysis, herbicide application, root cutting and mulching, hand weeding, autoclaving, or burial of seed or other plant material or a combination of these methods, as appropriate.

61. GM cotton is proposed to be allowed to set seed at the field trial sites. Harvested seed may be used to plant further trials, for laboratory experiments in Australia or overseas, or for the purpose of seed increase.

62. Trial sites may be replanted with GM cotton authorised under the same licence, or planted with an approved post-harvest crop as specified by the OGTR, or maintained as fallow.

5.1.4 Presence of sexually compatible plants in the receiving environment

63. Commercial cotton grown in Australia is either *G. hirsutum* or *G. barbadense*, with 99% being *G. hirsutum* (OGTR, 2024). The GM cotton lines proposed in this RARMP, are capable of crossing with both *G. hirsutum* and *G. barbadense*.

64. There are 17 native species of *Gossypium* in Australia, 12 of which are found in the relatively small coastal area in northern WA. Of the remaining, *G. sturtianum* is the most widely distributed and is scattered across the sub-tropical to warm temperate arid zones of Australia, in Qld, NSW, SA and WA. *G. australe* has a broad east coast – west coast distribution, but its indigenous range extends from southern areas of the NT to Katherine. *G. bickii* occurs largely within central NT, while *G. nelsonii* is distributed in a band from central NT to central Qld (OGTR, 2024).

65. Populations of cotton volunteers can be found on cotton farms, by roadsides where cotton seed is transported, or in areas where cotton seed is used as livestock feed (Eastick and Hearnden, 2006; Addison et al., 2007). Volunteer seedlings that emerge over winter are likely to be killed by frosts, while dry winters may promote volunteer survival and emergence in warmer months, with spring rains and irrigation promoting volunteer growth and development.

66. The likelihood that cultivated *G. hirsutum* could hybridise successfully with native Australian *Gossypium* species is low, due to genetic incompatibility. While hybrids between *G. hirsutum* and *G. sturtianum* have been produced under field conditions, the hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations (Brown et al., 1997; Brubaker et al., 1999).

5.1.5 Presence of similar genes and encoded proteins in the environment

67. The introduced genes for herbicide tolerance and insect resistance in MON 88701, MON 15985, COT102, MON 88813 and MON 88701 are derived from common soil-borne microorganisms and the genetic regulatory sequences are derived from plants, plant viruses and a common soil bacterium that are widespread and prevalent in the environment and thus humans and other organisms would commonly encounter their genes and encoded proteins. For details please refer to DIR 066/2006, DIR 124, DIR 145 and DIR 157 for MON 88701, MON 15985, COT102, MON 88813 and MON 88701 and DIR 147 for MON 88702.

68. Details of the presence of similar genes and encoded proteins in the environment for MON 96012 and MON 89151 are declared CCI.

Section 6 Relevant Australian and international approvals

6.1.1 Australian approvals

Approvals by the Regulator

69. Events MON 96012 and MON 89151 have not previously been authorised for release in Australia.

70. Event MON 15947 is a segregant of the commercial line MON 15985 released under DIR 066/2006, DIR 124 and DIR 145.

71. GM cottons MON 88702, MON 15985, COT102, MON 88913 and MON 88702 have previously been authorised by the Regulator for release in Australia (see Table 3).

72. Information on previous DIR licences for GM cottons is available from the GMO Record on the OGTR website. The Regulator has previously approved 37 field trials and 13 commercial releases of GM cotton. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Approvals by other government agencies

73. FSANZ has assessed the safety of the GM cotton events MON 15985, MON 88913, COT102, MON 88701 and MON 88702 (Table 6) and concluded that food derived from these GM cotton events is as safe for human consumption as food derived from conventional non-GM cotton (FSANZ). Events MON 96012, MON 15947 and MON 89151 have not been assessed by FSANZ. However, the Cry2Ab2 protein present in MON 15947 has been assessed by FSANZ as safe for human consumption (FSANZ, 2002).

74. The APVMA has approved MON 15985 as an insecticide and Cry1Ac, Cry2ab2 and Vip3 as active constituents. Events MON 15947 x MON 89151 x MON 96012 with or without MON 88702 would need to be submitted to the APVMA to be assessed for registration as an insecticide, and an associated request for an active constituent approval would also need to be submitted for MON 89151.

Table 6. Previous approvals of the GM cotton events by other Australian regulators

Event	FSANZ approvals	APVMA approvals
MON 96012	None	None
MON 15947	None	None (but Cry2ab2 approval for MON 15985)
MON 89151	None	None
MON 88702	Added to the Food Standards Code 1.5.2 (application A1154)	None
MON 15985	Added to the Food Standards Code 1.5.2 (application A436)	Approved as an insecticide (application 55786) and Cry1Ac and Cry2ab2 proteins approved as active constituents (application 56492)
COT102	Added to the Food Standards Code 1.5.2 (application A509)	Vip3A protein approved as an active constituent (application 69067)
MON 88913	Added to the Food Standards Code 1.5.2 (application A553)	None
MON 88701	Added to the Food Standards Code 1.5.2 (application A1080)	None

Source: FSANZ website and APVMA Public Chemical Registration Information System database.

6.1.2 International approvals

75. Events MON 96012 and MON 89151 have previously been authorised for small scale field trial release in the USA in 2022.

76. Events MON 15947 and MON 88702 have had overseas approvals to be used as food, feed and for environmental releases. See tables 7 and 8, respectively.

Table 7. International approvals for MON 15947

Country	Types of Approval	Year of Approval
Brazil	Food, Feed, Environment	2022
Canada	Feed	2022
Canada	Food	2022
USA	Environment	2005
USA	Food, Feed	2022
USA	Environment	2022

Source: International Service for the Acquisition of Agri-biotech Applications (ISAAA) GM approval database and the Organisation for Economic Co-operation and Development (OECD) BioTrack Product database.

Table 8. International approvals for MON 88702

Country	Types of Approval	Year of Approval
Canada	Food	2018
Canada	Feed	2018
Japan	Food	2019
Japan	Feed	2018
Japan	Environment	2019
Korea	Feed	2021

Country	Types of Approval	Year of Approval
Korea	Food	2021
Mexico	Food, Feed	2021
Philippines	Food, Feed	2021
Singapore	Food, Feed	2022
Taiwan	Feed	2019
Taiwan	Food	2019
USA	Environment	2021
USA	Food, Feed	2018
USA	Environment	2021

Source: ISAAA GM approval database and OECD BioTrack Product database.

77. The commercial GM cotton events MON 15985, COT102, MON 88913 and MON 88701 have had a large number of international approvals to be used as food, feed and for environmental releases (ISAAA GM approval database and the OECD BioTrack Product database).

