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Department of Health, Disability and Ageing

Office of the Gene Technology Regulator

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Risk Assessment and Risk Management Plan for

DIR 216

Commercial release of cotton genetically
modified for insect resistance and herbicide
tolerance (Bollgard® 3 ThryvOn® cotton with
XtendFlex® Technology)

Applicant: Bayer CropScience Pty Ltd

Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 216

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence for the intentional commercial-scale release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application has been prepared by the Regulator in accordance with the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that the proposed commercial release poses negligible risk to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Project title	Commercial release of cotton genetically modified for insect resistance and herbicide tolerance (Bollgard® 3 ThryvOn® with XtendFlex® Technology cotton) ¹
Parent organism	Cotton (<i>Gossypium hirsutum</i> L.)
Introduced genes and modified traits	4 insect resistance genes: • <i>mCry51Aa2</i> gene from <i>Bacillus thuringiensis</i> (Bt) • <i>cry1Ac</i> gene from Bt • <i>cry2Ab</i> gene from Bt • <i>vip3A</i> synthetic gene from Bt 3 herbicide tolerance genes: • <i>cp4 epsps</i> gene (two copies) from <i>Agrobacterium</i> sp. strain CP4 (glyphosate tolerance) • <i>bar</i> gene from <i>Streptomyces hygroscopicus</i> (glufosinate tolerance) • <i>dmo</i> gene from <i>Stenotrophomonas maltophilia</i> (dicamba tolerance) 4 selectable marker genes: • <i>nptII</i> gene from <i>Escherichia coli</i> (antibiotic resistance) • <i>aph4</i> gene from <i>E. coli</i> (antibiotic resistance) • <i>uidA</i> gene from <i>E. coli</i> (reporter) • <i>aad</i> gene from <i>E. coli</i> (antibiotic resistance)
Previous releases	The proposed GM cotton has been approved for field trials in Australia under DIR 147 and DIR 203 in Australia. It has been approved for commercial cultivation in the United States of America (USA) and for food and feed use in several other countries.

¹ The title of the project as supplied by the applicant is “Commercial release of cotton genetically modified for insect resistance and herbicide tolerance”

Proposed locations	Australia-wide
Principal purpose	Commercial release of the GM cotton

Risk assessment

The risk assessment process considers how the genetic modification and activities conducted with the GMO 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 information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short- and long-term risks were considered.

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

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

- the GM cotton has been produced by conventional breeding of 5 GM parental cotton lines, of which 4 have been approved for commercial release. The fifth has been approved for field trial in Australia. The risks associated with the GM parental cottons and combinations thereof, have been assessed previously as negligible and this RARMP has found no new information to change these conclusions.
- the genes and their products have been assessed as posing no increased risk of toxicity or allergenicity to humans, or toxicity to other organisms.
- the GM cotton has limited capacity to spread and persist in undisturbed environments and can be controlled using integrated weed management in agricultural and high intensity use areas.
- food made from the GM parental cotton lines has been approved by Food Standards Australia New Zealand (FSANZ) as safe for human consumption and this approval also covers food from offspring produced by conventional breeding.

Risk management

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 evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

The risk management plan concludes that risks from the proposed dealings can be managed to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence contains several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

<i>aad</i>	<i>3''(9)-O-aminoglycoside adenylyltransferase gene</i>
<i>aph4</i>	<i>hygromycin B phosphotransferase gene</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
<i>bar</i>	<i>bialaphos resistance (phosphinothricin N-acetyltransferase) gene</i>
BGI	Bollgard® II GM cotton
BG3	Bollgard® 3 GM cotton
BG3 RRF	Bollgard® 3 Roundup Ready Flex® GM cotton
BG3 XF	Bollgard® 3 XtendFlex® GM cotton
<i>Bt</i>	<i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
<i>cp4 epsps</i>	<i>epsps gene from Agrobacterium sp. strain CP4</i>
CP4 EPSPS	EPSPS protein from <i>Agrobacterium sp.</i> strain CP4
CRDC	Cotton Research and Development Corporation
Cry	Crystal protein
<i>cry1Ac</i>	<i>cry1Ac gene from B. thuringiensis</i>
Cry1Ac	Cry1Ac crystal protein from <i>B. thuringiensis</i>
<i>cry2Ab</i>	<i>cry2Ab gene from B. thuringiensis</i>
Cry2Ab	Cry2Ab crystal protein from <i>B. thuringiensis</i>
DIR	Dealing involving Intentional Release
<i>dmo</i>	<i>dicamba monooxygenase gene from Stenotrophomonas maltophilia</i>
DMO	Dicamba monooxygenase
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FMV	Figwort mosaic virus
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GUS	β-glucuronidase protein
ha	Hectare
HGT	Horizontal gene transfer
Hsp	Heat shock protein
IPM	Integrated Pest Management
IWM	Integrated Weed Management
m	metre
<i>mCry51Aa2</i>	<i>modified cry51Aa2 gene from B. thuringiensis</i>
NGS	Next Generation Sequencing
nptII	Neomycin phosphotransferase II
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin N-acetyl transferase
RARMP	Risk Assessment and Risk Management Plan
TGA	Therapeutic Goods Administration
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RRF	Roundup Ready Flex® GM cotton
USA	United States of America
USDA-APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
US EPA	United States Environmental Protection Agency
Vip	Vegetative insecticidal protein
<i>vip3Aa</i>	<i>vip3Aa gene from B. thuringiensis</i>
Vip3Aa	Vip3Aa crystal protein from <i>B. thuringiensis</i>
XF	XtendFlex® GM cotton

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.
5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

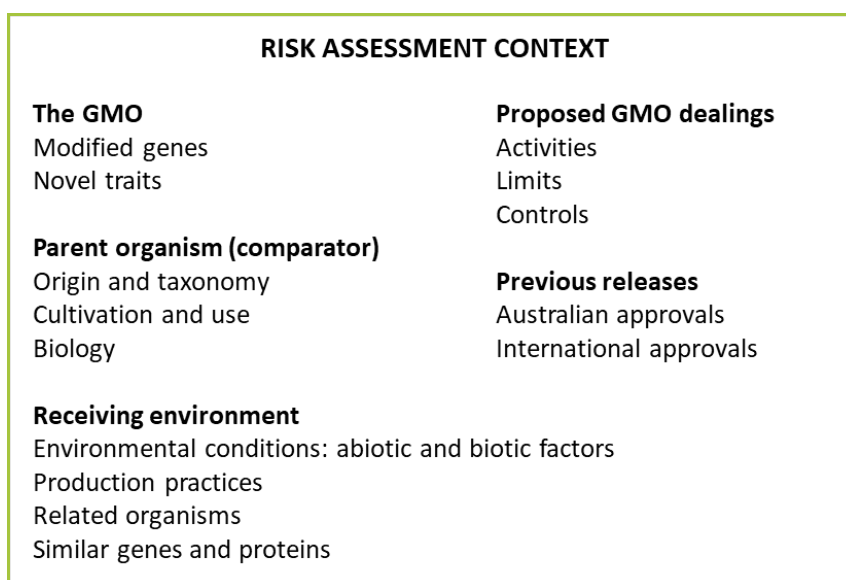


Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework

6. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Government authorities or agencies

prescribed in the Regulations, all Australian local councils, and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix B.

7. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is summarised in Appendix C. A total of 6 public submissions were received and their consideration is summarised in Appendix D.

1.1 Interface with other regulatory schemes

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

9. 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.

10. FSANZ assesses the safety of food produced using gene technology through administration of the Australia New Zealand Food Standards Code.

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

Section 2 The proposed release

12. Bayer CropScience Pty Ltd (Bayer) proposes commercial release of a GM cotton (Bollgard® 3 ThryvOn® cotton with XtendFlex® Technology) that contains 4 insect resistance genes, 3 herbicide tolerance genes, 4 selectable marker genes (3 antibiotic resistance genes and a visual marker gene).

13. The GM cotton is the result of conventional crossing of MON-88702-4 (also identified as ThryvOn®), Bollgard® 3, and XtendFlex® cottons. The parental GM cotton lines are identified by OECD unique identifiers MON-88702-4 and MON-15985-7 (Bollgard® II - BGII), SYN-IR102-7 (COT102), and MON-88701-3 and MON-88913-8 (Roundup Ready Flex - RRF). The GMO is identified as MON-88702-4 x MON-15985-7 x SYN-IR102-7 x MON-88701-3 x MON-88913-8.

14. For the remainder of the document Bollgard® 3 ThryvOn® cotton with XtendFlex® Technology will be referred to as 'the GMO'. XtendFlex® (RRF x MON-88701-3) will be referred to as XF and Bollgard® 3 (BGII x COT102) as BG3.

15. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by the States and Territories for marketing purposes. The GM cotton could be grown in all commercial cotton growing areas, and products derived from the GM plants would enter general commerce, including use in human food and animal feed.

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

- a) conduct experiments with the GMO
- b) breed the GMO
- c) propagate the GMO
- d) use the GMO in the course of manufacture of a thing that is not the GMO

- e) grow, raise or culture the GMO
- f) import the GMO
- g) transport the GMO
- h) dispose of the GMO

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

Section 3 The parent organism

17. The parent organism is upland cotton (*Gossypium hirsutum* L.), the most commonly cultivated cotton species worldwide. Detailed information about cotton is contained in a reference document, *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR, 2024), which was produced to inform the risk assessment process for licence applications involving GM cotton plants. This document is available from the Resources page on the [OGTR website](#). Baseline information from this document will be used and referred to throughout the RARMP.

18. Cotton is exotic to Australia and is grown as an agricultural crop in New South Wales (NSW) and Queensland (Qld), with recent expansion into northern Victoria (Vic), Western Australia (WA) and in the Northern Territory (NT). Cotton is grown as a source of textile and industrial fibre, cottonseed oil and linters for food use, and cottonseed meal for animal feed.

19. In establishing the risk context, details of the parent organism forms part of the baseline for a comparative risk assessment (OGTR 2013). Non-GM cotton is the standard baseline for biological comparison, although it should be noted that over 99.5 % of the Australian grown cotton is GM, expressing either insect resistance, herbicide tolerance, or both traits (OGTR, 2021).

