



Australian Government

Department of Health

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 147

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

Applicant: Monsanto Australia Limited

January 2017

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 147

Decision

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

The application

Application number	DIR 147
Applicant	Monsanto Australia Limited (Monsanto)
Project title	Limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance
Parent organism	Cotton (<i>Gossypium hirsutum</i> L.)
Introduced genes and modified traits	<ul style="list-style-type: none"> • Insect resistance: up to four genes derived from <i>Bacillus thuringiensis</i> • Herbicide tolerance: up to three genes derived from bacteria <i>Agrobacterium sp.</i>, <i>Stenotrophomonas maltophilia</i> and <i>Streptomyces hygrosopicus</i> • Selectable markers: up to three antibiotic resistance genes and one reporter gene derived from <i>Escherichia coli</i>
Proposed location	Maximum of 50 sites per year in New South Wales, Queensland, Northern Territory, Victoria and Western Australia
Proposed release size	Maximum area of 50 ha in 2017, 100 ha in 2018, and 250 ¹ ha per year in 2019 and 2020
Proposed release dates	March 2017 – July 2021
Primary purposes	<p>To breed and assess agronomic performance of the genetically modified (GM) cottons in all cotton growing areas of Australia and generate data for possible future commercial release.</p> <p>To conduct trait development, breeding and variety development trials to establish the stacked traits into elite germplasm suitable for Australian conditions.</p>

The licence permits Monsanto to conduct the field trials in cotton growing areas of Australia from March 2017 to July 2021. The licence allows planting up to 50 sites per year with a maximum combined area of 50 ha in 2017, 100 ha in 2018, and 250 ha per year in 2019 and 2020. The maximum planting size of individual trial sites is 2 ha in 2017, 10 ha in 2018, and 50 ha per year in 2019 and 2020. The licence holder

¹ The maximum area proposed in the original application was 500 ha per year in 2019 and 2020. The applicant later amended it to 250 ha.

may release GM cotton MON88702 both individually and crosses of GM cottons obtained by conventionally breeding MON 88702 insect resistant cotton with previously released GM cottons Bollgard II® (insect resistant), COT102 (insect resistant), Roundup Ready Flex® (herbicide tolerant) and MON 88701 (herbicide tolerant).

The purpose of the trial is to generate data for future submissions to regulatory agencies, to breed and develop varieties using elite germplasm suitable for use under Australian conditions, and for seed increase. Material from the GM cotton would not be used in human food or animal feed during the release.

The licence imposes a number of controls to restrict the spread and persistence of the GM cottons and the introduced genetic materials in the environment that have been considered during the evaluation of the application.

Risk assessment

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

Credible pathways to potential harm that were considered included exposure of people or animals to the GM plant material, dispersal of GM seed leading to spread and persistence of the GMOs, and transfer of the introduced genetic material to sexually compatible cotton plants. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to other desirable organisms, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed, the proposed limits and controls effectively contain the GMOs and their genetic material and minimise exposure; the introduced genetic modifications are unlikely to cause harm to people or the environment; and the introduced genes or highly similar genes are common in 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 or animal feed, to minimise dispersal of GM seed or GM pollen from trial sites, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs not required for testing or further planting, and to conduct post-harvest monitoring at trial sites to ensure all GMOs are destroyed.

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Abbreviations

The Act	<i>Gene Technology Act 2000</i>
aad	3''(9)-O-aminoglycoside adenytransferase
Aph4	hygromycin B phosphotransferase
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bt	<i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
Cry	Crystal protein
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FMV	Figwort mosaic virus
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
HGT	Horizontal gene transfer
LGA	Local government area
km	Kilometres
m	Metres
NLRD	Notifiable Low Risk Dealing
nptII	Neomycin phosphotransferase II
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNA	Ribonucleic acid
TGA	Therapeutic Goods Administration
<i>uidA</i>	<i>β-glucuronidase gene</i>
Vip	Vegetative insecticidal protein

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

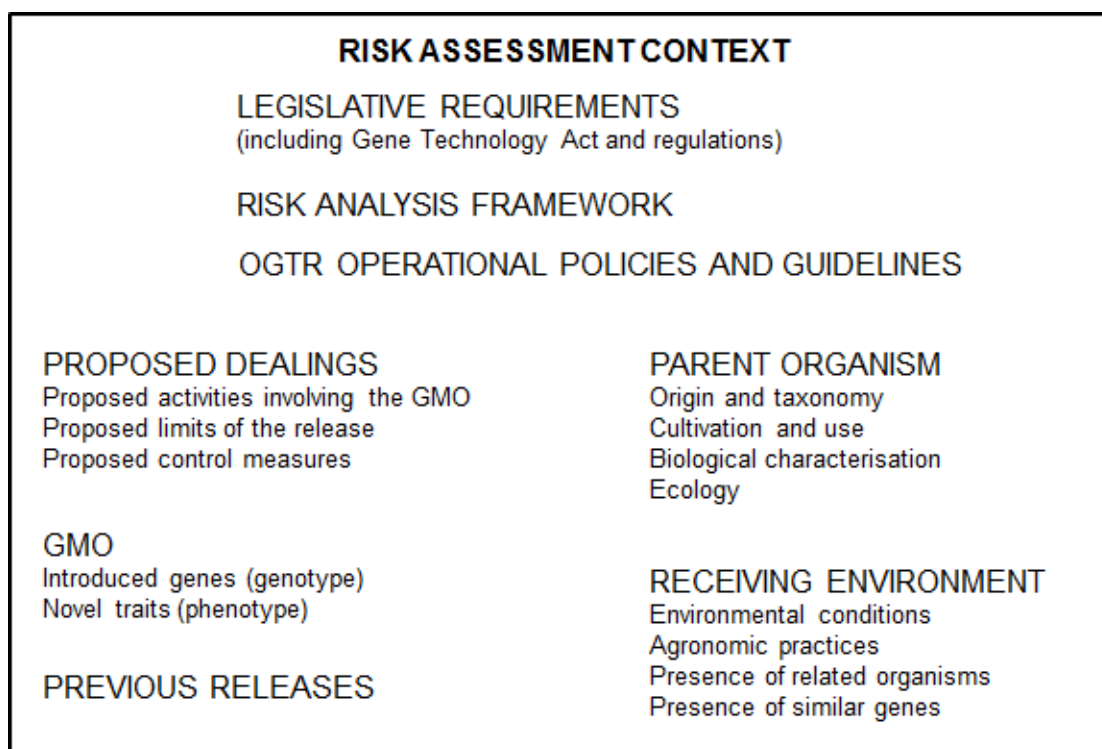


Figure 1 Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.
5. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as

well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. No public submission was received.

7. The *Risk Analysis Framework* (OGTR 2013) explains the Regulator’s approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the [OGTR website](#).

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed dealings

9. Monsanto proposes to release genetically modified (GM) insect resistant cotton MON 88702 and crosses with other previously released GM insect resistant and herbicide tolerant cotton into the environment under limited and controlled conditions.

10. The purpose of the release is to evaluate the agronomic performance, breeding and variety development of the GM cotton under Australian field conditions.

11. The dealings involved in the proposed intentional release include:

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- using the GMOs in the course of manufacture of a thing that is not a GMO
- growing the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs
- possession, supply or use of the GMOs for any of the purposes above.

12. These dealings are detailed further below.

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

13. The applicant proposes to conduct the field trials in cotton growing areas of Australia from March 2017 to July 2021.

14. The proposal is to plant up to 50 sites per year with a maximum combined area of 50 ha in 2017, 100 ha in 2018, and 250² ha per year in 2019 and 2020. The maximum planting size of individual trial sites is proposed to be 2 ha in 2017, 10 ha in 2018, and 50 ha per year in 2019 and 2020.

15. Sites for the proposed trial may be located in any of the local government areas listed in Table 1 in the states of New South Wales (NSW), Queensland (Qld), Victoria (Vic), Western Australia (WA) and Northern Territory (NT).

Table 1. Local government areas proposed for field trials of the GMOs

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

16. The field trials will be carried out and/or overseen by suitably qualified and experienced staff.

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

17. 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:

- locating the proposed trial sites at least 50 metres away from the nearest natural waterway
- separating the GMOs from other cotton crops by a 20 m wide pollen trap of other cotton potentially including commercially authorised GM cotton, or an exclusion zone of 1.5 km around the trial
- inspecting and cleaning all planting and harvest equipment used at the trial site before using for other purposes
- cleaning the trial site after harvest
- post-harvest monitoring of the trial site for at least 12 months and destroying any volunteer cotton until the site has been free of volunteers for six months
- destroying all plant material from the trial not required for analysis or further experimentation
- transporting and storing the GMOs in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- not allowing plant material from the GMOs to be used in human food or animal feed.