Chapter 2 Risk assessment

Section 1 Introduction

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

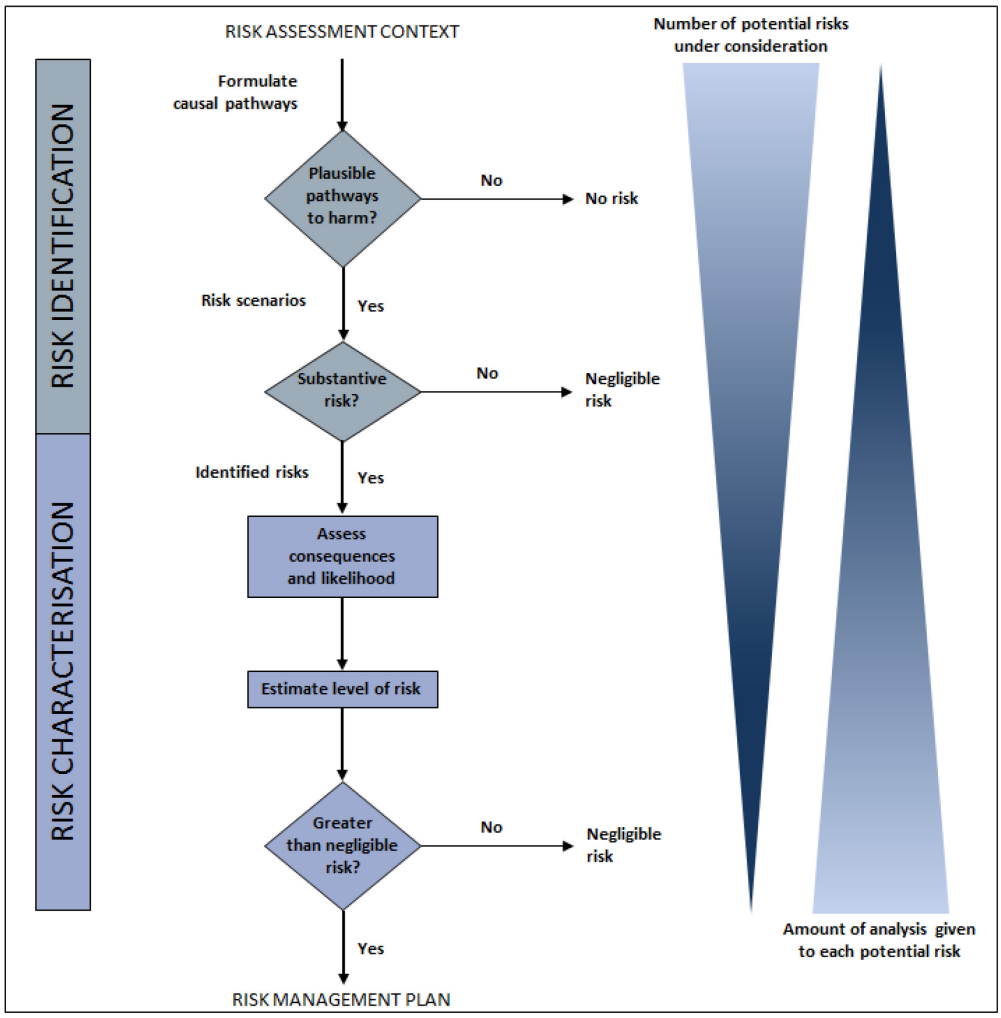

Figure 2. The risk assessment process

79. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

80. 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 plausible causal pathways that may give rise to harm for people or the environment from

dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 2) i.e. the risk is considered to be no greater than negligible.

81. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments 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

82. 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.

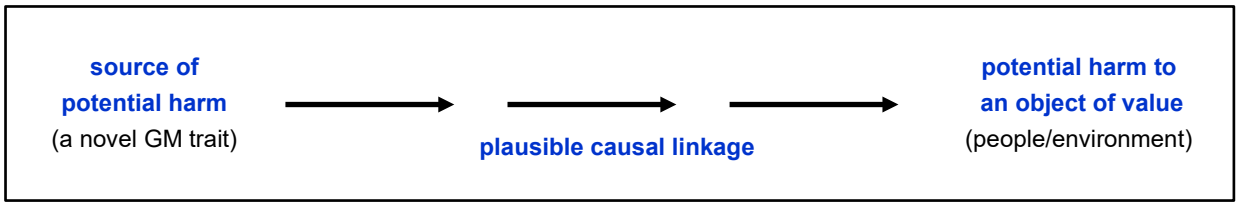

Figure 3. Risk scenario

83. 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

84. 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.

85. Details of risk sources for MON 96012 and MON 89151, including those relating to genes, proteins, genetic regulatory elements, sources and target HPPD inhibiting herbicide in MON 96012 are declared CCI. Risk sources for the remaining GM cotton events: MON 88701, MON 15985, COT102, MON 88813 and MON 88701 are described in the following paragraphs.

86. As discussed in Chapter 1, the GM cotton events that may be used in single and stacked combinations to generate up to 10 GM cotton lines (Table 4) have been modified by the introduction of genes conferring herbicide tolerance; *cp4 epsps* (MON 88913), *dmo* and *bar* (MON 88701) and CCI genes (MON 96012), and insect resistance; *Cry2Ab2* (MON 15947), *mCry51Aa2* (MON 88702), *Cry1Ac* and *Cry2Ab2* (MON 15985) and *Vip3Aa19* (COT102) and CCI genes (MON 89151). These introduced genes are considered further as potential sources of risk.

87. Some of the GM cotton events also contain antibiotic resistance selectable markers: *nptII* (MON 15985, MON 15947), *aad* (MON 15985), *aph4* (COT102) and/or the reporter gene *uidA* (MON 15947 and MON 15985). 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 the genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

88. The introduced genes are controlled by introduced regulatory sequences. These were derived from plants, bacteria and plant viruses. Regulatory sequences are naturally present in all plants, and the introduced sequences are expected to operate in similar ways to endogenous sequences. These 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.

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

2.2 Casual pathway

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

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

91. Although all of these factors are taken into account, some are not included in risk scenarios because they have been thoroughly considered in previous RARMPs. No new information has become available since then to change our consideration of these factors.

92. The potential HGT from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for DIR 108. Although the DIR 108 RARMP is for GM canola, the HGT considerations are the same for the current

RARMP: HGT events rarely occur and the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

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

2.3 Potential harm

94. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue, 2004; 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).

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

95. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 9 and examined in detail in Sections 2.4.1 - 2.4.3 (this Chapter).

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

Table 9. Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for herbicide tolerance and insect resistance	Cultivation of GMOs at trial sites ⬇ Exposure of people and/or other organisms at the trial site to the products of the introduced genes	• Adverse health effects in people and/or increased toxicity to non-target organisms.	No	• GM cottons would not be used in human food or animal feed. • The previously authorised GM cotton events containing the introduced genes have a history of safe use. • The proteins encoded by the introduced genes are unlikely to result in adverse health effects in humans or