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

21. Based on 2023/2024 estimates of commercial cropping areas and production volume in Australia, cotton is ranked seventh in area of production and sixth in total production among Australian crops (ABARES, 2024; Crop Data). In 2023/2024, the cotton production area in Australia was estimated at 474,000 hectares (ha); this area is forecast to decrease to 458,000 ha in 2024/2025, in part, due to marginal rainfall in cotton growing regions (ABARES, 2024).

Section 4 The GM parental cottons – nature and effect of genetic modification

22. The GMO is produced by conventional breeding of the following GM parental cottons:

- Insect resistant MON-88702-4 (ThryvOn®) cotton
- Insect resistant BG3 cotton (coventional crossing of BGII and COT102 cotton)
- Herbicide tolerant XF cotton (conventional crossing of MON-88701-3 and RRF cotton).

23. Both BG3 and XF cottons have been extensively evaluated in previous RARMPs for limited and controlled release and commercial release (Table 1). Therefore, this section will provide only summary information for these GM parental cotton lines.

Table 1 Previous releases of the parental cotton lines

Cotton (Commercial name)	OECD Unique Identifier	Previous licences ^a
ThryvOn [®]	MON-88702-4	DIRs 147, 203 (L&C)
BG3	MON-15985-7 (BGII)	DIRs 120, 203 (L&C) DIRs <u>012</u> , <u>059</u> , <u>066</u> , <u>124</u> ^b , <u>145</u> ^c (C)
	SYN-IR102-7 (COT102)	DIRs 017, 025, 034, 036, 058, 065, 073, 101, 120, 203 (L&C) DIRs <u>124</u> ^b , <u>145</u> ^c , <u>157</u> (C)
XF	MON-88701-3	DIRs 120, 203 (L&C) DIR <u>145</u> ^c (C)
	MON-88913-8 (RRF)	DIRs 035, 203 (L&C) DIRs <u>059</u> , <u>066</u> , <u>124</u> ^b , <u>145</u> ^c (C)

^a C: Commercial release; L&C: Limited and Controlled release

^b As part of BG3

^c Individually and with MON-15985-7, MON88701-3, SYN-IR102-7 and MON-88913-8 in BG3 XF

24. The insect resistant GM cotton line MON-88702-4 was evaluated for limited and controlled release under DIR 147, individually and in combination with the other parental cotton lines and was also included in DIR 203. It has not been approved for commercial cultivation in Australia and has few commercial cultivation approvals worldwide. Thus, the current RARMP will focus mainly on MON-88702-4, including the *mCry51Aa2* gene, the mCry51Aa2 protein and its metabolites.

4.1 The genetic modifications of the GM parents and the GMO

25. The introduced genetic material, source organisms and traits are summarised in Table 2.

26. Events MON-88702-4, COT102, MON-88701-3 and RRF were all produced by *Agrobacterium tumefaciens*-mediated transformation. The BGII event was produced using both *Agrobacterium tumefaciens*-mediated transformation and microprojectile bombardment (biolistics). These methods have been widely used in Australia and worldwide for introducing genes into plants. More information can be found in the document Methods of plant genetic modification on the OGTR Risk Assessment References webpage. The parental BG3 and XF cottons were subsequently produced by conventional crossing of these lines (see paragraph 22), and the GMO by conventional crossing of MON-88702-4, BG3 and XF. Detailed assessments of the genetic modification of the parental lines are provided in the DIR licences listed in Table 1 and further discussion of the GM parental cottons is in sections 4.2 – 4.4.

4.2 GM BG3 cotton

27. As noted, BG3 was approved for commercial release in Australia in 2014 (DIR 124). Both parental lines (BGII and COT102) were approved for commercial release in earlier licences and BG3 cotton combined with XF was approved (as BG3 XF) for commercial release in 2016 (DIR 145). See Table 1 for more information.

4.2.1 Genetic modification and introduced genes

28. BG3 contains 3 genes conferring insect resistance (*cry1Ac*, *cry2Ab*, *vip3Aa*), 4 marker genes (3 antibiotic resistance genes and a reporter gene) and regulatory elements (Table 2).

29. The RARMPs prepared for DIR 124 and DIR 145 contain extensive discussion of BG3, including the method of genetic modification, molecular stability, the introduced genes and regulatory elements and the proteins encoded by the introduced genes. These RARMPs considered the potential toxicity and allergenicity to humans, toxicity to animals (including non-target arthropods), effects on soil microorganisms and the presence of identical or similar genes and proteins in the environment. These risk assessments concluded that there was negligible risk of harm to people, other beneficial organisms or the environment from the introduced genes and their expression in the BG3 cotton.

30. The Regulator has not received reports of adverse effects on human health, animal health or the environment caused by BG3 as a crop. Literature searches did not find any information indicating adverse effects of BG3 cotton on human health and safety or the environment.

4.2.2 Other approvals of BG3

31. Regulatory systems in some countries do not require separate authorisation for environmental release of GMOs produced by conventional crossing between other already authorised GMOs. In all following discussions of international approvals, if a country with this type of system has approved the parental events, this will be noted.

32. To date BG3 cotton has been approved for direct use or processing in food in Japan and Mexico in 2014. BG3 RRF (also expresses the *cp4epsps* gene for glyphosate tolerance) was approved for direct use or processing in food in Japan (2014). Both GM parents of BG3 cotton have been approved in the United States of America (USA), Canada and China ([ISAAA GM approval](#) and [CropLife International](#) databases; accessed March 2025).

Table 2 Genetic elements in the GMO and their origin

Gene (Source)	Protein produced	Function	Promoter (source)	Terminator (source)	Additional elements (source)	Present in
<i>mCry51Aa2</i> (<i>Bacillus thuringiensis</i> (Bt))	Modified crystal protein 51Aa2	Resistance to hemipteran & thysanopteran insects	Hsp81-2 (<i>Arabidopsis thaliana</i>)	35S (CaMV)	E-FMV (Enhancer region of Figwort mosaic virus DNA)	MON-88702-4 (ThryvOn®)
<i>cry1Ac</i> (Bt)	Crystal protein 1Ac	Resistance to lepidopteran insects	35S (CaMV)	7S 3' (<i>Glycine max</i>)		BG3
<i>cry2Ab</i> (Bt)	Crystal protein 2Ab2	Resistance to lepidopteran insects	35S (CaMV)	<i>nos</i> (<i>Agrobacterium tumefaciens</i>)	PetHSP70 (<i>Petunia x hybrida</i>), Ctp2 (<i>A. thaliana</i>)	BG3
<i>vip3A</i> (Bt)	Vegetative insecticidal protein 3A	Resistance to lepidopteran insects	<i>actin2</i> (<i>A. thaliana</i>)	<i>nos</i> (<i>A. tumefaciens</i>)		BG3
<i>cp4 epsps</i> (<i>Agrobacterium</i> sp. strain CP4)	5-enolpyruvylshikimate-3-phosphate synthase	Tolerance to glyphosate	P-FMV/TSF2 (Figwort mosaic virus/ <i>A. thaliana</i>) P-35S/ACT8 (CaMV/ <i>A. thaliana</i>)	<i>rbcs-E9</i> (<i>Pisum sativum</i> – pea) <i>rbcs-E9</i> (<i>P. sativum</i>)	Ctp2 (<i>A. thaliana</i>) Ctp2 (<i>A. thaliana</i>)	XF
<i>bar</i> (<i>Streptomyces hygroscopicus</i>)	Phosphinothricin N-acetyl transferase (PAT)	Tolerance to glufosinate	35S (CaMV)	<i>nos</i> (<i>A. tumefaciens</i>)	HSP70 (<i>P. x hybrida</i>)	XF
<i>dmo</i> (<i>Stenotrophomonas maltophilia</i>)	Dicamba monooxygenase	Tolerance to dicamba	PC1SV (Peanut chlorotic streak caulimovirus)	E6 3' (<i>Gossypium barbadense</i>)	TEV (Tobacco etch virus) Ctp2 (<i>A. thaliana</i>)	XF
<i>aad</i> (<i>Escherichia coli</i>)	3''(9)-O-aminoglycoside adenylyltransferase	Marker - Antibiotic resistance (streptomycin)	Tn7 (native promoter of the <i>aad</i> gene from <i>E. coli</i>)			BG3
<i>nptII</i> (<i>E. coli</i>)	Neomycin phosphotransferase type II	Marker - Antibiotic resistance (kanamycin)	35S (CaMV)	<i>nos</i> (<i>A. tumefaciens</i>)		BG3
<i>uidA</i> (<i>E. coli</i>)	beta-glucuronidase (GUS)	Selective marker (colour reaction)	35S (CaMV)	<i>nos</i> (<i>A. tumefaciens</i>)		BG3
<i>aph4</i> (<i>E. coli</i>)	Hygromycin B phosphotransferase	Marker - Antibiotic resistance (hygromycin)	ubiquitin 3 (<i>A. thaliana</i>)	<i>nos</i> (<i>A. tumefaciens</i>)	ubi3 intron	BG3

33. FSANZ has approved both BGII and COT102 for human consumption (FSANZ, 2002, 2004). These approvals cover food produced from any offspring resulting from conventional breeding, including food produced from BG3 cotton.

4.3 GM XF cotton

34. The XF cotton, individually and in combination with BG3 (BG3 XF), was approved for commercial release in Australia in 2016 under DIR 145.

4.3.1 Genetic modification and introduced genes

35. As mentioned previously, XF was produced by crossing RRF with MON-88701-3. Detailed consideration of the genetic modifications of RRF and MON-88701-3 is available in the RARMPs for DIR 124 and DIR 145 (Table 1).

36. The XF cotton contains glyphosate (*cp4 epsps*), glufosinate (*bar*) and dicamba (*dmo*) herbicide tolerance genes and regulatory elements (Table 2).

37. The RARMP prepared for DIR 145 includes extensive discussion of the method of genetic modification, molecular stability, the introduced genes and regulatory elements and the proteins encoded by the introduced genes. It considered potential toxicity/allergenicity to humans, animals (including non-target arthropods), effects on soil microorganisms, the presence of identical or similar genes and proteins in the environment for XF. The risk assessment concluded that there was negligible risk of harm to people, other beneficial organisms or the environment from the introduced genes or their expression in GM cottons.

38. The Regulator has not received reports of adverse effects on human health, animal health or the environment caused by XF cotton alone or in combination with BG3 as a crop. Literature searches did not find any information indicating adverse effects of XF cotton on human health and safety or the environment.