² The maximum area proposed in the original application was 500 ha per year in 2019 and 2020. The applicant later amended it to 250 ha.

Section 4 The parent organism

18. The parent organism is upland cotton (*Gossypium hirsutum* L.), the most commonly cultivated cotton species worldwide. Cotton is exotic to Australia and is grown as an agricultural crop in NSW and Qld, with trial or small-scale cultivation in Vic, northern WA and the NT.

19. Cotton is grown as a source of textile and industrial fibre, cottonseed oil and linters for food use, and cottonseed meal for animal feed. A brief description of relevant biological information about the parent organism is provided in this section. More detailed information is contained in a reference document, *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR 2016), which was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the [OGTR website](#) or on request from the OGTR.

20. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (Figure 1, OGTR 2013). Non-GM cotton is the standard baseline for biological comparison, while noting that over 99.5% of currently grown cotton is genetically modified, 95% of those varieties contain stacked traits for insect resistance and herbicide tolerance (Cotton Australia 2015). In addition to non-GM cotton, information on GM cottons will be used as baselines where relevant.

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

5.1 Introduction to the GM cottons proposed for release

21. The GMOs proposed for release are the insect resistant GM cotton MON 88702, and MON88702 conventionally crossed with one or more of the following:

- insect resistant MON 15985 (Bollgard® II, referred to as BGII) cotton
- insect resistant COT102 cotton
- herbicide tolerant MON 88913 (Roundup Ready Flex®, referred to as RRF) cotton
- herbicide tolerant MON 88701 cotton.

Descriptions of BGII, COT102, RRF and MON88701, including detailed description of the genetic modifications can be found in the RARMPs prepared for those releases (Table 2) and will only be briefly summarised here. To date, the Regulator has not received reports of adverse effects on human health, animal health or the environment caused by these authorised releases. Information on previous international approvals for BGII, COT102, RRF and MON88701 GM cotton is available in the RARMP for DIR 145, available from the [GMO Record](#) on the OGTR website.

Table 2. Summary of Australian approvals for BGII, COT102, RRF and MON88701

GM cotton	DIR licence number	Approval type [†]	Comment
BGII	DIR 012/2002; DIR 059/2005; DIR 066/2006; DIR 124/2014	C	Approved individually and in combination with a herbicide tolerance trait.
COT 102 (VIP3A)	DIR 017/2002; DIR 025/2002; DIR 034/2003; DIR 036/2003; DIR 058/2005; DIR 065/2006; DIR 073/2007; DIR 101; DIR 124/2014	C; L&C	Approved individually or in combination with BGII or BGII x RRF; Bollgard III [®] or Bollgard III [®] x RRF.
RRF	DIR 059/2005; DIR 066/2006; DIR 124/2014	C	Approved individually and in combination with insect resistance traits.
MON 88701*	DIR 120; DIR 145	L&C; C	Approved individually and in combination with insect resistance and herbicide tolerance traits.

[†] C: Commercial release; L&C: Limited and Controlled release

22. Table 3 lists the GM cottons proposed for release and the traits and genes present in each. More details on the genes and their functions are provided in section 5.2.

Table 3 GMOs proposed for release – combination of genes

GMO	Gene combination	MON 88702	BGII	COT102	RRF	MON88701
		<i>mCry51Aa2</i>	<i>cry1Ac & cry2Ab</i>	<i>vip3Aa19</i>	<i>cp4 epsps</i>	<i>bar & dmo</i>
1	1 IR gene					
2	2 IR genes					
3	3 IR genes					
4	4 IR genes					
5	1IR & 1 HT gene					
6	1 IR & 2 HT genes					
7	1 IR & 3 HT genes					
8	2 IR & 1 HT genes					
9	2 IR & 2 HT genes					
10	2 IR & 3 HT					
11	3 IR & 1 HT genes					
12	3 IR & 2 HT genes					
13	3 IR & 3 HT genes					
14	4 IR & 1 HT genes					
15	4 IR & 2 HT genes					
16	4 IR & 3 HT genes					

IR = insect resistance gene/s; HT = herbicide tolerance gene

23. As the GM cottons BGII, COT102, RRF and MON88701 have been previously evaluated for release, the focus of this evaluation will be MON88702.

5.2 The introduced genes, encoded proteins and their associated effects

24. Table 4 lists each introduced gene, its source organism and its function in MON88702, BGII, COT102, RRF and MON88701.

Table 4. Genes present in the GM cottons proposed for release

GM event	Gene	Source	Function
MON 88702	<i>mCry51Aa2</i>	<i>Bacillus thuringiensis</i>	hemipteran and thysanopteran insect resistance
BGII	<i>cry1Ac</i>	<i>B. thuringiensis</i>	lepidopteran insect resistance
	<i>nptII</i>	<i>Escherichia coli</i>	selectable marker – antibiotic resistance
	<i>aad</i>	<i>E. coli</i>	selectable marker – antibiotic resistance
	<i>cry2Ab</i>	<i>B. thuringiensis</i>	lepidopteran insect resistance
	<i>uidA</i>	<i>E. coli</i>	selectable marker – reporter
COT102	<i>vip3Aa19</i>	<i>B. thuringiensis</i>	lepidopteran insect resistance
	<i>aph4</i>	<i>E. coli</i>	selectable marker – antibiotic resistance
RRF	<i>cp4 epsps</i>	<i>Agrobacterium sp.</i> strain CP4	glyphosate herbicide tolerance
	<i>cp4 epsps</i>	<i>Agrobacterium sp.</i> strain CP4	glyphosate herbicide tolerance
MON 88701	<i>dmo</i>	<i>Stenotrophomonas maltophilia</i>	dicamba herbicide tolerance
	<i>bar</i>	<i>Streptomyces hygroscopicus</i>	glufosinate herbicide tolerance

Introduced insect resistance genes

25. MON 88702 contains a modified *Cry51Aa2* (*mCry51Aa2*) gene. The gene was originally derived from *Bacillus thuringiensis* (Bt), a gram positive bacterium commonly present in soil. Bt produces a range of insecticidal proteins, including the crystal (Cry) proteins which are also known as delta-endotoxins. Cry proteins are expressed by Bt during sporulation as inactive crystalline protoxins. They become activated when the crystalline inclusions are ingested and cleaved by proteases in the insect midgut. Like other Cry proteins, *mCry51Aa2* was shown to be produced as a protoxin and has the same mode of action (Jerga et al. 2016).

26. In susceptible species, the activated toxins bind to specific receptors on the brush border membrane of the midgut epithelium, leading to formation of membrane pores (Bravo et al. 2007; Yu et al. 1997). Formation of the pores eventually leads to cell lysis, and impairs insect digestive process which is thought to be responsible for insect death (OECD 2007; Schnepf et al. 1998; Soberón et al. 2009).

27. Non-target organisms such as birds and mammals do not have specific receptors for Cry proteins and hence are not adversely affected (OECD 2007; Schnepf et al. 1998). The Cry proteins therefore undergo degradation by proteases.

28. In MON88702 cotton, the modified *mCry51Aa2* produces a Cry protein that aims to provide resistance against targeted hemipteran and thysanopteran insect pests. 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%.

29. BGII contains the *cry1Ac* and *cry2Ab* genes derived from Bt. The genes encode insecticidal proteins that are specifically toxic to the larvae of certain lepidopteran insect species, including significant pests of cotton.

30. COT102 contains the *vip3Aa19* gene, which is also derived from Bt. It encodes the insecticidal protein VIP3Aa19, which is toxic to the larvae of certain lepidopteran insect species.

Introduced herbicide tolerance genes

31. RRF contains two copies of the *cp4 epsps* gene, derived from the *Agrobacterium* strain CP4, a common soil-borne bacterium (Padgett et al. 1996). The gene encodes a glyphosate tolerant EPSP synthase enzyme and has been extensively used to develop GM plants with glyphosate tolerance (Dill 2005).

32. MON 88701 contains a *dmo* gene and a *bar* gene, which confer tolerance to herbicides containing dicamba (2- methoxy-3, 6-dichlorobenzoic acid) and glufosinate, respectively. The *dmo*

gene was derived from the aerobic, environmentally ubiquitous gram negative bacterium *Stenotrophomonas maltophilia* strain DI-6 (Herman et al. 2005). The mature protein is a dicamba mono-oxygenase that rapidly demethylates dicamba to the inactive metabolite 3,6-dichlorosalicylic acid (DCSA) and formaldehyde.