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
					toxicity in non-target organisms. • Some of the introduced genes and encoded proteins are widespread in the environment. • The limited scale and short duration of the trial minimise exposure to the GM plant material.
2	Introduced genes for herbicide tolerance and insect resistance	Cultivation of GMOs at trial sites ⬇ Dispersal of GM seed outside trial limits and establishment of volunteer GM cotton plants ⬇ Reduced effectiveness of weed management measures to control volunteer GM cotton plants	• Adverse health effects in people, and/or increased toxicity to non-target organisms and/or • Reduced establishment or yield of desirable plants	No	• The proposed controls would minimise dispersal and persistence of the GM cottons. • The introduced genes are not expected to change susceptibility of the GM cottons to the factors which limit the geographical range and persistence of cotton in Australia. • For GM cotton volunteers with herbicide tolerance, there are other herbicide options as well as weed management strategies available that can control volunteers. • The proteins encoded by the introduced genes are unlikely to result in adverse health effects in humans or toxicity in non-target organisms.
3	Introduced genes for herbicide tolerance and insect resistance	Cultivation of GMOs at trial sites ⬇ Cross-pollination with other cotton, including cotton with other herbicide tolerance and/or insect resistance traits ⬇ Establishment of hybrid GM cotton plants expressing the herbicide tolerance and/or insect resistance genes as volunteers ⬇ Reduced effectiveness of weed management measures to control the hybrid plants	• Adverse health effects in people and/or increased toxicity to non-target organisms and/or • Reduced establishment or yield of desirable plants	No	• The proposed controls would minimise opportunities for the GM cottons to hybridise with other cottons. • Hybrids between the GMOs and other cotton would likely be generated at low levels. • For multiple-herbicide tolerant hybrids, there are other herbicide options as well as other weed management strategies available that can control volunteers. • The proteins encoded by the introduced

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
					genes are unlikely to result in adverse health effects in humans or toxicity in non-target organisms.

2.4.1 *Risk scenario 1*

Risk source	Introduced genes for herbicide tolerance and insect resistance
Causal pathway	⬇ Cultivation of GMOs at trial sites ⬇ Exposure of people and/or other organisms at the trial sites to the products of the introduced genes ⬇
Potential harm	Adverse health effects in people and/or increased toxicity to non-target organisms

Risk source

97. The sources of potential harm for this postulated risk scenario are the GM cottons expressing the introduced herbicide tolerance and insect resistance gene or genes.

Causal pathway

98. Potential pathways of exposure to the introduced proteins are inhalation, dermal contact and ingestion. Workers who cultivate, harvest, gin, transport, experiment or conduct other dealings with the GM cotton would be exposed to cotton plant material. As the applicant proposes that only authorised staff deal with the GM cotton, other people are not expected to be exposed to the GM plants or plant material.

99. GM plant material that could potentially be airborne and inhaled includes pollen or cotton dust produced during the harvesting or ginning processes. However, cotton pollen is heavy, sticky and not easily dispersed by wind (OGTR, 2024), and dust masks and respirators are considered to be suitable to provide protection from cotton dust (AgHealth Australia) which may be an issue during cotton handling.

100. Workers could come into skin contact with the introduced proteins if they touch damaged plants where cell contents have been released.

101. There is little potential for human ingestion of the introduced proteins, as the applicant proposes that no GM plant material would be used as human food.

102. The applicant proposes the commercial use of lint (long cotton fibres) from the GM cottons. The processing of cotton lint for use in textiles has been reported to denature and/or remove any proteins, either endogenous or introduced into the cotton plant by genetic modification. Therefore, people wearing cotton clothing or using other products made from processed lint derived from GM cottons would not be exposed to the proteins produced by the introduced genes.

103. Other organisms may be exposed directly to the introduced proteins through ingesting the GM plants, or exposed indirectly through the food chain, or exposed through contact with dead plant material (soil organisms). Livestock would not be expected to ingest the introduced proteins as the GM cottons are not to be used as animal feed. Wild mammals and birds generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content (OGTR,

2024). A range of invertebrates would be expected to ingest GM cotton plant material during the release, including both target and non-target insects. The limited scale and duration of the proposed field trial would restrict the total number of organisms exposed to the proteins produced by the introduced genes.

Potential harm

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

105. Non-GM cotton produces natural toxins and anti-nutrients for defence against herbivory including gossypol and cycloprenoid fatty acids (OGTR, 2024). The applicant reports that the introduced genes are not known to affect any metabolic pathways other than those in which their modes of action are described. In addition, as discussed in Chapter 1, some of the proposed GM cotton events have previously been assessed to exhibit comparable gossypol and cycloprenoid fatty acid content to that in conventional and null-segregate cotton lines. Therefore, the GM cottons are not expected to have increased levels of these natural toxins and anti-nutrients.

106. The introduced insect resistance genes in MON 15947, MON 88702, MON 15985 and COT102 are derived from common soil-borne bacteria *B. thuringiensis*, which is widespread in the Australian environment. The World Health Organisation's International Programme on Chemical Safety evaluated the environmental safety of microbial *Bt* insecticides, and concluded that, because of the specificity of the mode of action of *Bt* toxins, *Bt* products are unlikely to pose any hazard to humans, other vertebrates, or the great majority of non-target invertebrates (International Programme on Chemical Safety 1999). The Regulator has previously assessed the *Bt* toxins in MON 88702 (DIR 147), MON 15985 (DIR 066/2006, DIR 124 and DIR 145) and COT102 (DIR 124, DIR 145 and DIR 157) to be highly specific to target lepidopteran species, in which the *Bt* toxins were assessed to pose negligible risk to human health and the environment. Therefore, the available information, including a long history of safe use of *Bt* sprays and GM cottons containing *Bt* proteins, indicates that the expressed mCry51Aa2, Cry1Ac and Cry2ab2 and Vip3Aa19 proteins are unlikely to exhibit insecticidal activity greater than that of *Bt* insecticidal sprays and are unlikely to be toxic or allergenic to people and other vertebrates.

107. The introduced herbicide tolerant genes in MON 88913 (DIR 066/2006, DIR 124 and DIR 145) and MON 88701 (DIR 145) have previously been assessed by the Regulator to pose negligible risk to human health and the environment. The proteins associated with the introduced genes in MON 88913 and MON 88701 have also been extensively assessed by regulatory bodies worldwide and in the literature. These past assessments concluded that there was no evidence that the proteins associated with the introduced herbicide tolerant genes were toxic or allergenic to humans or toxic to other organisms.

108. Potential toxicity and allergenicity of MON 96012 and MON 89151 have been assessed (information CCI). This assessment concluded that the proteins associated with the introduced genes in MON 96012 and MON 89151 are unlikely to be toxic or allergenic to people and other vertebrates, or toxic to non-target invertebrates. Some uncertainty exists in this area, in part due to the early-stage development of the events and gaps in the literature. However, as detailed in Chapter 1, the proposed limits and controls would restrict potential exposure of the GMOs to people, other vertebrates and non-target invertebrates, minimising potential risks arising from these uncertainties.

109. In addition, no adverse effects have been reported as a result of growing the insecticidal MON 15947, MON 88702, MON 15985 and COT102 or herbicide tolerant MON 88913 and MON 88702 GM cottons since their limited and controlled and/or commercial releases in Australia.

110. The proposed GM cotton lines may generate cottons containing multiple herbicide tolerant and/or multiple insecticidal proteins (Tables 3 and 4). It is possible that the combination of the stacked insecticidal gene products may potentially increase the range of sensitive insects. While the weight of

evidence supports high specificity of currently employed *Bt* insecticidal toxins in GM crops to their target species, there are some reports of toxicity of *Bt* toxins to non-target organisms (Hilbeck and Otto, 2015; Lang et al., 2019; Baranek et al., 2020). Some uncertainty exists in this area due to some of the GM cottons not being well characterised yet and due to data gaps in the literature investigating potential toxicity to non-target organisms. However, as discussed above, the proposed limits and controls would restrict exposure to the GMOs, minimising potential risks arising from these uncertainties.