4.3.2 Other approvals of XF cotton

39. Several countries have approved XF cotton for environmental release, as well as food and feed use (Table 3).

Table 3 International approvals of XF cotton^a

Country	Food - direct use or processing	Feed - direct use or processing	Cultivation - domestic or non-domestic use
Brazil	2018	2018	2018
Colombia	2016	2016	
Japan	2014	2014	
Mexico	2015		
South Korea	2016	2017	
Taiwan	2015		

^a Source: ISAAA GM approval database; accessed February 2025

40. Both GM parents of XF cotton (RRF and MON-88701-3) have been approved in the USA, Canada and China (ISAAA GM approval and CropLife International; accessed March 2025). Additionally, GM cotton produced by crossing events MON 88701-3 x RRF x BGII has been approved for food (including direct use or processing) in Mexico and Taiwan, and for food and feed (including direct use or processing) in Japan and South Korea (ISAAA GM approval database; accessed May 2025).

41. FSANZ approved both parental GM cotton lines of XF cotton for human consumption (FSANZ, 2005, 2013). FSANZ approval includes foods produced from cotton lines generated by conventional crossing of approved GM lines, including food produced from XF cotton.

4.4 GM MON-88702-4 cotton

42. The GM MON-88702-4 cotton was approved by the Regulator for limited and controlled release under licence [DIR 147](#) in 2017 and is discussed in detail in that RARMP. It has not been approved for commercial release in Australia and will therefore be discussed in more detail in this RARMP.

4.4.1 The introduced genes, regulatory elements and encoded proteins

43. The MON-88702-4 event contains a modified *cry51Aa2* (*mCry51Aa2*) gene derived from *Bacillus thuringiensis* (Bt), a gram-positive bacterium commonly present in soil. The Bt bacterium produces a range of insecticidal proteins, including the crystal (Cry) proteins, also known as delta-endotoxins. This also includes the Cry1Ac and Cry2Ab proteins included in this application. 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* protein encoded by *mCry51Aa2* gene, was shown to be produced in the bacteria as a protoxin and has the same mode of action (Jerga et al., 2016).

44. The MON-88702-4 event also contains non-coding regulatory elements that control expression of the introduced gene *mCry51Aa2*. These regulatory elements, listed in Table 4, are derived from plants, *A. tumefaciens* and from two plant viruses, namely Cauliflower mosaic virus and Figwort mosaic virus.

Table 4 Introduced regulatory elements in MON-88702-4

Element	Function	Source
E-FMV	<i>mCry51Aa2 enhancer</i>	Enhancer region of Figwort mosaic virus DNA
P-Hsp81-2	<i>mCry51Aa2 promoter</i>	Promoter sequence of the heat shock protein 81-2 gene from <i>Arabidopsis thaliana</i>
T-35S	<i>mCry51Aa2 terminator</i>	Sequence of 3' untranslated region of the 35S RNA of CaMV direct polyadenylation of mRNA in plants

4.4.2 The *mCry51Aa2* protein

45. In MON-88702-4 cotton, the modified *mCry51Aa2* gene expresses the *mCry51Aa2* protein that provides resistance against targeted hemipteran ('sucking bugs', including aphids) and thysanopteran (thrips) insect pest species (Baum et al., 2012). Compared to the native sequence, the amino acid sequence of the *mCry51Aa2* protein has 9 changes, namely 8 amino acid substitutions and one deletion of 3 amino acids, which results in a sequence similarity of 96.4%. These intentional modifications were made to increase its activity against target insect pests.

46. Individual Cry proteins have a narrow spectrum of insecticidal activity within a particular insect order (de Maagd et al., 2001). Activated Cry proteins bind to specific receptors on the brush border membrane of the midgut epithelium of susceptible species, leading to formation of membrane pores (Bravo et al., 2007; Yu et al., 1997). Formation of pores eventually leads to cell lysis and impairs the insect digestive process, which is thought to be responsible for insect death (OECD, 2007; Schnepf et al., 1998; Soberón et al., 2009).

47. Other 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). If consumed by birds and mammals, the Cry proteins would undergo degradation by proteases.

48. The applicant has provided data from studies in the USA that assessed the impact of *mCry51Aa2* protein on 11 representative non-target invertebrate species using a diet bioassay. The representative species were selected based on the results of a previous activity spectrum assessment (Bachman et al., 2017), and to ensure inclusion of different taxonomic groups, habitats and functions in the agroecosystem, the characteristics of the crop and the trait. The selected species include a pollinator, 8 beneficial insects and 2 decomposers from the soil biota. Test concentrations in this diet bioassay were based on the measured *mCry51Aa2* protein expression in the tissue type(s) to which the species are most likely to be exposed in the environment. Insects are continuously exposed to *mCry51Aa2* in their diet.

49. No toxicity was detected for 10 of the 11 representative species. However, the mCry51Aa2 had an adverse effect on insidious flower bug (*Orius insidiosus*), which is one of 4 Hemiptera species (the order of target pest) included in the diet bioassay. The mCry51Aa2 toxicity to *O. insidiosus* was also reported in the previous activity spectrum assessment (Bachman et al., 2017).

50. The applicant has conducted a comprehensive assessment of the impact of mCry51Aa2 on *Orius* spp. (*O. insidiosus* and *O. majusculus*) using a tier-based system developed by the US EPA. This sequential assessment included initial screening study at high concentrations with continuous feeding (tier 1), tri-trophic feeding studies (tier 2-3) and a field study (tier 4), designed to refine risk characterisation by progressing towards more realistic field-based exposure conditions. Where an effect was observed or a tier outcome was inconclusive, higher-tier testing can be conducted under exposure conditions reflective of the field concentrations.

51. The tier 1-3 studies demonstrated effects of mCry51Aa2 on *Orius* spp., or had outcomes that were inconclusive. Thus, a field study (tier 4) was conducted for these species, comparing the impact of MON-88702-4 on abundance of predatory Hemiptera to that of a commercial (non-GM) control cotton. This study evaluated *O. insidiosus*, and 3 closely related Hemiptera species at 6 sites in the USA in 2018. Insect samples were collected 10 times over the course of the season at each site. In combined site analyses, no significant differences ($p > 0.05$) in abundance of the predatory hemipterans were observed between MON-88702-4 and the control. It should be noted that *O. insidiosus* is not recorded in the [Atlas of Living Australia](#) (accessed March 2025) and *Orius* spp. are not listed as beneficial predatory species in Australian cotton fields (OGTR, 2024).

52. A field study conducted in Australia (2019-2020) assessed the impact of the GMO on the abundance of non-target arthropods with BG3 XF as a control, thus focussing on the effects of mCry51Aa2 in GM cotton. The field trials were conducted at 5 locations including one site in WA (winter season) and 4 sites in eastern Australia (summer season). The study evaluated key Australian beneficial arthropods including 9 Hemiptera, 2 Coleoptera (beetles), one Araneae (spiders) and one Neuroptera (lacewings) species, including 7 species listed as beneficial predatory insects in Australian cotton fields (OGTR, 2024). The sites were designed in a randomised complete block with 4 replicates and arthropod abundance was measured using beat sheet samples and a suction sample from each plot.

53. There were 8 significant ($p < 0.05$) differences that showed lower abundance of 4 arthropod species in the GMO than the control. However, these differences were limited to a single location or a sampling method and were not consistent across sites or sampling methods. It was concluded that the differences were not indicative of adverse impacts of the GMO compared to the BG3 XF control and that overall, the study suggests that the mCry51Aa2 protein in the GMO does not adversely affect non-target arthropods compared to the BG3 XF control.

4.4.3 Genetic modification and molecular characterisation

54. As mentioned previously, the MON-88702-4 cotton was produced using *Agrobacterium*-mediated transformation (Chapter 1; subsection 4.1.1).

55. The applicant provided data that indicate the introduced gene was stably incorporated, with no plasmid backbone and antibiotic resistance gene expression cassette sequences in MON-88702-4. This was achieved by transforming 2 separate T-DNA gene cassettes, one expressing mCry51Aa2 protein and the other expressing *aadA* (antibiotic selectable marker). Subsequently, selective breeding was used to remove the *aadA* expression cassette from the MON-88702-4 cotton line. Next Generation Sequencing (NGS) confirmed the presence of a single *mCry51Aa2* gene expression cassette and the absence of plasmid backbone, *aadA* expression cassette and any unintended sequences.

4.4.4 Germination and dormancy

56. Characteristics affecting germination and dormancy have the potential to affect the persistence of seed in the environment and therefore the potential for weediness. The applicant conducted laboratory seed germination and dormancy tests in the USA, with seed collected from 3 field test sites with 4 replicates per site (Barberis, 2017), comparing MON-88702-4 cotton to non-GM cotton derived from DP393

cotton as the control line. At each site, 4 commercial non-GM cottons were used as reference lines, selected from a group of 7 commercially available non-GM cotton varieties used across all trial sites. Combined-site analysis compared MON-88702-4 to the control and to the range from reference varieties.

57. Germination was tested under 6 controlled temperature treatments: 3 constant temperatures (10°C, 20°C and 30°C), and 3 alternating temperature treatments (10°C/20°C, 10°C/30°C and 20°C/30°C). Measures included percentage of germinated seed, viable hard seed, viable firm swollen seed and dead seed. The percentage of viable hard seed can be included as a measure of dormancy and potentially as an indicator of weediness.

58. Significant differences ($p < 0.05$) were observed in 10 of the 25 combined site analysis of MON-88702-4 compared to the conventional control. At 20°C, 10°C/20°C, and 20°C/30°C, MON-88702-4 showed lower germinated seed compared to the control. At 10°C, 10°C/20°C, and 20°C/30°C, MON-88702-4 had higher percentage hard seed compared to the control. At 10°C, MON-88702-4 had a lower percentage viable firm-swollen seeds, while at 10°C/20°C, MON-88702-4 had a higher percentage, compared to the control. However, in all cases, the means for each measure were within the reference ranges. The report concluded that these differences are unlikely to be biologically meaningful in terms of weediness potential of MON-88702-4 compared to conventional cotton.

59. Considering these results, it is unlikely that there are changes to germination and dormancy which would increase the weediness of MON-88702-4 cotton compared to non-GM cotton.

4.4.5 Approvals of MON-88702-4 cotton and its products

Australian approvals of MON-88702-4 cotton

60. MON-88702-4 has been approved for field trials in Australia, alone and in combination with the other GM parental cottons (DIR 147 and DIR 203).