33. The *bar* gene is isolated from *Streptomyces hygroscopicus* (Thompson et al. 1987), a common saprophytic, soil-borne microorganism that is not considered to be a pathogen of plants, humans, or other animals (OECD 1999). The *bar* gene encodes a phosphinothricin N-acetyl transferase (PAT) protein that confers tolerance to glufosinate (Thompson et al. 1987), the active component in a number of herbicides.

34. The herbicide tolerance genes have been described in scientific literature and extensively assessed in detail in a number of RARMPs and by regulatory authorities worldwide (CERA 2013). These assessments conclude that there is no toxicity or allergenicity associated with the proteins or the metabolites of the herbicides.

Introduced selectable marker genes

35. GMOs from crosses including BGII and COT102 will contain selectable marker genes in addition to genes conferring insect resistance (see Table 4). These selectable marker genes were originally isolated from the common gut bacterium *E. coli*. The *nptII* gene confers resistance to antibiotics such as kanamycin and geneticin, and the *aph4* gene confers resistance to the antibiotic hygromycin. The *aad* gene, which confers resistance to the antibiotics spectinomycin and streptomycin, is linked to a bacterial promoter that does not function in plants so the gene is not expected to be expressed in the GM cotton plants. The beta-glucuronidase (*uidA*) gene from *E. coli* encodes an enzyme enabling visual identification of plant tissues in which this gene is being expressed. These genes were used only as selective markers during early stages of development of the GM plants in the laboratory. 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.

5.3 Introduced regulatory elements

36. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants are mRNA terminators, including a poly-adenylation signal. Other regulatory sequences, such as enhancers, may contribute to the expression pattern of a given gene.

37. Some GM cottons proposed for release will contain short regulatory elements. These sequences are derived from plants (including thale cress, pea, petunia, pima cotton and soybean), a soil bacterium (*A. tumefaciens*) and plant viruses (Cauliflower mosaic virus, Figwort mosaic virus, Peanut chlorotic streak caulimovirus and Tobacco etch virus). The GM cottons proposed for release will contain some or all of these regulatory elements.

38. BGII, COT102, RRF and MON88701 containing these regulatory elements have been assessed as not presenting greater risk than cotton containing endogenous regulatory elements.

5.4 Method of genetic modification

39. MON 88702 was developed using *Agrobacterium*-mediated plant transformation. This method is widely used in Australia and overseas for introducing new genes into plants. Further information can be found in the document *Methods of plant genetic modification* available from the [Risk Assessment References](#) page of the OGTR website. Absence of *Agrobacterium* in MON 88702 was confirmed through PCR analysis.

40. The other GM cottons will be produced by conventional crossing of MON88702 with BGII, COT102, RRF and/or MON88701.

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

Introduced insect resistance proteins

41. The introduced VIP3Aa19 and Cry proteins for insect resistance are derived from Bt. Bt is naturally found worldwide in soil, on plant surfaces and in animals, and microbial preparations of Bt have been used as a commercial pesticide for over 60 years (OECD 2007). Thus, people and other organisms have a long history of safe exposure to Bt insecticidal proteins.

42. The potential for the introduced VIP3Aa19, Cry1Ac and Cry2Ab proteins to cause toxic or allergic reactions in humans or other organisms has been assessed previously (DIR 101 and 124).

43. Wild-type Cry51Aa2 has been shown to have insecticidal activity against the insect Western tarnished plant bug (*Lygus hesperus*; order: Hemiptera, family: Miridae) (Baum et al. 2012). mCry51Aa2 has been further modified to specifically target hemipteran and thysanopteran insect pests in cotton and has 96.4% sequence similarity to the wild-type Cry51Aa2.

44. The applicant has conducted diet bioassays to assess insecticidal activity on a diverse range of invertebrate species representing 12 families and seven orders found in the United States of America (US). A total of 17 species were selected, i.e. 3 target and 14 non-target invertebrates comprising both pest and non-pest species based on ecological and economic importance, representation of valued taxa or functional groups, taxonomic relatedness and ability to be reliably tested in a laboratory. The diet incorporation bioassays were designed to provide continuous exposure of mCry51Aa2 at doses well above the maximum predicted environmental concentration to each test species and provide a sufficient duration of exposure to evaluate the potential toxic effects of mCry51Aa2 on growth, development and survival. The US field trial data has been provided for two target pests for which established bioassays are not available.

According to the data provided by the applicant, hemipteran and thysanopteran insect pests were susceptible to the toxic effects of the mCry51Aa2 protein (Table 5). The LC₅₀ values represent the concentration of the mCry51Aa2 protein which kills 50% of the insects tested. The LC₅₀ value for *Lygus hesperus* was 3 ug/ml diet and in field trials, insecticidal activity was reported for *Pseudaatomoscelis seriatus* (Hemiptera) and *Frankliniella spp.* (Thysanoptera). No mCry51Aa2 expression data for MON88702 cotton was available.

45. The protein also showed some toxicity at high concentrations to one beneficial predatory species, insidious flower bug (*Orius insidiosus*; estimated LC₅₀> 400 µg/ml diet) and two coleopteran species, *Leptinotarsa decemlineata* (estimated LC₅₀: 25 - 400 µg/ml diet) and *Diabrotica undecimpunctata howardi* (estimated LC₅₀>200 µg/ml diet). No toxicity was detected for any of the other invertebrates tested.

46. Since Cry proteins are gut toxins, the effect of mCry51Aa2 on growth of the test organisms was tested and EC₅₀ (effective concentration 50%) was derived through measurement of body mass. Biological activity was detected for two coleopteran larvae subjected to an oral diet assay. After a twelve day exposure, a dose dependent decrease in body mass was observed and an effective concentration of 134 µg/ml and 7.82 µg/ml was estimated for *L. decemlineata* and *D. underimpunctata howardi*, respectively.

Table 5. Susceptibility of various invertebrate species to mCry51Aa2 protein

Species	Family	Order	Representative Function	Mean LC ₅₀ value (ug/mL diet)	Maximum concentration tested (ug/mL diet)	Activity
<i>Lygus hesperus</i>	Miridae	Hemiptera	Target pest	3	12	Yes
<i>Pseudaatomoscelis seriatus</i>	Miridae	Hemiptera	Target pest	Plant expression (50% reduction in nymphs)		Yes

Species	Family	Order	Representative Function	Mean LC ₅₀ value (ug/mL diet)	Maximum concentration tested (ug/mL diet)	Activity
				reaching adulthood)		
<i>Frankliniella spp.</i>	Thripidae	Thysanoptera	Target pest	Plant expression (lower damage rating scores)		Yes
<i>Leptinotarsa decemlineata</i>	Chrysomelidae	Coleoptera	Herbivore	Estimated 25-400	400	Yes ¹
<i>Diabrotica virgifera virgifera</i>	Chrysomelidae	Coleoptera	Herbivore	-	1000	No
<i>Diabrotica undecimpunctata howardi</i>	Chrysomelidae	Coleoptera	Herbivore	Estimated >200	200	Yes ²
<i>Epilachna varivestis</i>	Coccinellidae	Coleoptera	Herbivore	-	400	No
<i>Spodoptera frugiperda</i>	Noctuidae	Lepidoptera	Herbivore	-	400	No
<i>Helicoverpa zea</i>	Noctuidae	Lepidoptera	Herbivore	-	400	No
<i>Ostrinia nubilalis</i>	Crambidae	Lepidoptera	Herbivore	-	400	No
<i>Plutella xylostella</i>	Plutellidae	Lepidoptera	Herbivore	-	400	No
<i>Orius insidiosus</i>	Anthocoridae	Hemiptera	Predator	Estimated >400		Yes ³
<i>Coleomegilla maculata</i>	Coccinellidae	Coleoptera	Predator	-	400	No
<i>Apis mellifera</i>	Apidae	Hymenoptera	Pollinator	-	2000	No
<i>Pediobius foveolatus</i>	Eulophidae	Hymenoptera	Parasitoid	-	400	No
<i>Folsomia candida</i>	Isotomidae	Collembola	Decomposer	-	400	No
<i>Eisenia andrei</i>	Lumbricidae	Haplotaxida	Decomposer	-	400	No

¹ The corrected survival response was near 50% in treatment concentrations from 50 to 400 µg mCry51Aa2/mL diet treatment for *L. decemlineata*.