Conclusion

111. Risk scenario 1 is not identified as a substantive risk because the GM cottons will not be used in human food or animal feed; the previously authorised GM cotton events containing the introduced genes have a history of safe use; the proteins encoded by the introduced genes are unlikely to result in adverse health effects in humans or toxicity in non-target organisms; some of the introduced genes and encoded proteins are widespread in the environment; and the limited scale and short duration of the trial will minimise exposure to the GM plant material. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 *Risk Scenario 2*

Risk source	Introduced genes for herbicide tolerance and insect resistance
Causal pathway	Cultivation of GMOs at trial sites ⬇ Dispersal of GM seed outside trial limits and establishment of volunteer GM cotton plants ⬇ Reduced effectiveness of weed management measures to control volunteer GM cotton plants ⬇
Potential harms	Adverse health effects in people and/or increased toxicity to non-target organisms and/or Reduced establishment or yield of desirable plants

Risk source

112. The source of potential harm for this postulated risk scenario is GM cottons expressing introduced herbicide tolerance and insect resistance gene or genes.

Causal Pathway

113. The first step in the causal pathway for this risk scenario is dispersal of GM seed outside the trial limits. This could occur due to persistence of viable GM seeds at the trial site after the intended duration of the trial, or through physical movement of GM seeds to areas outside the trial site.

114. The applicant proposes a number of control measures to prevent persistence of GM seeds in the seed bank at the trial site. These include destroying GMOs that remain in the trial site after harvest, destroying any volunteers found prior to flowering, and post-harvest monitoring of each trial site for at least twelve months and until the site has been clear of volunteers for six months. It is not expected that expression of the introduced genes for herbicide tolerance or insect resistance would increase the ability of the GMOs to survive these standard control measures.

115. Cotton seed is not normally physically transported by runoff after rainfall or irrigation. The applicant proposes to select trial sites that are at least 50 m away from natural waterways.

116. Cotton seeds are enclosed in large, heavy bolls that remain attached to the plant. At maturity the bolls split open and the fibres can facilitate seed dispersal by wind over distances less than 100 m (OGTR, 2024). Wind dispersal of seed occurs during harvest. An extreme weather event such as a

cyclone could physically disperse cotton seeds over greater distances if the event occurred either soon after seed sowing, or late in the growth cycle as the bolls mature.

117. Cotton seeds do not possess specific adaptations, such as burrs or hooks, for dispersal on the exterior of animals. Wild mammals and birds generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content (OGTR, 2024). Therefore, wild animals are unlikely to disperse GM cotton seeds from the trial site. GM cotton seeds would not be used as livestock feed, so would not be dispersed by livestock.

118. Dispersal of cotton seeds by authorised people entering the trial site would be minimised by cleaning all equipment, used with the GM cotton before using it for any other purpose. The applicant proposes to contain GM plant materials during transportation and storage in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

119. The spread and persistence of cotton plants are limited by a number of biotic and abiotic factors, especially cold stress in southern Australia and water stress in non-irrigated environments throughout almost all of Australia. Feral cotton populations are sparse and ephemeral in all current cotton growing regions of Australia (OGTR, 2024). A study found that even when cotton was sown in cleared sites in northern Australian with high water availability, the cotton plants did not establish stable populations (Eastick & Hearnden 2006). Modelling of climatic factors limiting cotton persistence indicate that cotton has naturalisation potential only in the coastal regions of north-east Australia. A few small populations of naturalised cotton are reported in northern Australia, but these are not derived from modern cultivars (OGTR, 2024) and these tufted cottons may have a greater ability to survive outside agricultural settings than modern cotton cultivars.

120. A number of controls are proposed to physically separate the trial sites from commercial cotton crops. These controls have effectively restricted the spread of GM cotton seed in previous trials.

121. It is not expected that the expression of the introduced herbicide tolerance and insect resistance genes would allow cotton to overcome the biotic and abiotic factors that limit spread and persistence. Expression of the introduced insecticidal genes could reduce herbivory of the GM cottons by insects which are susceptible to the insecticidal proteins, and expression of the herbicide tolerance genes would reduce the range of herbicide classes that would be efficacious to kill the GM cottons. As noted in risk scenario 1, there is some uncertainty about the range of insects that may be susceptible to the introduced insecticidal genes and therefore what impact this may have on the GM cottons weediness potential in areas with high insect pressure. In addition, as discussed in Chapter 1, the applicant has reported that there is no evidence to suggest that the genetic modifications in the GM cotton events, including MON 96012 and MON 89151, have altered seed production characteristics or tolerance to abiotic or biotic stresses, other than herbicide tolerance and insect herbivory, that could enhance the potential for dispersal or persistence of the GM cottons proposed for release.

122. Management of the GM cotton volunteers, including crosses between GM lines, as listed in Table 4, would involve cultivation and/or herbicide spraying using herbicides other than those they are tolerant to. The 2023-2024 cotton pest management guide details which herbicides are currently recommended to the Australian cotton industry to control cotton volunteers (CRDC, 2023).

123. Management of the stacked herbicide tolerant MON 96012 cotton volunteers or GM lines 5-8 (Table 4) that may contain MON 96012, can be managed using herbicides other than the target herbicides of the introduced genes and/or by mechanical means.

Potential harms

124. The potential harms from this risk scenario are adverse health effects in people and/or non-target organisms, or reduced establishment or yield of desirable plants.

125. As discussed in Risk Scenario 1, the introduced proteins in the GM cotton are unlikely to be toxic or allergenic to people, or toxic to vertebrates, or toxic to invertebrates other than certain target lepidopteran species.

126. Risk Scenario 1 considered the potential for the introduced genes and proteins to lead to adverse health effects and did not identify any substantive risks.

127. The GM cottons could reduce the establishment or yield of desirable plants in agricultural settings if GM cotton volunteers grew in other crops. If this happened, the GM cotton volunteers could likely be controlled by similar weed management measures as volunteers from commercial cotton, such as application of alternative herbicides or mechanical cultivation.

128. The GM cottons could reduce the establishment or yield of desirable plants in the natural environment if the GM cottons spread and persisted as a weed in nature reserves, displacing native vegetation. However, as discussed above, cotton has limited potential to survive outside agricultural settings, and the introduced genes are not expected to increase the cotton's ability to spread and persist.

129. If the GM cottons established in intensive use areas, such as roadsides, then ephemeral GM cotton populations would be unlikely to cause harms other than those of commercial cotton and could be controlled by the same means.

130. Some uncertainty exists regarding potential weediness of the GM cotton events MON 96012 and MON 89151 and GM cotton lines 5-8 (Table 4) that may contain these GM cotton events. In part due to the early-stage characterisation of MON 96012 and MON 89151 and uncertainty as to whether expression of the insecticidal proteins in these GM cottons could increase the weediness potential of the GM cottons in areas with high insect pressure. However, as detailed in Chapter 1, cotton has a limited ability to persist outside of agricultural environments and the proposed limits and controls would restrict the potential for gene flow and dispersal of the GMOs outside of the trial sites, minimising potential risks arising from these uncertainties.