61. FSANZ assessed food from MON-88702-4 (A1154) cotton as being as safe for consumption as food derived from conventional cotton (FSANZ, 2018a). This assessment also includes foods derived from cotton lines generated by conventional crosses with MON-88702-4.

International approvals of MON-88702-4 cotton and its products

62. MON-88702-4 cotton has been approved internationally for cultivation, for food or for food and feed (including direct use and processing in each use), as summarised in Table 5.

Table 5 International approvals of MON-88702-4 cotton^a

Country	Food - direct use or processing	Feed - direct use or processing	Cultivation - domestic or non-domestic use
Canada	2018	2018	
Colombia	2020		
Japan	2019	2018	
Malaysia	2024	2024	2024 ^b
Mexico ^c	NA ^d	NA	
New Zealand	2018		
Philippines	2021 ^e	2021 ^e	
Singapore	2022	2022	
South Korea	2021	2021	
Taiwan	2019 ^f		
USA	2018	2018	2021

^a Source: ISAAA [GM Approval Database | ISAAA.org](https://www.isaaa.org) unless otherwise stated; accessed February 2025

^b for processing

^c Source: [CropLife International](https://www.crop-life.org/); accessed March 2025

^d NA=Date not applicable

^e expiring in 2026

^f validity: 17 April 2019 - 17 April 2024.

Section 5 The GMO – nature and effect of genetic modification

5.1 Introduction to the GMO

63. The GMO proposed for release is produced by conventional crossing between BG3, MON-88702-4 and XF cottons. Table 2 lists all the genetic elements present in the GM parental cottons used to produce the GMO and these have been discussed in Sections 4.2 – 4.4.

64. The GM plants are phenotypically similar to non-GM cotton. They will be limited by the same abiotic factors as non-GM cotton, sexually compatible with the same plants and their products used identically to non-GM cotton. The difference between the GMO and non-GM cotton is that the GMO is tolerant to glyphosate, glufosinate and dicamba herbicides and resistant to lepidopteran, hemipteran and thysanopteran insect pests of cotton. No additional marker genes are introduced in the GMO, only those that were present in BG3 or BG3 XF.

65. Agricultural management of the GMO differs from non-GM cotton with respect to insect pest management and in the application of herbicides to control weeds in the crop. Any GMO volunteers in subsequent crops would need to be controlled by mechanical means or use of herbicides other than those to which the crop is tolerant.

66. The RARMP for DIR 147 identified additional information that may be required to assess an application for a large scale or commercial release of the GMO, or to justify a reduction in containment conditions. This included:

- Molecular, biochemical and phenotypic characterisation of the GM cotton MON-88702-4 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 cotton and the potential for increased weediness.

67. The applicant has provided the additional information for MON-88702-4 alone, and for the GMO (discussed below).

5.2 Characterisation of the GMO

68. The applicant has provided data from both Australian and USA field trials for the GMO. Field trials in Australia provided protein expression data and phenotypic data. The USA field trials included data for protein expression, cottonseed composition and phenotypic measures (see Section 5.2.3).

5.2.1 Molecular Stability

69. NGS was used to confirm the presence and intactness of each of the insert genes in the GMO. The DNA sequencing data obtained from the GMO was analysed by comparing to the previously determined sequences for each of the GM parental lines. Results of this analysis confirmed that the GMO contains a single complete copy of DNA inserts of each of the parental GM cotton lines.

5.2.2 Protein expression

Field Trials - USA

70. In the USA field trials, concentrations of expressed proteins from the introduced genes in the GMO and GM parental lines were measured in leaves, roots and seeds samples (Table 6).

Table 6 Protein expression for the GMO and GM parental cotton lines in US field trials

Protein	Line ^a	Leaf ^{b,c} (OSL1) (µg/g dw)	Leaf (OSL4) (µg/g dw)	Root (µg/g dw)	Seed (µg/g dw)	Pollen (µg/g dw)
mCry51Aa2	GMO	1200 ± 420	1100 ± 430	210 ± 39	170 ± 34	5.1 ± 1.9
		580 - 1900	560 - 1700	150 - 290	120 - 270	3.8 – 8.3
	MON-88702-4	1500 ± 350	1300 ± 450	170 ± 30	140 ± 23	3.9 ± 1.7
		870 - 1900	740 - 2200	130 - 230	97 - 190	2.7 – 7.0
Cry1Ac	GMO	51 ± 19	33 ± 21	1.5 ± 1	8.2 ± 1.8	0.41 ± 0.092

Protein	Line ^a	Leaf ^{b,c} (OSL1) (µg/g dw)	Leaf (OSL4) (µg/g dw)	Root (µg/g dw)	Seed (µg/g dw)	Pollen (µg/g dw)
Cry2Ab	BGII	15 - 90	11 - 85	0.57 - 5.2	6.4 - 12	0.33 - 0.56
		47 ± 15	21 ± 9.9	0.83 ± 0.34	9.3 ± 0.82	0.40 ± 0.069
		12 - 69	9.7 - 42	0.4 - 1.7	8 - 11	0.29 - 0.46
	GMO	880 ± 560	460 ± 160	150 ± 32	340 ± 49	0.56 ± 0.26
		310 - 2100	290 - 940	100 - 190	240 - 410	0.33 - 0.96
		710 ± 340	330 ± 85	130 ± 27	340 ± 64	0.54 ± 0.080
Vip 3Aa19	BGII	260 - 1600	230 - 530	94 - 190	260 - 500	0.46 - 0.65
		66 ± 22	31 ± 9	<LOQ ^d (NA ^e)	<LOQ (NA)	<LOQ (NA)
		43 - 110	20 - 48	NA - NA	NA - NA	NA - NA
	COT102	120 ± 38	38 ± 14	<LOQ (NA)	<LOQ (NA)	<LOQ (NA)
		54 - 210	20 - 66	NA - NA	NA - NA	NA - NA
DMO	GMO	360 ± 140	150 ± 60	25 ± 7.8	51 ± 22	<LOQ (NA)
		160 - 700	76 - 330	13 - 46	17 - 97	NA - NA
		320 ± 110	140 ± 65	24 ± 7	29 ± 11	<LOQ (NA)
	MON-88701-3	180 - 610	69 - 290	12 - 35	10 - 54	NA - NA
PAT	GMO	11 ± 5.1	5.7 ± 2.1	2.9 ± 0.92	14 ± 3.8	<LOQ (NA)
		4.2 - 19	3 - 11	1.9 - 6.0	5.0 - 19	NA - NA
		8.5 ± 3.5	4.7 ± 1.6	2.6 ± 0.91	10 ± 3.6	<LOQ (NA)
	MON-88701-3	3.7 - 18	2.1 - 7.3	0.91 - 4.6	0.54 - 16	NA - NA
CP4 EPSPS	GMO	3800 ± 1100	1700 ± 690	260 ± 70	370 ± 100	5.0 ± 0.14
		1500 - 5600	830 - 3300	130 - 400	160 - 540	4.8 - 5.1
		2400 ± 860	1000 ± 380	240 ± 79	280 ± 61	4.3 ± 0.60
	RRF	830 - 3800	420 - 1900	130 - 410	170 - 390	3.4 - 4.9

^a For each protein, expression in the test line (the GMO) was compared to the appropriate GM parental cotton, listed in the table

^b Leaf – OSL1 and OSL4, collected at 2-4 node stage and cutout stages, respectively; root and pollen collected at peak bloom stage; seed collected at maturity

^c Data are shown as the mean ± standard deviation, followed by the range of values recorded across all samples

^d LOQ=limit of quantitation (lowest value of concentration that can be quantified with acceptable precision and accuracy)

^e NA=not applicable

71. In general, the mean concentrations of all expressed proteins are higher in all tissues tested in the GMO than in the comparable GM parental line. The exceptions are mCry51Aa2 and Vip3Aa19 (lower than parental line) in leaf tissues, Cry1Ac (lower than parental line) in seed tissue and Cry2Ab (same as parental line) in seed tissue. In addition, the protein concentrations of Vip3Aa19 in root, seed and pollen tissues, and DMO and PAT in pollen tissues were below the limit of quantitation (LOQ).

72. Although there are differences in protein expression between the GMO and parental lines, the means for all proteins in the GMO are within the ranges observed for the parental lines. The exception is CP4 EPSPS in pollen, for which the mean for the GMO is slightly above the range observed for the parental cotton.

Field Trials - Australia

73. Protein expression data for the GMO was collected from Australian field trials between 2018 and 2020, as summarised in Tables A1 and A2, Appendix A. In 2018-2019, samples were obtained from a single site in WA (winter 2018) and from 4 sites across NSW and Qld (summer 2018-19). In 2019-2020, samples were obtained from one site in WA (winter 2019) and 4 sites across NSW and Qld (summer 2019-20). Data were analysed separately for the WA site, while NSW and Qld site data were combined in each season. In each year, each site had 4 plots in a randomised complete block design. Protein expression levels were determined in bolls (Boll-1 and Boll-2), seeds, leaves (over season leaf (OSL), OSL1 – OSL4), pollen, and squares (Square 1-4).

74. For the insect resistance proteins, across all seasons, highest expression was measured for Cry1Ac in OSL1 and Square 1; Cry2Ab in Square 1 and seed; mCry51Aa2 in square samples (all) and for Vip3Aa19 in OSL1 (all leaf samples high) and Square 1 samples.

75. For herbicide tolerance proteins, highest expression was measured for CP4 EPSPS in leaf samples, mainly OSL1 and 2, across all seasons; for DMO in leaf (2018, 2018-19) or square samples (2019, 2019-20) and for PAT in seed and/or leaf samples across all seasons.

76. The most consistent expression pattern was observed for mCry51Aa2, with highest expression in square samples, but also high expression in leaf samples across all seasons. Additionally, pollen expression was lowest for all proteins, across all seasons.

77. Comparing between seasons, expression of Cry and Vip proteins was lower in the 2018-19 samples than for 2018, 2019 or 2019-20 samples and mCry51Aa2 expression was higher in 2019 samples than all others. For herbicide tolerance proteins, expression was generally highest in 2019 samples, with 2019-20 also high for CP4 EPSPS and DMO proteins.