² The corrected survival response was 64% in the 200µg mCry51Aa2/mL diet treatment which was the highest concentration tested for *D. undecimpunctata howardi*.

³ The survival response was 67% in the 400µg mCry51Aa2/mL diet treatment which was the highest concentration tested for *O. insidiosus*.

Introduced herbicide tolerance and selectable marker proteins

47. Data relating to the potential for causing toxic or allergic reactions of the introduced CP4 EPSPS, PAT, DMO and selectable marker proteins as well as their metabolic products to humans or other organisms has been detailed previously (see Section 5.1 for details of RARMPs).

5.6 Characterisation of the GM cottons

5.6.1 Phenotypic characterisation

48. MON88702 GM cotton plants grown in the glasshouse have shown normal cotton phenotype. The genetic modification provides insect resistance to some cotton pests and is not known to affect other metabolic pathways within the cotton plant. Further phenotypic data would be collected during the proposed field trials.

49. Crosses of MON88702 with BGII, COT102, RRF and/or MON88701 have not been tested in the field. However, BGII, COT102, RRF and MON88701 GM cottons have been previously assessed and the Regulator has not received reports of the GM cottons having unexpected or harmful phenotypes when planted in the field, noting that both BGII and COT102 GM cottons are toxic to certain insect pests. As MON88702 will be conventionally bred with BGII, COT102, RRF, MON88701, changes in phenotype compared to the parents are not expected to occur.

Section 6 The receiving environment

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

51. Important 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 2016). Discussions in the document include

- relevant abiotic factors
- relevant biotic factors
- relevant agronomic practices in commercial cotton cultivation and
- presence of sexually compatible plants.

52. Factors important for the proposed release of each of these subject areas are highlighted in the sections below.

6.1 Relevant abiotic factors

53. Factors restricting where cotton can be grown in Australia are water availability (through rainfall or irrigation), soil suitability and, most importantly, 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.

54. The local government areas where the release is proposed to take place (see Table 1, Section 3.1) have a broad range of climate types, as defined by the Köppen Classification system used by the Australian Bureau of Meteorology. Proposed planting areas include subtropical, grassland and temperate climate types, with areas of tropical climate in northern Qld and northern WA. The proposed cotton growing areas in WA and coastal Qld may experience the effects of tropical cyclones between November and April. There is a possibility of extreme weather events and flooding moving into inland Qld and northern NSW (Bureau of Meteorology). The applicant has stated that they will select trial sites that are not prone to flooding.

6.2 Relevant biotic factors

55. Cotton is susceptible to competition from weeds. The most important pests of cotton in southern Australia are the caterpillars of *Helicoverpa armigera* and *Helicoverpa punctigera* and the spider mite *Tetranychus urticae*; other serious pests include cotton aphid (*Aphis gossypii*), green mirid (*Creontiades dilutus*), silverleaf whitefly (*Bemisia tabaci* b-biotype), thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*) and the green vegetable bug (*Nezara viridula*) (Farrell & Johnson 2005). A number of fungal pathogens are important diseases of cotton in Australia. These issues are managed by various strategies in commercial cotton cultivation.

Several other aphids and bugs (order Hemiptera) and thrips (order Thysanoptera) have been listed as pest species in cotton in Australia (Williams et al. 2011). These are sap sucking insects which are not easily controlled by current GM insect resistant cottons. Currently, a range of narrow to broad spectrum insecticides is applied at various stages of plant growth to control these pests (CRDC &

CottonInfo 2016). Several insect species including beetles belonging to Coleoptera are listed as beneficials in cotton growing areas in Australia (CRDC & CottonInfo 2016; Williams et al. 2011).

56. A variety of invertebrates, vertebrates and microorganisms are present at each proposed trial site and are expected to be exposed to the introduced genes, their encoded proteins and end products.

6.3 Relevant agricultural practices

57. The limits and controls of the proposed release are outlined in Sections 3.1 and 3.2 of this Chapter. The GM cotton would be grown at field trial sites, either as an irrigated or dryland crop. Seed may be planted in a variety of configurations. Small areas may be hand-planted or planted with a small plot cone-seeder. Larger areas would be planted with commercial equipment. The crops would be maintained in a similar fashion to commercial cotton crops. Some GM plants would be treated differently with respect to weed management within the crop (as some are glyphosate tolerant) and pesticide application (as some are not expected to need as many pesticide applications as non-GM cotton). All application of chemicals would occur in accordance with APVMA requirements. Harvesting of cotton bolls would occur either by hand (for small plantings) or with commercial equipment.

58. The GM cotton would be allowed to set seed at the field trial sites. Harvested seed may be used to plant further trials as authorised, for laboratory experimentation in Australia or overseas, or for seed production. Any seed harvested from the field trials, which is not kept for evaluation or future planting, would be destroyed.

59. After harvest, sites would be replanted to the GM cottons proposed for release or planted to an approved post-harvest crop. The crop would be chosen from the approved post-harvest crops list on the [OGTR website](#).

6.4 Presence of sexually compatible plants in the receiving environment

60. Cotton is proposed for release in all cotton growing areas in Australia where it is grown as a large-scale commercial crop as well as some areas where cotton trials are conducted. Over 99.5% of currently grown cotton is genetically modified, 95% of those varieties contain stacked traits for insect resistance and herbicide tolerance (Cotton Australia 2015). Commercial cotton grown in Australia is either *Gossypium hirsutum* or *Gossypium barbadense*, with 99% of cotton planted in 2006 being *G. hirsutum* (OGTR 2016). The GM *G. hirsutum* proposed for release is capable of crossing with both species of commercially grown cotton.

61. Ephemeral 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 (Addison et al. 2007; Eastick & Hearnden 2006).

62. There are 17 native species of *Gossypium* in Australia, most of which can be found in the NT and the north of WA (OGTR 2016). Generally, they are found in native vegetation and not in disturbed/modified habitats such as agricultural areas (Groves et al. 2002). Well established genetic incompatibility prevents crossing of native cotton species with cultivated cotton in the natural environment (OGTR 2016).

6.5 Presence of similar genes and encoded proteins in the environment

63. The introduced *cry* and *vip* genes were originally isolated from Bt which occurs naturally in Australia. Also, microbial Bt sprays are used as insecticide sprays in Australia, particularly in organic agriculture and domestic gardening ([Australian Pesticides and Veterinary Medicine Authority](#)). Therefore, these genes and their encoded proteins are widespread in the Australian environment.

64. The introduced genes for herbicide tolerance are derived from common soil-borne microorganisms. The regulatory sequences (promoters, terminators, leader sequences) are derived from plants (cotton, soybean, pea, thale cress, petunia), plant viruses (peanut chlorotic streak caulimovirus, tobacco etch virus, cauliflower mosaic virus, figwort mosaic virus) and a common soil

bacterium (*Agrobacterium tumefaciens*). All the source organisms for the introduced genetic elements are widespread and prevalent in the environment and thus humans and other organisms would commonly encounter their genes and encoded proteins.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

Approvals by the Regulator

65. Neither MON 88702 nor crosses of MON88702 with BGII, COT102, RRF and/or MON88701 have been approved previously in Australia.

66. However, GM cottons BGII, COT102 RRF and MON88701, individually and in combination, have been approved by the Regulator for release in Australia (see Table 2).

67. Information on previous DIR licences for GM cottons is available from the [GMO Record](#) on the OGTR website. The Regulator has previously approved 36 field trials and 11 commercial releases of GM cotton. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.

Approvals by other government agencies

68. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Department of Agriculture and Water Resources, Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

69. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has not assessed MON88702 GM cotton with regard to its use in food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from BGII, VIP3A, RRF and MON 88701 GM cottons (under applications A436, A509, A553 and A1080, respectively; assessments are available from the [FSANZ website](#)). The applicant does not intend to use materials from the GM cottons generated in the proposed release in human food.

70. 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 intends to apply herbicide to the GM cottons during the trial, which is also subject to regulation by the APVMA.

71. GM cotton seed would be imported into Australia from both North and South America by Monsanto at various times. An import permit for MON 88702 has been granted by the Department of Agriculture and Water Resources for growing under quarantine conditions.

7.2 International approvals

72. MON 88702 has been approved for small scale, contained field trials in the United States since 2011. The applicant has applied to the US EPA for an Experimental Use Permit which would allow planting of MON88702 on more than 10 acres (approximately 4 ha).