Conclusion

131. Risk scenario 2 is not identified as a substantive risk because the proposed controls would minimise dispersal and persistence of the GM cottons; the introduced genes are not expected to change susceptibility of the GM cottons to the factors which limit the geographical range and persistence of cotton in Australia; for GM cotton volunteers with herbicide tolerance, there are other herbicide options as well as weed management strategies available that can control volunteers; and the proteins encoded by the introduced genes are unlikely to result in adverse health effects in people and toxicity other non-target organisms. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 *Risk Scenario 3*

Risk source	Introduced genes for herbicide tolerance and insect resistance
Causal pathway	Cultivation of GMOs at trial sites ⬇ Cross-pollination with other cotton, including cotton with other herbicide tolerance and/or insect resistance traits ⬇ Establishment of hybrid GM cotton plants expressing the herbicide tolerance and/or insect resistance genes as volunteers ⬇ Reduced effectiveness of weed management measures to control the hybrid plants ⬇
Potential harms	Adverse health effects in people and/or increased toxicity to non-target organisms and/or Reduced establishment or yield of desirable plants

Risk source

132. The source of potential harm for this postulated risk scenario is GM cotton expressing introduced insect resistance gene(s).

Causal pathway

133. The first step in the causal pathway for this risk scenario is pollen from the GM cottons fertilising sexually compatible plants. Cotton is predominantly self-pollinating, with pollen that is large, sticky and heavy and generally not dispersed by wind. Pollen can be transported by insect pollinators, mainly via honeybees, but gene flow studies have shown that outcrossing occurs at low levels and decreases rapidly with distance (OGTR, 2024). For *G. hirsutum* cotton, the only sexually compatible plants are other *G. hirsutum* plants or *G. barbadense* plants, as native *Gossypium* species are not sexually compatible with *G. hirsutum* cotton. It is not expected that the introduced herbicide tolerance or insect resistance genes would alter the pollen dispersal characteristics of the GM cottons proposed for release or their ability to cross with other cotton species.

134. The applicant has proposed to restrict pollen flow by surrounding the trial sites either with a 20 m pollen trap of commercial cotton, or a 1.5 km exclusion zone where no cotton crops are planted. In addition, the applicant has proposed to destroy any post-harvest cotton volunteers on the trial site before flowering. These controls would minimise the potential for pollinators to transfer pollen from GM cottons to related plants outside the trial sites.

135. Some outcrossing is expected to occur between the GM cottons and other cotton plants grown in close vicinity of the GM cottons, e.g. non-GM, other GM cottons authorised in the trial and commercial GM cotton plants in the pollen trap. These hybrids may contain multiple stacked combinations of the herbicide tolerance and/or insect resistance traits. As the cotton plants grown in the trial sites and pollen traps are expected to produce a small proportion of hybrid seeds, the applicant has proposed that all cotton planted in the trial sites and in the pollen trap will be handled as if they are the GMOs. The limits and controls proposed for the GM cottons would minimise dispersal and persistence of any hybrid seed and plants (see Risk Scenario 2).

Potential harms

136. The potential harms from this risk scenario are adverse health effects in people and/or non-target organisms, or reduced establishment or yield of desirable plants.

137. As discussed in Risk Scenario 1, the introduced proteins in the GM cotton are not expected to be toxic or allergenic to people, or toxic to vertebrates, or toxic to invertebrates other than certain species in specific insect orders. The same considerations as discussed in Risk Scenario 1 would apply if the introduced proteins are expressed in hybrids with non-GM or commercially released GM cotton.

138. The potential for the GM cottons to reduce establishment or yield of desirable plants was discussed in Risk Scenario 2. Cotton plants, including hybrids, expressing the introduced proteins are unlikely to spread and persist in nature reserves or to survive standard weed management practices for cotton volunteers in agricultural settings.

Conclusion

139. Risk scenario 3 is not identified as a substantive risk because the proposed controls would minimise opportunities for the GM cottons to hybridise with other cotton; hybrids between the GMOs and other cotton would likely be generated at low levels; for multiple-herbicide tolerant hybrids, there are other herbicide options as well as weed management strategies available that can control volunteers; and the proteins encoded by the introduced genes are unlikely to result in adverse health effects in people or toxicity to other non-target organisms. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

140. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Risk Analysis Framework (RAF).

141. Uncertainty is addressed by approaches such as balance of evidence, making 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.

142. 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.

143. For DIR 203, uncertainty is noted particularly in relation to:

- molecular, biochemical and phenotypical characterisation of the GM cottons MON 96012 and MON 89151, including potential for increased toxicity, allergenicity and weediness, and changes in anti-nutrient levels
- potential for the stacked GM cottons to lead to adverse health effects in people or toxicity to non-target organisms
- potential for the stacked genetic modifications to increase plant competitiveness and survival.

144. 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.

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

Section 4 Risk evaluation

146. 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.

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

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

148. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- none of the GM plant material would enter human food or animal feed
- no reported adverse health effects on people handling the GM plants in glasshouse and previous field trials
- the insecticidal proteins are expected to be toxic to a limited range of insect species
- the herbicide tolerant proteins are derived from bacterium widespread in the environment, some of the proteins have been applied to GM crops globally, with a long history of safety
- 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 cotton plants and their genetic material.

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

Chapter 3 Risk management plan

Section 1 Background

149. 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.

150. 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.

151. 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.

152. 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

153. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM cotton. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.2), the proposed controls (Chapter 1, Section 2.3), 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

154. 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 size, location and duration of the trial, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the licence.

3.1 Limits and controls on the release

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

3.1.1 Consideration of limits proposed by the applicant

156. The release will be limited to a maximum of 25 sites per year in cotton growing areas in Australia in the states of NSW, Qld, WA, NT and Vic. The maximum combined size of the trial sites each year will not

exceed 10 ha in 2024, 50 ha per year in 2025-2027 and 100 ha per year in 2028-2029. The limited size and duration of the trial limits exposure to the GM cottons (Risk Scenario 1).

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

157. The applicant states that the GM cottons and their products would not be used for human food or animal feed. A licence condition prohibits the use of GM plant material in human food or animal feed. This measure would minimise exposure of people or animals to the GM cottons by consumption (Risk scenario 1).

158. The applicant has proposed to sell lint from GM cotton grown in the trial. As discussed in Risk Scenario 1, cotton lint is free of detectable levels of DNA and protein, and exposure (if any) to the introduced genes and proteins would be negligible. Therefore, the licence does not impose conditions on transport and sale of lint from GM cotton, other than the prohibition of use in food or feed described above.

159. The applicant proposes that only authorised and trained personnel would be permitted to deal with the GMOs. Standard licence conditions require all people dealing with the GMOs to be informed of relevant licence conditions. These measures would limit the potential exposure of people to the GMOs (Risk Scenario 1).