5.2.3 Phenotypic characterisation of the GM cottons

Australian phenotypic characterisation of the GMO

78. Data for phenotypic analysis of the GMO were generated from 2018-2019 and 2019-2020 field trials in Australia. Details of the treatments in the Australian trials are as follows:

- Test: the GMO
- Control: BG3 XF (BGII x COT102 x MON-88701-3 x RRF) in a similar background line
- Each season included data from one WA site and 2 sites each in Qld and NSW
- Due to lower germination rate identified, at some sites the test lines were planted at higher rates of seeds per metre
- No chemical sprays were applied for insect control
- All other crop managements were carried out according to the commercial agronomic practices for cotton farming, including irrigation and water management, cultivation and bed preparation, weed control and plant nutrition.

79. Measures assessed were:

- Stand counts - early (21 days after planting - DAP), final (7 days prior to harvest)
- Plant vigour rating
- Plant height (every 21 days from 21 DAP)
- Number of nodes (every 21 days from 21 DAP)
- Nodes above white flower (NAWF) – 3 samples, taken weekly from 7 days after flowering (DAF)
- Cotton seed weight per plot and as kg/ha
- Lint yield per plot and kg lint/ha
- Fibre quality measures - length, uniformity, short fibre index, strength, elongation and micronaire
- Seed measures - seed index (g/100 seeds), seed number per 50 bolls on acid-delinted seed (boll sample collected 4 days prior to harvest).

80. Both combined site data (5 sites) and individual site data were analysed for 2018-2019 and 2019-2020 sites. There were few significant differences between test and control plants in either season or for individual site data (Table A3, Appendix A). Differences were:

- Plant stand counts (plants/m) were lower for test plants at one site in 2018-2019 due to susceptibility of the test cultivar to the fungal disease Verticillium wilt, but no significant difference ($p < 0.05$) was apparent for combined site comparisons
- Plant stand counts (plants/m) were higher for test plants at one site in 2019-2020, corresponding with a higher planting rate, but no significant difference was apparent for combined site comparisons
- Yield (kg lint/ha) was significantly lower for test plants than control plants at 2 sites in 2019-2020, but no significant difference was apparent for combined site comparisons.

81. The applicant suggested that the differences, including lower stand count and lower yield at individual sites, may have been influenced by the lower disease tolerance (e.g. to Verticillium wilt) in the

test plant genetics. However, there were not consistent differences observed between the test and control plants, and their similarity in terms of growth rate and habit indicates that there was no difference in the overall agronomic performance or the phenotype of the test cotton plants compared to the control plants.

United States phenotypic characterisation of the GMO

82. The applicant has provided phenotypic and environmental interactions measures for 2016 season in the USA.

83. These studies compared the GMO to the control (non-GM line DP393) and commercial non-GM reference lines. Four reference lines were planted at each site, chosen from a group of 14 possible commercial lines used across all sites. Eight sites were set up as a randomised block design, with 4 replicates per site.

84. The GMO was compared to the control in a combined-site analysis for 8 phenotypic characteristics: early stand count, days to flowering, final stand count, plant height, total bolls, first position fruit retention, seed cotton yield, and seed weight.

85. The GMO was also compared to the control in a combined-site analysis for environmental interaction assessments (Table 7).

Table 7 Environmental measures collected for US field trials of the GMO

Measure class	Measure ^a
Abiotic stressors	drought, flooding, hail injury, heat, nutrient deficiency, soil compaction, sandstorm, wet soil and wind
Disease stressors ^b	blights, rots, rusts, leaf spots and wilts
Arthropod stressors	9 non-lepidopteran arthropods

^a abiotic, disease and arthropod measures were by observation

^b thirteen disease stressors assessed

86. Data were analysed from individual sites, as well as combined data across 8 sites. For some parameters, there were significant differences ($p < 0.05$) observed at individual sites, but not for combined site analyses. Likewise, a significant difference found for combined site data may not mean that each site showed a significant difference when analysed individually.

87. In the combined site analysis significant differences between the GMO and the control were observed in 2 phenotypic characteristics for the GMO: higher first position fruit retention, which showed significant differences at 3 individual sites, and lower seed weight, which showed significant differences at 6 sites (Table 8).

Table 8 US phenotypic characterisation of the GMO

Characteristic (units)	Mean (SE) GMO ^a	Mean (SE) Control ^{a,b}	Reference Range ^c
Early Stand Count (plants/2 rows)	177.8 (5.11)	178.5 (4.94)	148.5 - 244.6
Days to flowering ^d	57.1 (0.82)	57.4 (0.98)	50.6 - 64.1
Final Stand Count (plants/2 rows)	170.7 (5.23)	166.1 (5.15)	144.4 - 230.4
Plant height (cm)	94.7 (4.34)	94.8 (4.57)	80.0 - 166.0
Total bolls (bolls/plant)	10.0 (0.50)	9.8 (0.57)	3.7 - 11.8
First position fruit retention (%)	51.6 (3.60) *	46.0 (3.16)	10.4 - 61.0
Yield (kg/ha)	3486.2 (221.82)	3526.5 (210.91)	1142.5 - 4460.5
Seed weight (g)	7.3 (0.10) *	8.2 (0.15)	6.4 - 10.2

*Indicates a statistically significant difference between test and the conventional control ($\alpha=0.05$) using ANOVA.

^a N = 32 for test/control means. SE= standard error.

^b Control is non-GM commercial cotton line

^c Minimum and maximum mean values among 14 references, where each mean was combined over all the sites at which the reference was planted.

^d Days after planting

88. In addition to this, 4 individual sites showed significant differences between the GMO and the control for phenotypic characteristics for, including higher days to first flower (one site), higher total bolls (2 sites) and lower yield (one site), but these differences were not seen for the combined site analysis.

89. For combined site data, test and control means for all phenotypic measures were within the range for reference material across all sites. This indicates that although there were some statistically significant differences in these parameters, there were no biologically meaningful differences.

90. There were no differences observed between GMO and the control for any of the assessed abiotic, disease or arthropod stressors.

5.2.4 Compositional analysis

91. Seed collected from the GMO grown at field trial sites in the USA was analysed for proximates (protein, total fat, and ash), amino acids (18), fatty acids (7 components), carbohydrates by calculation, fibre (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total detergent fibre (TDF)), minerals (calcium and phosphorus), vitamin E and anti-nutrients (gossypol and cyclopropenoid fatty acids), as well as moisture content (Table A4, Appendix A).

92. Of the 40 measures, 26 showed statistically significant ($p < 0.05$) differences between the GMO and non-GM control line (DP393). However, the means for all 26 measures were within the ranges available from the International Life Sciences Institute (ISLI) [Crop Composition Database](#) (accessed February 2025) and within ranges cited in literature, as provided by the applicant.

93. In summary, although there were differences in cottonseed composition between the GMO and conventional cotton, the mean values for all measures were within the range of expected variation between lines and locations.

94. FSANZ has assessed each of the GM parental cotton lines and determined that the cotton lines are compositionally equivalent to conventional cotton and that food derived from these lines is considered to be as safe for human consumption as food derived from conventional cotton cultivars. FSANZ approval includes foods produced from cotton lines generated by conventional crossing of approved GM lines, therefore food derived from the GMO is included in these approvals.

5.3 Toxicity and allergenicity of the proteins encoded by the introduced genes

5.3.1 Introduced herbicide tolerance and selectable marker proteins

95. The GMO contains glyphosate (*cp4 epsps*), glufosinate (*bar*) and dicamba (*dmo*) herbicide tolerance genes from XF and selectable marker genes from BG3. These introduced genetic materials, source organisms and traits are summarised in Table 2.

96. 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 have been detailed in their respective RARMPs listed in Table 1, most recently in DIR 124 and DIR 145. These assessments concluded that the proteins pose negligible risk to human or other organisms. Additional information about the marker genes is available in the [Marker genes in GM plants](#) reference, available on the OGTR website.

5.3.2 Introduced insect resistance proteins

97. The introduced Vip3Aa19 and Cry proteins for insect resistance are derived from Bt, which is naturally found in the environment including in soil, on plant surfaces and on animals. Microbial biopesticide formulations of Bt have been used commercially for several decades (OECD, 2007). Extensive toxicity testing of the Bt biopesticides that contain numerous Cry proteins has not provided any evidence for adverse effects on human or animal health (Koch et al., 2015; McClintock et al., 1995).

98. 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), with the conclusion that these proteins pose negligible risk to humans or other organisms.

99. The applicant has not supplied data related to the toxicity or allergenicity of the mCry51Aa2 protein. However, Health Canada recently assessed toxicology and allergenicity studies of mCry51Aa2 supplied by Monsanto Canada Inc. The full assessment can be found in the [Health Canada website](#). The following is the summary of this assessment.

100. The mCry51Aa2 toxicity studies on CD-1 mice found no adverse effects at high doses (5000 mg/kg body weight). *In silico* analysis showed no relevant similarity to known toxins, and *in vitro* tests indicated the mCry51Aa2 protein was rapidly digested in the gastrointestinal tract.

101. Heat stability tests demonstrated that the mCry51Aa2 protein was inactivated during cotton oil processing, meaning consumers would not be exposed to active protein. Additionally, cotton seed products including refined oil and cellulose contain negligible amounts of protein, thus dietary exposure to mCry51Aa2 is minimal.

102. Allergenicity assessment, including sequence comparisons and digestibility studies, showed no evidence of the mCry51Aa2 matching known allergens or being available to provoke allergic reactions.

103. Overall, cottonseed oil and linters derived from MON-88702-4 cotton are not expected to have higher toxicity or allergenicity than conventional cotton.

5.4 Effects of the GMO on non-target invertebrates

104. No adverse effects of BG3 and XF on non-target invertebrates were identified in the RARMPs for DIR 124 and DIR 145. As previously discussed earlier in Section 4.4.2 of this Chapter, the GMO does not adversely affect non-target arthropods.

105. Synergistic, additive and antagonistic interactions between the Cry1Ac and Cry2Ab proteins were reviewed in previous RARMPs (DIR 059/2005, DIR 066/2006, DIR 124 and DIR 145), with the conclusion that Cry1Ac and Cry2Ab acted independently without any consistent evidence of any of these effects. In addition, a study examining interactions between Cry1Ac and Vip proteins in their effects on Lepidoptera species found no evidence of any interactions between Cry1Ac and Vip3Aa (Lemes et al., 2014).