Chapter 2 Risk assessment

Section 1 Introduction

73. 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 context established for the risk assessment (see chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

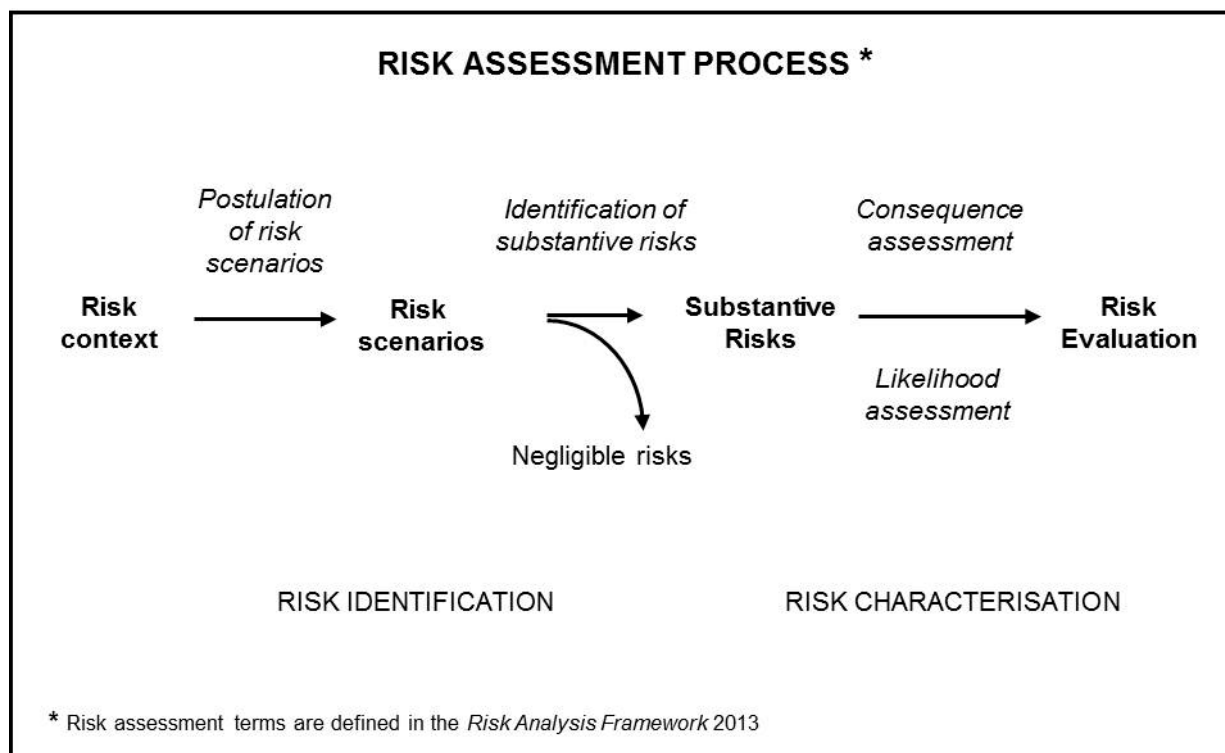


Figure 2 The risk assessment process

74. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

75. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

76. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al. 2014). In addition, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

77. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood

assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

Section 2 Risk Identification

78. Postulated risk scenarios are comprised of three components:

- i. The source of potential harm (risk source).
- ii. A plausible causal linkage to potential harm (causal pathway).
- iii. Potential harm to an object of value, people or the environment.

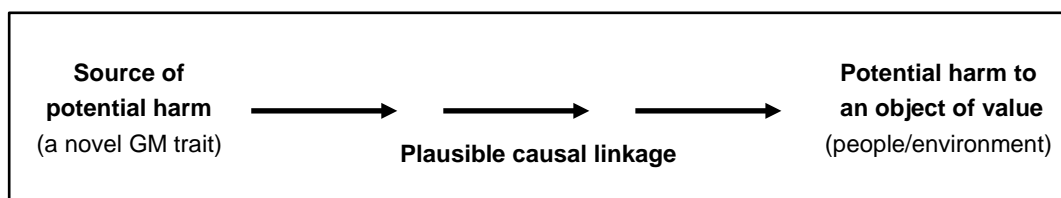


Figure 3 Risk scenario

79. In addition, the following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO and
- characteristics of the parent organism(s).

2.1 Risk source

80. 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.

81. The GM cottons proposed for release will be modified by the introduction of up to four genes for insect resistance. *Cry1Ac*, *cry2Ab* and *vip3A*; present in BGII and COT102 have been evaluated both individually and in combination in previous assessments in Australia and overseas (see Chapter 1, Section 5.1 for details). Therefore, these will not be assessed here. However, the *mCry51Aa2* gene and its encoded protein in MON88702 will be considered further as potential source of risk as will be its potential to interact with the other three insecticidal proteins in BGII and COT102.

82. Some GM cottons proposed for release will be modified by the introduction of up to three genes conferring herbicide tolerance. These genes have been evaluated both individually and in combination in previous assessments in Australia and overseas (see Chapter 1, Section 5.1 for details). None of the herbicide tolerances is known to interact with the biochemical pathways involved in the insect resistance in BGII and COT102 or could reasonably be expected to interact with those in MON88702. Therefore, the introduced herbicide tolerance genes will not be assessed here.

83. In addition, some of the GM cottons may contain the *nptII*, *aad*, and/or *aph4* antibiotic resistance selectable marker genes. Some GM cottons may contain the *uidA* reporter gene. These genes and their products have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas (see Chapter 1, Section 5.1 for details). As these genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

84. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from plants, bacteria and plant viruses. Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology 2003). As described in Chapter 1, these sequences have been widely used in other GMOs, including BGII, COT102, RRF and MON88701. These have been trialled or grown in Australia or overseas without credible reports of adverse effects. Hence, risks from these regulatory sequences will not be further assessed for this application.

85. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the 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, the range of unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. These types of effects also occur spontaneously and in plants generated by conventional breeding (Bradford et al. 2005; Ladics et al. 2015; Schnell et al. 2015). In general, the crossing of plants, each of which will possess a range of innate traits, does not lead to the generation of progeny that have health or environmental effects significantly different from the parents (Steiner et al. 2013; Weber et al. 2012). Therefore, although unintended effects resulting from the introduced genes will be considered further, unintended effects resulting from the process of genetic modification will not be considered further in this application.

2.2 Causal pathway

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

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

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

88. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a

licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

89. Potential harms from GM plants include:

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

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

2.4 Postulated risk scenarios

91. Three risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 6 and more detail of these scenarios is provided later in this Section. Postulation of risk scenarios considers impacts of the GM cotton or its products on people undertaking the dealings, as well as impacts on people and the environment if the GM plants or genetic material were to spread and/or persist.

92. 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 6. Summary of risk scenarios from the proposed dealings with the GM cottons

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
1	GM cotton expressing the introduced insect resistance gene or genes	Cultivation of GMOs at trial sites ↓ Exposure of people or other organisms at the trial site to the introduced proteins	Toxicity or allergenicity in people or toxicity to desirable organisms	No	<ul style="list-style-type: none"> • GM cottons would not be used in human food or animal feed. • The mode of action of Cry proteins is well understood and is not known to influence the levels of natural cotton toxicants. • Bt products are not known to be harmful to people or other vertebrates. • The mCry51Aa2 protein has been demonstrated to be toxic to certain hemipteran, thysanopteran and coleopteran insects, whereas invertebrates outside these orders were not adversely affected. • mCry51Aa2 alone or in combination with Cry1Ac, Cry2B and VIP3 is not expected to have a greater insecticidal spectrum than insecticides used in cotton or Bt sprays. • The limited scale and short duration of the trial minimise exposure to the GM plant material.
2	GM cotton expressing introduced insect resistance gene or genes	Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM plants	<ul style="list-style-type: none"> • Toxicity or allergenicity in people or toxicity to desirable organisms • Reduced establishment or yield of desirable plants 	No	<ul style="list-style-type: none"> • The proposed controls would restrict the potential for spread and persistence of the GM cottons. • The introduced insect resistance genes are not expected to change susceptibility of the GM cottons to the factors which limit cotton in Australia.
3	GM cotton expressing introduced insect resistance gene or genes	Pollen from GM plants fertilise sexually compatible plants ↓ GM hybrid seed germinates ↓ GM hybrids spread and persist	<ul style="list-style-type: none"> • Toxicity or allergenicity in people or toxicity to desirable organisms • Reduced establishment or yield of desirable plants 	No	<ul style="list-style-type: none"> • Cotton is predominantly self-pollinating and has limited ability to outcross. • The proposed limits and controls would restrict the potential for gene flow.