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

160. The applicant has proposed to restrict the potential for gene flow at the trial sites by surrounding the planting zones with a 20 m wide pollen trap or a 1.5 km exclusion zone. The plants within the pollen trap may be non-GM cotton or commercial GM cottons that would also be authorised under this licence, and would be managed so as to flower at the same time as the GMOs. Pollen trap plants may provide sufficient forage for incoming pollinating insects so that they do not visit the GM plants, and any insects that reach the GM plants are expected to deposit most GM pollen on pollen trap plants while exiting the trial site. As discussed in Risk scenario 3, cotton is predominantly self-pollinating and outcrossing rates decrease rapidly with distance. A 20 m pollen trap around GM cotton was found to be an effective buffer under Australian conditions (Llewellyn et al., 2007). Therefore, using a 20 m pollen trap would minimise gene transfer to cotton plants outside the trial sites (Risk Scenario 3). The licence specifies that the pollen trap must have reasonably dense and vigorous growth to avoid the occurrence of large gaps and to ensure there are sufficient pollen recipient plants. As discussed in the RARMP for DIR 120, in the absence of a pollen trap, a 1.5 km isolation distance from other cotton is considered appropriate to restrict the potential for gene flow. This isolation distance is included in the licence as two distinct areas; a 100 m monitoring zone surrounding the outer edge of the planting area and a 1.4 km isolation zone around the monitoring zone. No cotton is to be grown in these areas while the GMOs are growing. The combination of a monitoring zone and an isolation zone is considered effective to restrict gene transfer from GM cotton trial sites to other cotton (Risk Scenario 3).

3.1.4 Consideration of proposed controls regarding persistence of the GMOs

161. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or further plantings. It is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed, to limit persistence of the GMOs. Licence conditions require that the 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 nine months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been cleaned.

162. The applicant proposes that the GM cotton would be destroyed using one or more of the following methods: herbicide application, root cutting and mulching, uprooting, autoclaving or burial of seed or other plant material to a depth of at least 1m. All of these methods are considered effective in destroying one or more life stages of the GM cotton, so are included in the licence. To ensure the effectiveness of destruction by burial, a licence condition specifies how this must be carried out, including a requirement that the GM material must be sufficiently irrigated at time of burial to encourage decomposition. The applicant has

proposed that the burial site remain undisturbed for a period of at least 12 months after burial. This is considered appropriate to avoid dispersal of the material before it decomposes.

163. Cotton seeds have low dormancy levels and do not generally form a viable seed bank, however, dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture (OGTR, 2024). In order to promote cotton seed germination or decomposition, a licence condition requires tillage in the spring or summer following the harvest, and provision of adequate soil moisture, so that soil temperature and moisture will be suitable for cotton seed germination. These measures would restrict the persistence of a GM cotton seed bank after the duration of the trial (Risk Scenario 2).

164. The applicant proposes that each trial site will be monitored post-harvest every 35 days for a minimum of twelve months and until the site has been clear of volunteers for at least six months. During this period any cotton volunteers will be destroyed before flowering. These measures would restrict the persistence of GMOs after completion of the trial (Risk Scenario 3).

165. The applicant may plant both GM and non-GM cotton in the trial site and intends to treat all cotton from the trial site and the pollen trap as if it were GM cotton. This measure would minimise exposure to and dispersal of hybrid seed resulting from outcrossing between the GM cottons and other cotton (Risk Scenario 3).

3.1.5 Consideration of proposed controls to limit the dispersal of the GMOs

166. The applicant has proposed that the trial sites would be located at least 50 m from any natural waterways. This would reduce the likelihood of plant material being washed away from the planting areas (Risk Scenario 3). It is a standard licence condition that trial sites be located at least 50 m from waterways to limit the dispersal of viable plant material in the event of flooding. There is also a condition in the licence requiring immediate notification of any extreme weather event affecting the properties during the release to allow assessment and management of any risks.

167. The applicant has proposed to clean all equipment used with the GMOs before using the equipment for other purposes. Equipment used on the trial sites would be cleaned on site. The licence imposes a condition that the GM cotton would be ginned separately from other cotton crops and the gin would be cleaned after use to prevent GM cotton seed mixing with other seed. These measures are appropriate to restrict potential dispersal of GM cotton seed outside the trial sites (Risk Scenario 2).

168. The applicant has stated that netting to restrict access to birds, baiting/trapping to control rodents and fences with lockable gates are not proposed for the trial sites, as the incidence of cotton boll and seed movement by animals and birds is unlikely. This is consistent with the discussion in Risk Scenario 2, in which is was concluded that dispersal by wild animals is unlikely. In the instance that a trial site is located in close proximity to grazing animals, the applicant has indicated that fencing will be used to restrict access and prevent potential dispersal of the GMOs. The licence does not require fencing, however a condition preventing the GM cottons being used as animal feed is imposed in the licence and therefore the use of fencing would be one way to comply with this condition.

169. The applicant has proposed that GMOs will be transported and stored according to the Regulator's current *Guidelines for the Transport, Storage and Disposal of GMOs* (OGTR website). These protocols restrict the potential for dispersal of GM seeds outside the trial sites (Risk Scenario 3).

170. The applicant has proposed that imported GM cotton seed would be transported to accredited PC2 glasshouses where it will be sown under NLRDs. Transport may occur between/among accredited PC2 glasshouses/laboratories, accredited facilities and field sites. Transport of GM cotton seed and plant material may also be carried out for export for testing purposes. Anyone handling the GMOs would be trained in the relevant licence conditions and a signed statement taken to that effect. If a courier is used and it is not possible to train the particular driver, such dealing will occur under an approved NLRD authorisation in accordance with applicable requirements of the Regulations. This is considered appropriate but is not included in the licence as it would be conducted under a separate valid authorisation.

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

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

- limit the duration of the field trial to between September 2024 and September 2029
- limit the field trial to a maximum of 25 sites per year with a maximum combined area of 10 ha in 2024, 50 ha per year in 2025-2027 and 100 ha per year in 2028-2029
- locate the trial sites at least 50 m away from natural waterways
- restrict gene flow via pollen by using one of the following measures:
    i. surround the trial site with a 20 m pollen trap of non-GM cotton or commercial GM cotton authorised under this licence
    ii. surround the planting area with a 100 m monitoring zone and 1.4 km isolation zone in which no other cotton plants may be grown
- ensure that pollen trap plants flower for the same period of time as the GM cottons
- treat any plants in the planting area or pollen trap as if they were the GMOs
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering
- clean all equipment used with the GMOs before using it for any other purpose
- gin the GMOs separately from any other cotton crops
- use tillage and irrigation to promote germination of any cotton seeds remaining in the trial site after harvest
- monitor the trial site and any area onto which the GMOs may have been dispersed to for at least 12 months after harvest and destroy any cotton volunteers until no volunteers are detected for a continuous 6 month period
- destroy all GMOs from the trial that are not required for testing or future planting
- transport and store the GMOs in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- not allow GM plant material to be used for human food or animal feed.

3.2 Other risk management considerations

172. 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

173. 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 and
- the capacity of the applicant to meet the conditions of the licence.

174. The conditions include a requirement for the applicant to inform the Regulator of any information that would affect their suitability.

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

3.2.2 Contingency plan

176. The applicant is required to submit a contingency plan to the Regulator before planting the GMOs. This plan must detail measures to be undertaken in the event of any unintended presence of the GM cottons outside permitted areas.