106. However, recent studies reported that some combinations of these proteins showed synergistic effects against Lepidoptera pests of cotton (Liu et al., 2024). For example, Cry1Ac and Cry2Ab interacted synergistically against cotton bollworm (*Helicoverpa armigera*) and Egyptian bollworm (*Earias insulana*), and the combination of Cry2Ab and Vip3Aa19 proteins showed synergistic effects on fall armyworm (*Spodoptera frugiperda*) larvae (Liu et al., 2024).

107. The applicant has provided data from diet incorporation bioassays that evaluated the potential for interaction between mCry51Aa2 and Cry1Ac and Cry2Ab, or Vip3Aa using 2 cotton pests. Toxicity to cotton bollworm (*Helicoverpa zea*), targeted by the Cry1Ac, Cry2Ab and Vip3Aa19 proteins, was evaluated using tissue from BGII (Cry1Ac, Cry2Ab), COT102 (Vip3Aa19) and the GMO (mCry51Aa2, Cry1Ac, Cry2Ab and Vip3Aa19) with a non-GM control. The results from this comparison demonstrated that the activity of Cry1Ac, Cry2Ab and Vip3Aa19 against this target species was not altered in the GMO, i.e. the presence of mCry51Aa2 did not affect the activity of these toxins in the GMO.

108. Toxicity to the Western tarnished plant bug (*Lygus hesperus*) targeted by the mCry51Aa2 protein was evaluated using tissue from MON-88702-4 (mCry51Aa2) and the GMO (mCry51Aa2, Cry1Ac, Cry2Ab and Vip3Aa19), with a non-GM control. This comparison showed that the activity of mCry51Aa2 on the target organism was not altered in the GMO, i.e. the presence of the Cry1Ac, Cry2Ab and Vip3Aa19 did not affect the activity of mCry51Aa2 in the GMO.

109. Overall, these results suggest that the BG3 proteins and mCry51Aa2 protein have no synergistic or antagonistic effect on toxicity to cotton bollworm and Western tarnished plant bug.

5.5 Experience from cultivation of the GMO

110. Field data from trials conducted with the GMO in Australia and the USA have been discussed in earlier this section (Sections 5.2.2-5.2.4). The results indicated that the GMO did not show increased weediness potential, and its composition was substantially equivalent to that of conventional cotton.

111. The GMO has been approved for field trials in Australia under DIR 147, and has been cultivated on over 172,800 ha (2021-2023) commercially in the USA (data supplied by applicant). The applicant states that no adverse consequences have been recorded from the field trials or commercial release.

5.6 Approvals of the GMO

112. As mentioned previously, FSANZ has approved use of oil and linters from each of the parental cottons and these approvals include material from lines generated by conventional crossing of the parents, hence the GMO is automatically approved.

113. The BG3 has been registered by the APVMA for use as an insecticidal substance. The applicant will seek APVMA approval for the cultivation of MON-88702-4 for use as an insecticidal substance, prior to commercial release of the GMO in Australia. The applicant has APVMA registrations for the use of glyphosate-, glufosinate- and dicamba-containing herbicides on herbicide tolerant cottons including RRF and MON-88701-3 (information supplied by the applicant).

114. The parental GM lines for the GMO, including MON-88702-4, have received non-regulated status from United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) in the USA (USDA-APHIS, 2002, 2004, 2005, 2012, 2021). Also, the parental GM lines have been considered safe for human consumption and approved for environmental release in Canada ([Health Canada & CFIA](#)).

115. The GMO has been approved for food (including direct use or processing) in Colombia and Taiwan, and for food and feed (including direct use and processing) in South Korea (ISAAA [GM approval database](#); accessed February 2025).

116. Approvals for the GMO and its parental GM lines (BG3, XF and MON-88702-4) have been provided in earlier sections of Chapter (Section 4.2-4.4).

Section 6 The receiving environment

117. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO is 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, the presence of plants that are sexually compatible with the GMO, and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

118. The applicant has proposed to release the GMO in all commercial cotton growing areas, Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia but especially, agricultural areas that are suitable to cultivate cotton. Commercial cotton production occurs mainly in NSW, and Qld, but it is also grown in Vic, WA and the NT. The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

119. The cultivation practices, and abiotic and biotic factors for cotton are discussed in detail in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR, 2024). The following summary is based on this document.

6.1 Relevant agronomic practices

120. It is anticipated that the agronomic practices for cultivation of the GMO will not differ significantly from industry best practices used in Australia, where over 99% of commercial cotton grown is GM and is likely have one or more traits found in the GMO. All cotton plants would be grown following standard cotton agricultural management practices and would receive applications of water and fertilisers, similar to current commercially grown non-GM and GM cotton crops; and herbicide applications similar to current commercially grown GM cotton crops.

121. The control of cotton volunteers is usually achieved by mechanical means such as cultivation, or use of a range of herbicides, preferably as part of Integrated Weed Management (IWM) practices. Seven mode of action (MoA) groups of herbicides, including glufosinate, are currently registered for cotton volunteer control in Australia. Control of volunteer cotton by herbicides is most effective on seedling cotton and only

one herbicide (fluroxypyr) is registered for control of large (15- to 30 node) volunteer cotton (CRDC, 2024). Currently, dicamba and glyphosate are not registered for use in volunteer cotton control in Australia (CRDC, 2024). For further information, refer to the [Public Chemical Registration Information System \(PubCRIS\)](#) on the APVMA website.

122. The use of Integrated Pest Management (IPM) is preferred to control insect pests. This involves using a range of tactics throughout the season to manage pest and beneficial insect populations in and around farms. Use of insecticides is only one part of this system and use of IPM is important to slow the development of insecticide resistance (CottonInfo, 2016; CRDC, 2024).

6.2 Relevant abiotic factors

123. The abiotic factors relevant to the growth and distribution of commercial cotton 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.

6.3 Relevant biotic factors

6.3.1 Presence of sexually compatible plants in the receiving environment

124. Commercial cotton grown in Australia is either *G. hirsutum* or *G. barbadense*, of which over 99% is *G. hirsutum*. The GMO proposed in this RARMP is capable of crossing with both *G. hirsutum* and *G. barbadense*.

125. In the natural environment for successful hybridisation to occur parent plants must occur in close proximity, flower at the same time, have pollen from one plant deposited on the stigma of the other, fertilisation must occur, and progeny must survive to sexual maturity. Additionally, for hybrids to persist in the environment, any progeny seed would have to be viable. Cotton is largely self-pollinating, and no self-incompatibility mechanisms exist. Where cross-pollination does occur, it is likely facilitated by honeybees. Cotton does not reproduce by asexual mechanisms, although root cuttings can be propagated under laboratory conditions (OGTR, 2024).

126. Cotton (*G. hirsutum*) is not recorded in the Australian government's Weeds of National Significance list on the [Weeds Australia website](#) (accessed February 2025), or the Noxious Weed List for Australian States and Territories (Invasive Plants and Animals Committee, 2015). Most reports of *G. hirsutum* volunteers or naturalised populations are from tropical regions of Australia, and cotton-growing areas throughout Qld and NSW ([Atlas of Living Australia](#) accessed June 2025). Persistence of feral populations is limited, as *G. hirsutum* has negligible ability to invade non-disturbed habitats and any such populations are not routinely subjected to control measures such as the use of herbicide and cultivation (OGTR, 2024). Records of feral cotton presence do not indicate a marked change in the number of records or the pattern of occurrence ([Australia's Virtual Herbarium](#) accessed March 2025) since the previous comprehensive review in the RARMP for DIR 124 (OGTR 2014). If gene transfer from the GM cottons to feral cotton were to occur, the presence of herbicide tolerance genes in those feral cottons would not be expected to provide a selective advantage in the absence of herbicide application. The presence of insect resistance genes is unlikely to provide a selective advantage in the absence of high pest insect pressure.

127. 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 and Hearnden, 2006). Volunteer seedlings that emerge over winter are likely to be killed by frosts, while dry winters may promote volunteer survival and emergence in warmer months, with spring rains and irrigation promoting volunteer growth and development.

128. As well as the parental GM cottons (Table 1), several other insect resistant and/or herbicide tolerant GM cotton lines have been approved for commercial release in Australia (Table 9). These form part of the risk context for this DIR licence application.

Table 9 Australian approvals for the commercial release of other insect resistant and or herbicide tolerant GM cotton lines^a.

GM cotton	Genes	DIR licence number	Comment
Liberty Link®	<i>bar</i>	062/2005	Glufosinate herbicide tolerance
Widestrike™	<i>cry1Ac</i> (synthetic) <i>cry1F</i> (synthetic) <i>pat</i>	091	Insect resistance and glufosinate herbicide tolerance
GlyTol®	<i>2mepsps</i>	143	Glyphosate herbicide tolerance
GlyTol TwinLink Plus®	<i>2mepsps</i> <i>bar</i> <i>cry 1Ab</i> <i>cry 2Ae</i> <i>vip3Aa19</i>	143	Glyphosate and glufosinate herbicide tolerance and 3 insect resistance

^aFor the licences relating to the parental lines of the GMO, see Table 1 (Section 4.1)

129. To date, the Regulator has not received any reports of adverse effects caused by these authorised releases, although it should be noted that not all of these have been planted on a commercial scale.

6.3.2 Presence of related native plants in the receiving environment

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

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

6.3.3 Presence of other biotic factors

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

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

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

135. Cotton is susceptible to competition from weeds. Problematic weeds range from large plants such as Noogoora burr (*Xanthium occidentale*), Bathurst burr (*Xanthium spinosum*), thornapples (*Datura* spp.) and sesbania (*Sesbania canabina*), to vines such as cowvine and bellvine (*Ipomoea* spp.), yellow vine or spineless caltrop (*Tribulus* spp.), to grasses such as nut grass (*Cyperus rotundus*) (CRDC, 2013). Some weed species are alternate hosts for diseases of cotton, e.g. many weeds are hosts for *V. dahliae* (CRDC and CottonInfo, 2017). These weed species have not been recorded globally as resistant to glyphosate, glufosinate or dicamba (Heap, 2025).

136. In Australia, at least 20 glyphosate-resistant and one dicamba-resistant weed species have been reported, but no glufosinate-resistant weed species have been recorded (Heap, 2025). To date, at least 11 and 5 weed species globally are reported to have resistance to dicamba and glufosinate, respectively (Heap, 2025).