2.4.1 Risk scenario 1

<i>Risk source</i>	GM cotton expressing introduced insect resistance gene or genes
<i>Causal pathway</i>	↓ Cultivation of GMOs at trial sites ↓ Exposure of people or other organisms at the trial sites to the introduced proteins ↓
<i>Potential harm</i>	Toxicity or allergenicity in people or toxicity to desirable organisms

Risk source

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

Causal pathway

94. 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.

95. 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 2016), and people who enter cotton gins typically wear protective face masks ([International Fibre Centre website](#)).

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

97. There is little potential for human ingestion of the introduced proteins, as the applicant proposes that no GM plant material would be used as food. The applicant proposes to sell lint (long cotton fibres) from the GM cottons. Cotton lint does not enter human food. Processed cotton lint contains over 99% cellulose and does not contain detectable protein or DNA (OGTR 2016). Therefore, people wearing cotton clothing or using other products made from GM cotton would not be exposed to the introduced proteins.

98. Non-human 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 2016). A range of invertebrates would be expected to ingest GM cotton plant material during the release. 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

99. 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).

100. Non-GM cotton produces natural toxins for defence against herbivory including gossypol and cycloprenoid fatty acids (OGTR 2016). The introduced mCry51Aa2 protein in MON88702 GM cotton belongs to a protein family with a well understood mode of action that would not alter cotton metabolic pathways. Therefore, the GM cottons are not expected to have increased levels of natural toxins.

101. The introduced insect resistance genes were isolated from Bt, 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 available information, including a long history of safe use of Bt sprays and GM cottons containing Bt proteins, suggests that neither the introduced mCry51Aa2 protein alone nor when combined with on Cry1Ac, Cry2B and/or VIP3A would be toxic or allergenic to people and other vertebrates.

102. An oral toxicity study and a field trial were conducted by the applicant to test the effect of mCry51Aa2 protein on undesirable insects, i.e. target organisms and other pests, and on desirable invertebrates, i.e. neutral and beneficial invertebrates. The Cry51Aa2 protein is intended to target cotton pests in the insect orders Hemiptera and Thysanoptera. The experimental findings confirm insecticidal activity of the protein on hemipteran and thysanopteran cotton pests (Table 5). The mCry51Aa2 protein also showed some toxicity to two coleopteran insect pests of other crops, the Colorado potato beetle (*Leptinotarsa decemlineata*) and Southern corn rootworm (*Diabrotica undecimpunctata howardi*). The native Cry51Aa2 protein has also shown activity against a coleopteran insect (Baum et al. 2012).

103. The introduced mCry51Aa2 protein also showed some toxicity against a beneficial hemipteran insect, *Orius insidiosus* which feeds on herbivorous insect pests found in the US. The protein was not toxic to other representative desirable invertebrates, including honeybees, earthworms, predatory wasp and ladybird, at doses well above the maximum predicted environmental concentration. However, some uncertainty remains over the insecticidal spectrum of the mCry51Aa2 protein.

104. When mCry51Aa2 is combined with other GM insect resistant cottons creating a plant containing up to four insect resistance proteins, it is possible that additive or synergistic effects could occur, potentially increasing the range of sensitive insects. Some uncertainty exists in this area due to data gaps, mainly in relation to toxicity of mCry51Aa2 to non-target organisms in cotton growing areas in Australia. However, hemipteran and thysanopteran insect pests in commercial cotton are currently controlled by the application of insecticides (CRDC & CottonInfo 2016). It is not expected that the insecticidal activity of mCry51Aa2 alone or in combination with the other three insect resistance proteins would be greater than that of these insecticides. Also, it is unlikely that the combination of insect resistance proteins in the GM cottons could be toxic to a wider range of insects than microbial Bt insecticidal products, which contain a combination of insect resistance proteins.

105. **Conclusion:** Risk scenario 1 is not identified as a substantive risk because the GM cottons will not be used in human food or animal feed; mCry51Aa2 is expected to be toxic to a limited range of insect species; the native Cry51Aa2 protein is already present and widespread in the Australian environment (both individually and in combination with other Bt proteins); and the limited scale, short duration and the proposed controls of the trial restrict exposure of people and other organisms to the GM cottons. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

<i>Risk source</i>	GM cotton expressing introduced insect resistance gene or genes
<i>Causal pathway</i>	↓ Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM cottons ↓
<i>Potential harms</i>	Toxicity or allergenicity in people or toxicity to desirable organisms or Reduced establishment or yield of desirable plants

Risk source

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

Causal Pathway

107. 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.

108. 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, cultivating the site after harvest to encourage germination of remaining seed, 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 gene for insect resistance would increase the ability of the GMOs to survive these standard control measures.

109. 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 and are not prone to flooding. Well managed sites would be selected that may have proximity to irrigation channels or holding ponds that do not flow into natural waterways.

110. 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 2016). 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.

111. Wild mammals and birds generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content (OGTR 2016). Therefore, wild animals are unlikely to disperse GM cotton seeds from the trial site. GM cotton seeds would not be used as stock feed, so would not be dispersed by stock.

112. Dispersal of cotton seeds by authorised people entering the trial site would be minimised by cleaning all equipment, including clothing, used with the GM cotton before using it for any other purpose. GM seed cotton would be ginned separately from any other cotton crop in an approved facility to avoid accidentally mixing GM cotton seed with other cotton seed, then dispersing the mixed cotton seed. 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*. Only plant material needed for experimentation would be transported between PC2 or accredited facilities and field trial sites.

113. 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 2016). 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 climactic factors limiting cotton persistence indicate that cotton has naturalisation potential only in the coastal regions of north-east Australia (Rogers et al. 2007). A few small populations of naturalised cotton are reported in northern Australia, but these are not derived from modern cultivars (OGTR 2016), and these tufted cottons may have a greater ability to survive outside agricultural settings than modern cotton cultivars.

114. It is not expected that the expression of the introduced insect resistance genes would allow cotton to overcome the biotic and abiotic factors that limit spread and persistence. Expression of the introduced genes could reduce herbivory of the GM cottons by insects which are susceptible to the insecticidal proteins (see Risk Scenario 1). There is no evidence to suggest that the genetic modifications have altered seed production characteristics or tolerance to abiotic or biotic stresses other than insect herbivory that could enhance the potential for dispersal or persistence of the GM cottons proposed for release.

115. In addition, a number of controls are proposed to physically separate the trial sites from commercial cotton. These controls have effectively restricted the spread of GM cotton seed in previous trials.

Potential harms

116. The potential harms from this risk scenario are toxicity or allergenicity in people or toxicity to desirable organisms, or reduced establishment or yield of desirable plants.

117. 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.

118. Risk Scenario 1 considered the potential for the introduced genes and proteins to lead to toxicity or allergenicity, and did not identify any substantive risks.

119. 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 be controlled by similar weed management measures as volunteers from commercial cotton, such as application of alternative herbicides or mechanical cultivation.

120. 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 its ability to spread and persist.

121. 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.

122. **Conclusion:** Risk scenario 2 is not identified as a substantive risk because the proposed controls would restrict the spread and persistence of the GM cottons; cotton has limited ability to survive outside agricultural settings and the genetic modifications are not expected to change this; and the GM cottons are susceptible to standard weed control measures used on commercial cotton volunteers. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

<i>Risk source</i>	GM cotton expressing introduced insect resistance gene(s)
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Pollen from GM cottons fertilising sexually compatible plants</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">GM hybrid seed germinates</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">GM hybrids spread and persist</p> <p style="text-align: center;">↓</p>
<i>Potential harms</i>	<p style="text-align: center;">Toxicity or allergenicity in people or toxicity to desirable organisms</p> <p style="text-align: center;">or</p> <p style="text-align: center;">Reduced establishment or yield of desirable plants</p>

Risk source

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

Causal pathway

124. 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, chiefly honeybees, but gene flow studies have shown that outcrossing occurs at low levels and decreases rapidly with distance (OGTR 2016). 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 cotton. It is not expected that the introduced insect resistance genes would alter the pollen dispersal characteristics of the GM cottons proposed for release.

125. 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.

126. 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 or GM comparator cotton plants at the trial sites and commercial cotton plants in the pollen trap. 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

127. The potential harms from this risk scenario are toxicity or allergenicity in people or toxicity in desirable organisms, or reduced establishment or yield of desirable plants.

128. 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.

129. 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.

130. **Conclusion:** Risk scenario 3 is not identified as a substantive risk because cotton has limited ability to outcross; the proposed limits and controls would minimise pollen flow to sexually compatible plants; and hybrids between the GM cottons and commercial cotton are not expected to show increased levels of toxicity or weediness. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

131. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis³. Uncertainty in risk assessments arises from sources such as incomplete knowledge and inherent biological variability. Uncertainty is addressed by approaches including balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

132. 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.