177. Before planting the GMOs, the applicant 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.

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

178. 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 applicant is required to provide a list of people and organisations covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

179. The licence requires the applicant 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 and
- any unintended effects of the trial.

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

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

3.2.5 Monitoring for compliance

181. 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 site.

182. 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.

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

Section 4 Issues to be addressed for future releases

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

- Additional molecular, biochemical and phenotypical characterisation of the GM cottons MON 96012 and MON 89151, including potential for increased toxicity to non-target insects and weediness.
- Data investigating potential effects of the stacked herbicide resistance and/or stacked insecticidal proteins on toxicity to non-target insects and weediness.

Section 5 Conclusions of the RARMP

185. The risk assessment concludes that the proposed limited and controlled release of the GM cottons poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

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

References

ABARES (2023). Australia Crop Report.

Addison, S.J., Farelll, T., Roberts, G.N., and Rogers, D.J. (2007). Roadside surveys support predictions of negligible naturalisation potential for cotton (*Gossypium hirsutum*) in north-east Australia. Weed Research *47*, 192-201.

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

Baranek, J., Pogodziński, B., Szipluk, N., and Zielezinski, A. (2020). TOXiTAXi: a web resource for toxicity of Bacillus thuringiensis protein compositions towards species of various taxonomic groups. Scientific Reports *10*, 19767.

Bravo, A., Gill, S.S., and Soberón, M. (2007). Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon *49*, 423-435.

Brown, A., Brubaker, C., and Kilby, M. (1997). Assessing the risk of cotton transgene escape into Australian Gossypium species. In The Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations (Bureau of Resource Sciences), pp. 83-94.

Brubaker, C.L., Brown, A.H.D., Stewart, J.M., Kilby, M.J., and Grace, J.P. (1999). Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. Euphytica *108*, 199-214.

CRDC (2013). WEEDpak. (Narrabri, Australia: Cotton Research and Development Corporation).

CRDC (2023). Cotton Pest Management Guide 2023-24. (Cotton Research & Development Corporation).

CRDC and CottonInfo (2017). Cotton Pest Management Guide 2017-18. (Narrabri, Australia: Cotton Research and Development Corporation (CRDC) and CottonInfo).

Eastick, R.J., and Hearnden, M.N. (2006). Potential for weediness of *Bt* cotton in northern Australia. Weed Science *54*, 1142-1151.

EPA (2001). Biopesticides registration action document - *Bacillus thuringiensis* plant-Incorporated protectants.

EPA (2018). White paper on resistance in Lepidopteran pest of *Bacillus Thuringiensis* (*Bt*) plant incorporated protectants in the United States. (Environmental Protection Agency).

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

FSANZ (2002). Final assessment report – Application A436 – Oil and linters derived from insect-protectant cotton containing event 15985. (Food Standards Australia New Zealand).

FSANZ (2004). Final assessment report - Application A509 - Food derived from insect-protected cotton line COT102. (Food Standards Australia New Zealand).

FSANZ (2005). Final assessment report - Application A553 - Food derived from glyphosate tolerant cotton line MON 88913. (Food Standards Australia New Zealand).

FSANZ (2013). Supporting Document 1 - Application A1080 – Food derived from Herbicide-tolerant Cotton Line MON88701. (Food Standards Australia New Zealand).

FSANZ (2018). Supporting document 1 - Safety assessment – Application A1154 - Food derived from insect-protected cotton line MON88702. (Food Standards Australia New Zealand).

Gill, S.S., Cowles, E.A., and Pietrantonio, P.V. (1992). The mode of action of *Bacillus thuringiensis* endotoxins. Annual Review of Entomology *37*, 615-636.

Hilbeck, A., and Otto, M. (2015). Specificity and Combinatorial Effects of *Bacillus Thuringiensis* Cry Toxins in the Context of GMO Environmental Risk Assessment. Frontiers in Environmental Science *3*.

ISAAA (2018). Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. *ISAAA Brief No. 54*.

Jerga, A., Chen, D., Zhang, C., Fu, J., Kouadio, J.K., Wang, Y., Duff, S.M.G*., et al.* (2016). Mechanistic insights into the first Lygus-active β-pore forming protein. Archives of Biochemistry and Biophysics *600*, 1-11.

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

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

Lang, A., Lee, M., Dolek, M., Berchtold, J., and Otto, M. (2019). Laboratory tests with Lepidoptera to assess non-target effects of *Bt* maize pollen: analysis of current studies and recommendations for a standardised design. Environmental Sciences Europe *31*, 39.

Llewellyn, D.J., Tyson, C., Constable, G.A., Duggan, B., Beale, S., and Steel, P. (2007). Containment of regulated genetically modified cotton in the field. Agriculture, Ecosystems & Environment *121*, 419-429.

OECD (2007). Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis* - derived insect control protein (Paris, France: Organisation for Economic Co-operation and Development).

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

OGTR (2024). The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton) v4.0. (Canberra, Australia: Office of the Gene Technology Regulator).

Philips, J.G., Martin-Avila, E., and Robold, A.V. (2022). Horizontal gene transfer from genetically modified plants - Regulatory considerations. Frontiers in Bioengineering and Biotechnology *10*, 971402.

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

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R*., et al.* (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews *62*, 775-806.

Soberón, M., Gill, S.S., and Bravo, A. (2009). Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells? Cellular and Molecular Life Sciences *66*, 1337-1349.

Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. Toxicological Sciences *71*, 2-8.

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

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

Yu, C.G., Mullins, M.A., Warren, G.W., Koziel, M.G., and Estruch, J.J. (1997). The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Applied and Environmental Microbiology *63*, 532-536.

Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received several submissions from prescribed experts, agencies and authorities[3] on the consultation RARMP. All issues raised in submissions relating 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. Advice received is summarised below.

Submission	Summary of issues raised	Comment
1	No advice or comments on the RARMP.	Noted.
2	No specialist scientific expertise available to provide informed comment, however, acknowledges the RARMP determines the risk to the health and safety of people and to the environment to be negligible.	Noted.
3	No advice or comments on the RARMP, recommends contacting agricultural users within their region to get their thoughts on the growing of cotton in the future.	Noted.
4	Notes that the draft licence prohibits the use of GM plant material in human food and animal feed. No comments at this stage.	Noted.
5	Agreed that the risk assessment identifies all plausible risk scenarios by which the proposed dealings could potentially give rise to risks relating to the health and safety of people or the environment. Agreed that the measures to limit and control the release, particularly to restrict persistence and gene flow of the GM cotton, are appropriate for the trial. Did not identify additional information that should be considered. Agreed with the overall conclusion of the RARMP.	Noted.
6	Noted that the RARMP was comprehensive and thorough and the draft licence conditions seem appropriate and commensurate with the level of risk that the release may pose.	Noted.
	Extreme weather events (cyclones and mass flooding) may spread GM cotton seed long distances from the planting site. What criteria are used to identify flood-prone areas in the RARMP?	The RARMP does not define what a flood prone area is, however, there are various LGA, State, and Territory resources available that provide flood risk information and historical flood mapping. In addition, there are specific conditions in the licence that assist in managing flood risks, such as requiring the licence holder to