6.3.4 Presence of the introduced or similar genes and encoded proteins in the receiving environment

137. The introduced genes for insect resistance and herbicide tolerance in the GMO are derived from common soil-borne microorganisms. The genetic regulatory sequences that have been used in the development of GMOs, are derived from plants, plant viruses and a common soil bacterium. All these source organisms are present in Australia with the exception of Figwort mosaic virus (FMV) (Atlas of Living Australia accessed May 2025). However, FMV-derived regulatory elements are commonly used as promoters in the development of GM plants. Hence, the introduced or similar genes and regulatory sequences in the GMO are also present in the Australian environment (Table 2). The introduced selective marker genes are prevalent in naturally occurring bacteria found in the Australian environment in soil, and in animal and human digestive systems. Thus, humans and other organisms would commonly encounter these genes and their encoded proteins (Table 2).

Chapter 2 Risk assessment

Section 1 Introduction

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

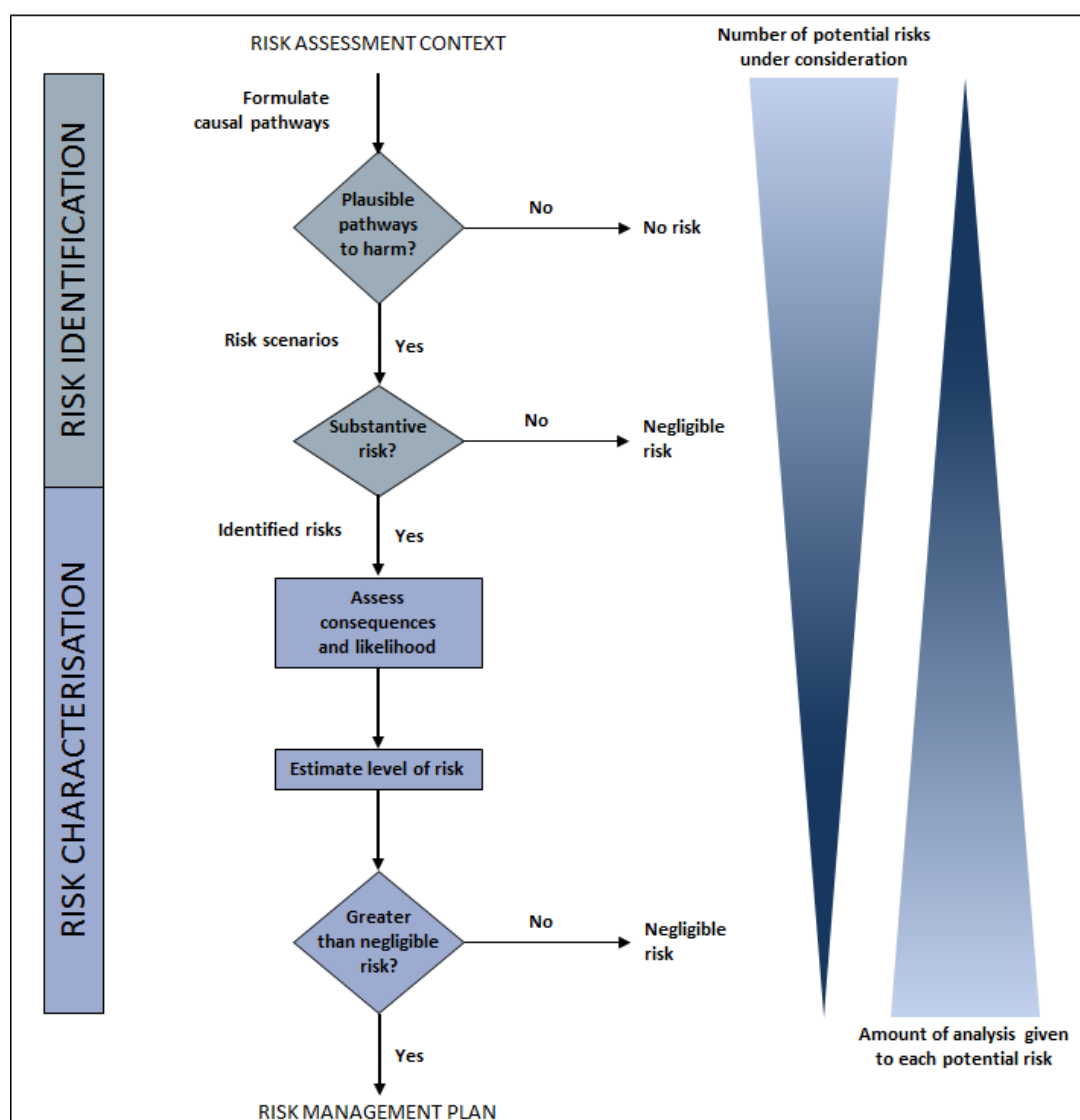


Figure 2 The risk assessment process

139. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013). Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

140. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 2), i.e. the risk is considered no greater than negligible.

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

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

Section 2 Risk Identification

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

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

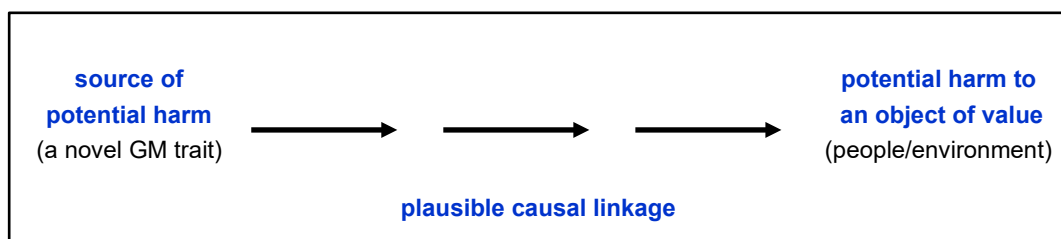


Figure 3 Components of a risk scenario

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

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

2.1 Risk source

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

146. As discussed in Chapter 1, the GMO has been modified by the introduction of 3 genes for herbicide tolerance and 4 genes for insect resistance. It also contains 4 marker genes, including 3 antibiotic resistance genes and one reporter gene.

The introduced genes for herbicide tolerance

147. The *cp4 epsps* and *bar* genes from the RRF parental cotton confer tolerance to the glyphosate and glufosinate herbicides, respectively. The *dmo* gene from MON-88701-3 parental cotton confers tolerance to dicamba herbicide. These genes and their encoded proteins have been evaluated in a number of

commercial cotton licence RARMPs (Table 1) and were assessed as posing negligible risk to human or animal health and the environment by the Regulator. Furthermore, they have been assessed and approved by other regulatory agencies in Australia and overseas (see Chapter 1, Section 4.3).

148. The RARMP for DIR 145 considered these 3 herbicide tolerance genes in combination with respect to potential for weediness. No substantive risk was identified, as glyphosate, glufosinate and dicamba are not used commonly to control volunteer cotton or other weeds. No new information was found to change this conclusion (see Chapter 1, Section 4.3.1) and there are no additional herbicide tolerance genes in the GMO. Therefore, these genes will not be considered further as potential sources of risk for this application.

The introduced genes for insect resistance

149. The *cry1Ac*, *cry2Ab* and *vip3Aa* genes, which confer resistance to lepidopteran insects, along with their encoded proteins, have been assessed, individually and in combination, in several commercial cotton licence RARMPs (Table 1, Chapter 1). The RARMP for DIR 124 also considered the potential for interaction (e.g. synergistic or antagonistic) between these proteins that may lead to increased harm. These previous RARMPs concluded that these genes and their proteins pose negligible risk to human health or the environment. There are no credible reports of adverse effects on human health or the environment since those RARMPs were published. In addition, FSANZ has approved the use of material derived from GM cottons containing the *cry1Ac*, *cry2Ab* and/or *vip3Aa* genes and their proteins. Therefore, these 3 genes will not be individually considered further as potential sources of risk for this application.

150. The *mCry51Aa2* gene, which encodes the mCry51Aa2 protein and confers resistance to hemipteran and thysanopteran insect pests, has not been assessed previously for commercial release in Australia. This introduced gene is considered further as a potential source of risk. Additionally, the potential for interaction (e.g. synergistic or antagonistic) between mCry51Aa2 protein and Cry1Ac, Cry2Ab and Vip3Aa proteins will be considered and whether any such interaction may lead to increased harm. Interactions with other insect resistance genes from sexually compatible plants will also be considered.

Selectable marker and reporter genes

151. The GMO also contains 3 selectable marker genes that confer antibiotic resistance (*nptII*, *aph4* and *aad*) and a reporter gene (*uidA*). These 3 marker genes all originated from the BG3 parental line. These genes and their products have been extensively characterised and have been assessed by the Regulator as posing negligible risk to human health or to the environment, and by other regulatory agencies in Australia and overseas. As noted in Chapter 1 (Section 6.3.4), these marker genes and their related products are prevalent in the Australian environment. Further information about these genes can be found in [DIR 012/2002 RARMP](#) and in the document *Marker genes in GM plants* available from the [Risk Assessment References page](#) on the OGTR website. As these genes have not been found to pose a substantive risk to either people or the environment, they will not be further considered as potential sources of harm.

The regulatory sequences

152. The introduced genes are controlled by introduced regulatory sequences. These are derived from plants, bacteria and plant viruses (see Chapter 1, Table 2). Regulatory sequences are naturally present in plants and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein and dietary DNA has no toxicity (Society of Toxicology 2003). As discussed in Chapter 1 and in previous RARMPs, these sequences have been widely used in other GMOs, including the parental GM lines that are grown commercially, without reports of adverse effects. Hence, risks from these regulatory sequences will not be further assessed for this application.

Unintended effects

153. The genetic modifications have the potential to cause unintended effects in several ways, including altered expression of endogenous genes due to random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the proteins encoded by the introduced genes, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, the range of unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. 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 (Weber et al. 2012; Steiner et al. 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further in this application.

2.2 Causal pathway

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

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

155. Although all these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs or are not expected to give rise to substantive risks (see sections 2.2.1 to 2.2.3 below).

2.2.1 Gene transfer to sexually compatible relatives

156. As discussed in Chapter 1, Section 6.3.1, *G. hirsutum* is sexually compatible with all GM and non-GM *G. hirsutum* varieties, as well as *G. barbadense*. Therefore, some cross-hybridisation with these plants is inevitable. However, gene transfer to Australian native cotton species is not expected due to genetic incompatibility.