133. For DIR 147, uncertainty is noted particularly in relation to:

- Molecular, biochemical and phenotypical characterisation of the GM cotton MON 88702 including potential for increased toxicity, allergenicity and weediness
- Potential toxicity to an increased range of insects of the combination of the insecticidal proteins in the GM cottons and their potential for increased weediness.

134. 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 GM cottons.

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

Section 4 Risk Evaluation

136. 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.

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

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

138. 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 6 and include:

- none of the GM plant material or products will enter human food or animal feed supply chains
- widespread presence of the introduced genes and their encoded proteins in the environment
- the protein encoded by *mCry51Aa2* is expected to be toxic to a limited range of insect species

³ A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the [Risk Assessment References](#) page on the OGTR website or via Free call 1800 181 030.

- limited ability of the GM cottons to establish populations outside cultivation
- limited ability of the GM cottons to transfer the introduced genetic material to other cotton plants
- limits on the size, location and duration of the release proposed by Monsanto
- suitability of controls proposed by Monsanto to restrict the spread and persistence of the GM cottons and their genetic material.

139. 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 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

140. 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.

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

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

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

Section 2 Risk treatment measures for substantive risks

144. 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, the proposed containment measures, and the receiving environment, and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

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

3.1 Licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by Monsanto

146. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by Monsanto in their application. These are taken into account in the three risk scenarios postulated for the proposed DIR in Chapter 2. Many of the proposed control measures are considered standard for GM crop trials and have been imposed by the Regulator in previous DIR licences. The appropriateness of these controls is considered further below.

147. The release will be limited to a maximum of 50 sites per year in cotton growing areas in Australia in the states of NSW, Qld, Vic, WA and NT. The maximum size of individual trial sites will not exceed 2 ha in

2017, 10 ha in 2018 and 50 ha per year in 2019 and 2020 each. The total planting area each year will not exceed a maximum combined area of 50 ha in 2017, 100 ha in 2018, and 250 ha per year in 2019 and 2020 each. The limited size and duration of the trial limits exposure to the GM cottons (Risk Scenarios 1 and 2).

148. The applicant has proposed that only authorised and trained personnel would be permitted to deal with the GMOs. A standard licence condition requires that all persons dealing with the GMOs must be informed of any applicable licence conditions. This measure would limit the potential exposure of humans to the GMOs (Risk Scenarios 1).

149. The applicant has proposed that the trial site will be surrounded by a 20 m wide pollen trap or a 1.5 km exclusion zone to control pollen flow from the GMOs to cotton plants outside the trial site. The plants within the pollen trap would be non-GM cotton or commercially approved GM cotton and would be managed so as to flower at the same time as the GMOs. 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) and the licence permits pollen trap plants to be either non-GM or GM cotton approved for commercial release in Australia.

150. As an alternative to a 20 m pollen trap, a 1.5 km exclusion zone was proposed by the applicant to minimise gene flow from the GM cotton plants to other cotton crops. As discussed in the RARMP for DIR 120, a 1.5 km exclusion zone in combination with a 100 m monitoring zone is considered appropriate. The combination of a monitoring zone and an exclusion zone is considered effective to restrict gene transfer from GM cotton trial sites to other cotton (Risk Scenario 3).

151. The applicant does not propose using any of the GM plant material for human or animal consumption. Therefore, a condition in the licence prohibits material from the trial from being used for human food or animal feed. This control will restrict exposure of humans and other organisms to the GMOs (Risk Scenario 1) and the potential for GM cotton seed to be dispersed outside the trial limits (Risk Scenario 2).

152. 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.

153. The applicant has proposed to clean all equipment used with the GMOs before using the equipment for other purposes. Equipment used on the trial site 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).

154. After the trial site has been harvested, the applicant proposed to destroy all GMOs except for plant material and seed required for testing through destructive analysis and cotton seed required for further authorised planting. 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 2016). The applicant has proposed post-harvest cultivation of the trial sites 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).

155. The applicant has proposed 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). 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).

156. The applicant has proposed to select the trial sites that are a minimum of 50 m from a natural waterway and not prone to flooding. The sites may be in close proximity to irrigation channels or holding ponds that would not flow into natural waterways. The licence includes standard DIR licence conditions that require: that the site be at least 50 m from the nearest natural waterway or man-made waterway that flows into a natural waterway; that any area where the GMOs may have dispersed, including irrigation channels, be monitored after harvest; and immediate notification of any extreme weather conditions affecting the site during the proposed release. These measures will minimise the likelihood for the GM cotton establishing outside the proposed release site, including as a result of extreme weather events (Risk Scenario 2).

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

158. The applicant has proposed that imported GM cotton seed would be transported to either PC2 or accredited facilities or to a field site. Transport may occur between/among PC2 glasshouse/laboratory, 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 Notifiable Low Risk Dealing (NLRD) authorisation in accordance with applicable requirements of the Gene Technology Regulations 2001. This is considered appropriate but is not included in the licence as it would be conducted under a separate valid authorisation.

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

159. 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 March 2017 and July 2021
- limit the field trial to a maximum of 50 sites per year with a maximum combined area of 50 ha in 2017, 100 ha in 2018, and 250 ha per year in 2019 and 2020
- limit the maximum planting size of individual trial sites to 2 ha in 2017, 10 ha in 2018, and 50 ha per year in 2019 and 2020
- locate the trial site at least 50 m away from natural waterways
- restrict gene flow via pollen by using one of the following measures:
 - surround the trial site with a 20 m pollen trap of non-GM cotton or GM cotton approved for commercial release
 - surround the planting area with a 100 m monitoring zone and 1.5 km exclusion 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 cotton in the planting area or pollen trap plants 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 crop

- 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

160. 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

161. 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.

162. The conditions include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

3.2.2 Contingency plan

164. Monsanto 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.

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

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

166. 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, Monsanto is required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

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

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

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

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

3.2.5 Monitoring for compliance

169. 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.

170. 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.

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

Section 4 Issues to be addressed for future releases

172. 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 cotton MON 88702 including potential for increased toxicity especially to Australian non-target insects, allergenicity and weediness
- Additional data on the potential toxicity to an increased range of relevant insects of the combination of the insecticidal proteins in the GM cottons and their potential for increased weediness.

Section 5 Conclusions of the RARMP

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

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

References

- Addison, S.J., Farrell, T., Roberts, G.N., 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., de Heer, C. (2006) Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical Reviews in Toxicology* **36**: 219-251.
- Baum, J.A., Sukuru, U.R., Penn, S.R., Meyer, S.E., Subbarao, S., Shi, X. et al. (2012) Cotton Plants Expressing a Hemipteran-Active *Bacillus thuringiensis* Crystal Protein Impact the Development and Survival of *Lygus hesperus* (Hemiptera: Miridae) Nymphs. *Journal of Economic Entomology* **105**: 616.
- Bradford, K.J., van Deynze, A., Gutterson, N., Parrott, W., Strauss, S.H. (2005) Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* **23**: 439-444.
- Bravo, A., Gill, S.S., Soberon, M. (2007) Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon* **49**: 423-435.
- CERA - Center for Environmental Risk Assessment , CERA, ed. GM crop database. ILSI Research Foundation, Washington DC. http://www.cera-gmc.org/?action=gm_crop_database.
- Cotton Australia (2015) Cotton Annual 2015 Australian cotton industry statistics. Cotton Australia.
- CRDC and CottonInfo (2016) Cotton Pest Management Guide 2016-17. Australian Government Cotton Research and Development Corporation and CottonInfo.
- Dill, G.M. (2005) Glyphosate-resistant crops: history, status and future. *Pest Management Science* **61**: 219-224.
- Eastick, R., Hearnden, M. (2006) Potential for Weediness of *Bt* Cotton (*Gossypium hirsutum*) in Northern Australia. *Weed Science* **54**: 1142-1151.
- Farrell, T., Johnson, A. (2005) *Cotton pest management guide 2005/06*. NSW Department of Primary Industries; Cotton Catchment Communities CRC.
- Felso, A.S. (2000) Insecticidal genes part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.
- Groves, R.H., Hosking, J.R., Cooke, D.A., Johnson, R.W., Lepschi, B.J., Mitchell, A.A. et al. (2002) The naturalised non-native flora of Australia: its categorisation and threat to agricultural ecosystems. Report to Agriculture, Fisheries and Forestry Australia by the CRC for Weed Management Systems, unpublished.
- Herman, P.L., Behrens, M., Chakraborty, S., Chrastil, B.M., Barycki, J., Weeks, D.P. (2005) A Three-component Dicamba O-Demethylase from *Pseudomonas maltophilia*, Strain DI-6: Gene isolation, characterisation and heterologous expression. *Journal of Biological Chemistry* **280**: 24759-24767.