[3] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
		notify the Regulator of any extreme weather events, locating the trial sites at least 50 m away from the nearest waterways, and enacting the contingency plan if GMOs spread outside of the trial sites.
	The RARMP states that seed dispersal by wind can occur over distances less than 100 m, however trial sites are proposed to be located at least 50 m away from the nearest natural waterways. The distance from waterways needs to be greater than the potential for seed dispersal, even without flooding/cyclone concerns.	Cotton seed is not normally physically transported by runoff after rainfall or irrigation, therefore a 50 m distance from natural waterways is considered appropriate. Seeds within the cotton boll are not expected to disperse easily, as the cotton boll will easily catch on surrounding vegetation (OGTR Cotton Biology document). Severe windstorms may result in dispersal of cotton bolls over larger distances. Licence conditions require that Planting Areas and Pollen Traps must be Cleaned within 28 days of harvest. These conditions limit the time during which viable seed is present at the trial site and minimises the risks of spread and persistence. Additionally, it is considered that cotton seeds are unlikely to remain viable after soaking or in water (OGTR Cotton Biology document). This available information and past extensive experience of monitoring GM cotton trials, give confidence that a distance of 50 m from natural water ways is sufficient to manage risk.
	The potential for hybridisation of GM cotton with native Australian *Gossypium* species wasn't explained thoroughly in the RARMP. The cited papers documenting genetic incompatibilities between the GM cotton and native Australian species were not conducted in all regions of Australia. Risk could be minimised by including native *Gossypium* species in the 1.5 km exclusion zone.	The OGTR Cotton Biology document discusses the potential for cross pollination between *Gossypium hirsutum* with Australian native *Gossypium* species in detail. It indicates that cross-pollination of *G. hirsutum* with Australian native *Gossypium* species is extremely low due to genetic incompatibility. Additionally, if cross pollination with Australian native *Gossypium* species occurred, the likelihood of fertile hybrids occurring and surviving to reproductive maturity and back-crossing to the parental species is effectively zero.
	Lack of knowledge of diversity of invertebrates in some regions, so hard to assess the impact the GM cotton will have on non-target invertebrates. Suggests addressing knowledge gaps with additional invertebrate monitoring requirements during the GM trial.	Uncertainty, including potential toxicity to non-target organisms of single and stacked combinations is discussed in Chapter 2, Section 3 of the RARMP. This section notes that 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.
	Suggest that the HPPD, and PPO-inhibiting herbicide tolerances be indicated in full for the first instance rather than in abbreviated form in the RARMP.	Noted and corrected.

Submission	Summary of issues raised	Comment
7	Further consideration is needed on: Impact of the worldwide distribution of GM materials within prime specialised agricultural areas and the impact of multi-national company on the operation and viability of a predominately family-owned industry.	Matters related to marketing or trade issues, consumer preferences and coexistence regimes are outside the Regulator's legislative responsibility. These issues are the responsibility of the State and Territory governments and industry.
	The level of engagement with peak body stakeholders.	The consultation RARMP was published on the OGTR website, in the Gazette and the Australian newspaper and was open for consultation for 30 days. An invitation to comment on the consultation RARMP was also sent to prescribed government agencies, LGAs and public subscribers. Additionally a report commissioned by the Regulator in 2020 providing advice on GM crops containing multiple herbicide tolerant traits, including discussions surrounding stakeholder engagement can be found here.
	Residual chemical accumulation due to frequency and concentrations of herbicide use.	The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products in Australia. Any use of herbicides in DIR-203 is subject to regulation by the APVMA.
	That a trial timeline of 5 years will exponentially exacerbate environmental risk.	The RARMP concluded that the proposed field trial poses negligible risks to human health and safety or the environment. Licence conditions regarding limits (including duration) and controls have been imposed to maintain the context in which the risks have been assessed.
8	Broadly agrees with the conclusions of the RARMP, but notes two issues:	Noted.
	Suggest monitoring of the trial sites for two seasons post-cotton field experiment (rather than the proposed 12 months) and to require irrigation of the trial locations in the following spring to account for volunteer emergence across two spring seasons.	Cotton has low dormancy levels and is unlikely to form a seed bank, except in conditions of low soil temperature and/or low soil moisture. The licence requires that post-cleaning inspection activities be conducted for at least 12 months after harvest, with a 6 month period where no volunteers are detected prior to site sign-off. Licence conditions require tillage in the spring or summer following the harvest, with adequate soil moisture, to ensure conditions suitable for cotton seed germination. The licence holder must provide evidence of compliance with these conditions to the Regulator before applying to sign off a site. Past extensive experience of monitoring GM cotton trials indicate that these conditions are appropriate to minimise the persistence of the GMOs at trial sites.
	Raised concerns about the lack of a requirement to conduct ecotoxicological testing to scientifically assess the safety of the GM	Many of the *Bt* derived proteins assessed in this application have previously been assessed in GM cotton for limited and controlled or commercial

Submission	Summary of issues raised	Comment
	cotton. The introduced genes are sourced from ubiquitous and naturally occurring genes from bacteria, however these are modified during plant transformation and are broadly and constitutively expressed as activated toxins (not protoxins) across the plant. The exposure scenarios for non-target insects may therefore differ from the original studies, particularly in the case of 'stacked' genes from a variety of sources. Consider using these field trials to carry out ecotoxicological testing to scientifically assess safety of the GM cotton.	release by the Regulator (see RARMP for details). Many *Bt* toxins expressed by *B. thuringiensis* are in their inactive protoxin forms, limiting their toxicity to target pests that have alkaline midgut environments, specific proteases, and specific receptors for the toxins to bind to when activated. Some Cry proteins expressed by *B. thuringiensis,* including Cry2 proteins, do not undergo the protease-mediated C-terminal cleavage step as they appear to be naturally truncated. Chapter 1, Section 4 of the RARMP has been updated to include this information. This application also contains a new lepidopteran resistant GM cotton event (MON 89151). Details relating to proteins in MON 89151 have been declared Confidential Commercial Information (CCI) under Section 185 of the Act. The APVMA regulates efficacy and toxicity of plant produced toxins, including consideration of effects on non-target organisms in the environment. More information can be found in public release summaries available on the APVMA website. Uncertainty about the potential toxicity to non-target organisms of single and stacked combinations is discussed in Chapter 2, Section 3 of the RARMP. As noted in risk scenario 1, while the weight of evidence supports high specificity of *Bacillus thuringiensis* toxins to target pests, some studies report toxicity to non-target organisms, and this is an area of uncertainty. Chapter 2, Section 3, lists 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. In the context of the limits and controls for DIR 203, this data is not required to manage the risks of this particular trial.
9	Accepts that, overall, the application has negligible risks to the health and safety of people and the environment and are satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.	Noted.

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	Where will the fields sites be located, and will they be located on farms where cotton is already grown?	Before planting at a site, the licence holder must send a notification to the Regulator including details about where the trial sites will be located. This information will then be published on our website, on the DIR-203 webpage as well as on the Crop field trial map.
2	Noted typos in the consultation RARMP and consultation RARMP summary	Noted and corrected.