International Programme on Chemical Safety (1999) Environmental Health Criteria 217: *Bacillus thuringiensis*. Report No: E, <http://www.inchem.org/documents/ehc/ehc/ehc217.htm>, United Nations Environment Programme; International Labour Organisation; World Health Organization.

Jerga, A., Chen, D., Zhang, C., Fu, J., Kouadio, J.L., Wang, Y. 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., Smith, J. (2014) Applying a weed risk assessment approach to GM crops. *Transgenic Research* **23**: 957-969.

Ladics, G.S., Bartholomaeus, A., Bregitzer, P., Doerrler, N.G., Gray, A., Holzhauser, T. et al. (2015) Genetic basis and detection of unintended effects in genetically modified crop plants. *Transgenic Research* **24**: 587-603.

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

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

OECD (2007) Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis* - derived insect control protein. Report No: 42, Organisation for Economic Co-operation and Development, Paris, France.

OGTR (2013) *Risk Analysis Framework*. Office of the Gene Technology Regulator, Canberra, Australia.

OGTR (2016) The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton) v3.0. Office of the Gene Technology Regulator, Canberra, Australia.

Padgett, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L. et al. (1996) Chapter 4: New weed control opportunities: development of soybeans with a Roundup Ready gene. In: *Herbicide-resistant crops: agricultural, environmental, economic, regulatory and technical aspects*, Duke, S.O., ed. CRC Press Boca Raton. 53-84.

Rogers, D.J., Reid, R.E., Rogers, J.J., Addison, S.J. (2007) Prediction of the naturalisation potential and weediness risk of transgenic cotton in Australia. *Agriculture, Ecosystems & Environment* **119**: 177-189.

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N. 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. et al. (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews* **62**: 775-806.
- Soberón, M., Gill, S.S., 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.
- Standards Australia Ltd, Standards New Zealand, CRC for Australian Weed Management (2006) *HB294:2006 National Post-Border Weed Risk Management Protocol*. Available online.
- Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L. et al. (2013) Evaluating the potential for adverse interactions within genetically engineered breeding stacks. *Plant Physiology* **161**: 1587-1594.
- Thompson, C.J., Movva, N.R., Tizard, R., Crameri, R., Davies, J., Lauwereys, M. et al. (1987) Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. *EMBO Journal* **6**: 2519-2523.
- Weber, N., Halpin, C., Hannah, L.C., Jez, J.M., Kough, J., Parrott, W. (2012) Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. *Plant Physiology* **160**: 1842-1853.
- Williams, S., Wilson, L., and Vogel S. (2011) Pests and Beneficials in Australian Cotton Landscapes. Cotton Catchment Communities CRC.
- Yu, C.G., Mullins, M.A., Warren, G.W., Koziel, M.G., Estruch, J.J. (1997) The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. *Applied Environmental Microbiology* **63**: 532-536.

Appendix A Summary of submissions from prescribed experts, agencies and authorities⁴

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

Abbreviations: **GM: Ch:** chapter, **DAFWA:** Department of Agriculture and Food, Western Australia, **GTTAC:** Gene Technology Technical Advisory Committee, **FSANZ:** Food Standards Australia New Zealand, **GM:** genetically modified, **GMO:** genetically modified organism, **RARMP:** Risk Assessment and Risk Management Plan, **Sec:** Section

Sub.No:	Summary of issues raised	Comment
1	Agrees with the overall conclusions of the RARMP and supports the requirement for further research on non-target organisms, including beneficial species, prior to future releases with reduced limits and controls	Noted
	Suggests considering the relevance of uncertainty relating to growing GM cotton in Northern Australia	There is a low level of uncertainty for GM cotton which may be planted in northern Australia, in a commercial context. This low level of uncertainty is much less relevant in the context of a limited and controlled field trial which has a range of measures in place to restrict spread and persistence of GM plants.
2	Supports the conclusion that the proposed dealing poses negligible risk to human health and safety and the environment. Notes that the dealing involves only one new gene (MON88702) and other genes have been trialled in Australia with no reports of harm to human health and safety and the environment.	Noted
	Suggests to include a study to investigate the impact of the GM cotton on Australian <i>Orius</i> species insects into the licence	Since this information was not considered to be a requirement for this limited and controlled field trial, it was not included in the licence. However, data requirements for future commercial release are highlighted in Ch 3. These requirements include information on potential for increased toxicity on a range of relevant insects.
3	Agrees with the conclusion of the consultation RARMP that risks of the proposed release to the environment are negligible.	Noted

⁴ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub.No:	Summary of issues raised	Comment
	The RARMP should address the reliability and/or validity of the studies on non-target organisms, including the fact that toxicity was not expressed in accordance with the relevant international guidelines and the guidelines used for the experiments were not specifically named.	Risk assessment was conducted using OGTR Risk Analysis Framework which is based on international best practice for regulatory risk analysis of GMOs. The RARMP identifies uncertainty and future requirements regarding the range of insects that may be adversely affected by the introduced Cry51Aa2 protein, both alone and in combination with other proteins.
	The RARMP should address how the lack of Cry51Aa2 expression data was considered in the risk assessment.	The RARMP highlighted the lack of expression data (Ch 1 Sec 5.5). Ch 2 risk scenario 1 concludes that the risk of toxicity was negligible in the context of limited and controlled release. Ch 2 Sec 3 identified the lack of characterisation of the GM cotton as a source of uncertainty. Ch 3 Sec 4 includes requirements for characterisation of the GM cotton for any future release.
	Suggests that <i>in planta</i> expression of <i>cry51Aa2</i> be made available for the assessment of a commercial release application.	Noted. Ch 3 Sec 4 includes requirements for characterisation of the GM cotton for any future release.
	Sought clarification on how non-target organism Collembola (springtail) was exposed to the protein and the validity of the test.	The test method was adapted from OECD guidelines. A bioassay was carried out by incorporating mCry51Aa2 protein in inactivated-yeast medium for feeding to confined populations of springtails. The endpoints of the bioassay were an assessment of mortality and reproductive success.
	Sought clarification on the number of sites, maximum planting size of individual trial sites and total trial size.	The wording in the RARMP was changed to reflect that the application was amended. These are outlined in Ch 1 Sec 3. Note that the applicant has proposed a number of LGAs from which trial sites will be selected. There are also limits on both the number and size of sites which must be observed.
	Seeks clarification and discussion on the feasibility of monitoring and implementing the licence conditions	The DIR is in line with previously approved cotton field trials. The Regulator has not observed or received reports of adverse effects from these trials. The licence includes a range of conditions about inspection of trial sites and reporting to the Regulator and the OGTR has a monitoring and inspection program which effectively manages compliance with licence conditions.
	Agrees that it is not expected that the introduced insect resistance gene would alter the pollen dispersal or weediness characteristics of the GM cottons. However, recommends a more in depth assessment of a commercial release application.	Noted
	Agrees that there is uncertainty regarding the insecticidal spectrum of the Cry51Aa2 protein and that the limits and controls restrict exposure of non-target organisms and thus decrease the likelihood of harm. However, notes that uncertainty and exposure is expected to increase in the event of commercial release and these must be thoroughly addressed.	Noted

Sub.No:	Summary of issues raised	Comment
	Raised the uncertainty of combination toxicity of up to four insecticidal proteins present in the new cotton to non-target insects and to birds in case of a commercial release. Recommends that the combination toxicity of the four proteins be determined for future commercial applications.	Noted. Ch 3 Sec 4 includes requirements for additional data on the potential toxicity to an increased range of relevant insects of the combination of the insecticidal proteins in the GM cottons and their potential for increased weediness for a larger scale release.
4	Noted that the licence prohibits the use of the GM plant material in human food or animal feed.	Noted
5	Supports the conclusion that DIR 147 as the proposed release poses negligible risk to people or the environment.	Noted
6	States the application is in line with previous applications for GM cotton. Questions the number of variants being tested which seem to be commercially rather than scientifically motivated.	Noted. The aim of the field trial is to breed and assess agronomic performance of the GM cottons in all cotton growing areas of Australia and generate data for possible future commercial release. The proposed data collection and the limits and controls qualify the release as a field trial.