157. Also noted in Chapter 1, Section 6.3.1, gene transfer from the GMO to feral cotton is unlikely to confer an advantage in the absence of cultivation or herbicide use. Therefore, only gene transfer to cultivated *G. hirsutum* and *G. barbadense* will be considered further.

2.2.2 Segregation of genes present in the GMO

158. It should be noted that the GMO was generated by conventional crossing between 5 GM cotton lines, so the introduced genes are located in different regions of the plant genome and may segregate independently of one another. Therefore, after any initial outcrossing of the GMO to other cotton, subsequent generations of cotton volunteer plants may contain all the genes from the GMO, genes from

one of the GM parental cottons, genes from combinations of some of the parental lines of the GMO, or none of the genes from the GMO. The resulting cottons will have equivalent or less insect resistance and herbicide tolerance than a GMO volunteer plant with all genes, so the assessment for weediness as a result of gene transfer of the introduced genes to other cottons is not affected. Therefore, segregation of the inserted genes will not be considered further.

2.2.3 Gene transfer by HGT

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

2.2.4 Unauthorised activities

160. 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

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

- harm to the health of people or desirable organisms², including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

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

2.4 Postulated risk scenarios

163. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 10 and examined in detail in Sections 2.4.1 – 2.4.3. Postulation of risk scenarios considers potential impacts of the introduced *mCry51Aa2* gene in the GMO, or its products, on people undertaking the dealings, as well as impacts on people and the environment exposed to the GMO or its

² Desirable organisms are those that are valued and should be protected, while undesirable organisms cause harm and should be controlled (OGTR, 2013). This is determined by legislation, government policies, national and international guidance material, and widely acceptable community norms. Undesirable plants that cause economic, social or environmental harm, or harm to human/animal health, are called weeds. Animals that cause harm are known as pests.

products as the result of the commercial use or the spread and persistence of plant material, including pollen.

164. In the context of the activities proposed by the applicant and considering both the short and long term, none of the 3 risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 10 Summary of risk scenarios from dealings with the GMO

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced insect resistance <i>mCry51Aa2</i> gene	Commercial cultivation of the GMO expressing the <i>mCry51Aa2</i> gene ↓ Exposure of humans and other desirable organisms by ingestion of, or contact with, GM cotton plant material or products, or inhalation of GM cotton pollen	Increased toxicity or allergenicity for humans OR Increased toxicity to other desirable organisms, including non-target invertebrates	No	- There is limited exposure of humans to the expressed protein. - The mCry51Aa2 protein has no demonstrated toxicity or allergenicity for humans. - The mode of action of Cry proteins, including mCry51Aa2, is well understood and is not known to influence the levels of endogenous cotton toxins. - Consumption of cotton by livestock is limited. - The mCry51Aa2 protein is toxic to insects from specific orders and other animals or invertebrates outside these orders are not expected to be adversely affected.
2	Introduced insect resistance <i>mCry51Aa2</i> gene	Commercial cultivation of the GMO expressing the <i>mCry51Aa2</i> gene ↓ Persistence of the GMO seeds in agricultural areas or dispersal of the GMO to nature reserves or intensive use areas ↓ Establishment of population of volunteer GMO in agricultural areas, nature reserves or intensive use areas	Increased toxicity or allergenicity to people OR Increased toxicity to other desirable organisms, including non-target invertebrates OR Reduced establishment or yield of desirable agricultural crops OR Reduced establishment or yield of desirable plants in the environment OR Reduced utility or quality of the environment OR Increased reservoir for pest or pathogens	No	- GMO volunteers can be controlled by standard weed management measures. - The spread and persistence of cotton is restricted by a range of biotic and abiotic factors other than herbivory by insect pests. - As cotton is unlikely to spread and persist in the environment, it is unlikely to provide a reservoir for pests or pathogens. - As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the mCry51Aa2 protein.
3	Introduced insect resistance <i>mCry51Aa2</i> gene	Commercial cultivation of the GMO expressing the <i>mCry51Aa2</i> gene ↓	Increased toxicity or allergenicity in people or toxicity to desirable organisms	No	- Cotton is predominantly self-pollinating and has limited ability to outcross. - Hybrid cottons are unlikely to spread and persist in the

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
		Pollen from GM plants dispersed outside the cropping areas ↓ Outcrossing with sexually compatible plants ↓ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	OR Reduced establishment or yield of desirable agricultural crops OR Reduced utility or quality of the environment OR Increased reservoir for pest or pathogens		environment as the spread and persistence of cotton is restricted by abiotic and biotic factors. - As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the mCry51Aa2 protein. - No synergistic or antagonistic effects have been shown between mCry51Aa2 with other 3 insect resistance proteins in the GMO. - Gene transfer from the GMO to other commercially approved GM cottons is unlikely to produce hybrids with a greater potential to spread and persist in the environment than the GM parental lines or the GMO.

2.4.1 Risk scenario 1

<i>Risk Source</i>	Introduced insect resistance <i>mCry51Aa2</i> gene
<i>Causal pathway</i>	Commercial cultivation of the GMO expressing the <i>mCry51Aa2</i> gene ↓ Exposure of humans and other desirable organisms by ingestion of, or contact with, GM cotton plant material or products, or inhalation of GM cotton pollen ↓
<i>Potential harm</i>	Increased toxicity or allergenicity for humans OR Increased toxicity to other desirable organisms including non-target invertebrates

Risk source

165. The source of potential harm for this postulated risk scenario is the introduced insect resistance *mCry51Aa2* gene.

Causal pathway

166. The applicant proposes that the GMO would be cultivated on a commercial scale in all Australian cotton producing areas. The insect resistance gene *mCry51Aa2* is expressed in leaf, root, seed, pollen, square and boll tissues of the GMO (Chapter 1, Section 5.2.2). People and other desirable organisms may be exposed to the mCry51Aa2 protein through contact, consumption of plant material, plant products, or by inhalation of pollen.

167. The introduced gene and expressed protein are unlikely to be present in cotton products such as cottonseed oil, fibres and linters (FSANZ, 2018b). The public, who consume cottonseed oil and cottonseed linters, or have contact with cotton fabrics, are not expected to be exposed to the introduced gene and its products. Therefore, the people most likely to be exposed to the introduced gene and its product will be workers involved in breeding, cultivating, harvesting, transporting and processing the GMO.

168. Expression of the insect resistance gene in the GMO, or in a GM volunteer, may expose other organisms, including livestock, to the GM plant material through contact or ingestion. The mCry51Aa2 protein may also occur at low levels in the soil as a result of plant material remaining after harvest.

169. Livestock may be exposed to cotton in the form of white cottonseed – seeds left after fibre removal during ginning – and cottonseed meal in feed rations, or through limited grazing of stubble. However, the amount of cotton plant material (both GM and non-GM) that is consumed by livestock is, by necessity, limited due to the presence of endogenous toxins such as gossypol.

170. Other organisms, including wild mammals, birds, soil microbes and non-insect invertebrates would also be exposed to the GMO material in agricultural areas where the GMO is cultivated. The non-target invertebrates may include non-pest insect species that consume the GMO plant, for example, insect predators of pest species, parasitoids, pollinators, or soil biota. These organisms may be exposed to the mCry51Aa2 protein through contact, ingestion or indirectly by feeding on herbivores that have ingested the GMO.

Potential harm

171. If humans and other organisms were exposed to the GMO, the potential harms are increased toxicity or allergenicity to humans or increased toxicity to desirable organisms.

172. The mCry51Aa2 protein is unlikely to be toxic to humans, other vertebrates, or the majority of the non-target invertebrates that lack the receptors to which the mCry51Aa2 protein binds (Chapter 1, Section 4.4.2 and Section 5.3.2). As also discussed in Chapter 1, Section 5.3.2, the mCry51Aa2 toxicity studies on CD-1 mice found no adverse effects at high doses. The mCry51Aa2 protein has no sequence similarity to known protein allergens and toxins, and is rapidly degraded in simulated gastric fluid (FSANZ, 2018b). FSANZ assessed the safety of human food derived from MON-88702-4 cotton expressing mCry51Aa2 protein and concluded that it is as safe for human consumption as food derived from conventional cotton (FSANZ, 2018a). It is therefore not expected that the GMO expressing mCry51Aa2 protein would be toxic or allergic to people, or toxic to other desirable organisms.

173. For livestock, cotton (*G. hirsutum*) tissue, particularly the seeds, can be toxic if ingested in excessive quantities due to the presence of endogenous anti-nutritional and toxic factors including gossypol and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). The presence of gossypol and cyclopropenoid fatty acids in cottonseed limits its use as a protein supplement in animal feed. Ruminants are less affected by these components, which are detoxified by digestion in the rumen, but its use as stockfeed is limited to a relatively small proportion of the diet (Blasi & Drouillard 2002).

174. The presence of the *mCry51Aa2* gene is not expected to affect the levels of endogenous toxins. This is supported by data provided by the applicant (Chapter 1, Section 5.2.4) showing that gossypol and cyclopropenoid fatty acid levels in seed from the GMO are within the recorded range of non-GM cottons and ranges cited in literature. It is not expected that exposure of animals, either through intentional feeding of cottonseed to livestock or occasional, opportunistic feeding of other animals would have an increased risk from exposure to endogenous toxins as a result of the genetic modification.

175. Exposure of non-target invertebrates, particularly arthropods, to the expressed mCry51Aa2 protein may result in death, slowed growth rate, or reduced fecundity if the protein is toxic to these organisms. Arthropods that feed on hemipteran and thysanopteran insects could be adversely affected due to loss of a food source. However, as discussed in Chapter 1, Section 4.4.2, mCry51Aa2 protein shows high degree of specificity to target hemipteran and thysanopteran species and is not expected to harm invertebrates outside these orders. Data from an Australian field study (Chapter 1, Section 4.4.2) indicate that the GMO does not affect the abundance of 13 key beneficial non-target arthropods compared to the BG3 XF control, including non-target insects within the same order as target species.

176. The degradation and persistence of Cry proteins in soil are primarily influenced by microbial activity, which is affected by factors such as soil type, pH, and temperature and other physicochemical and biological properties (APVMA, 2011). For instance, Cry proteins are adsorbed on clay particles and humic substances in soil, which may protect Cry proteins from microbial degradation and allow them accumulate and persist (APVMA, 2011). This adsorption is likely to persist at pH levels below the proteins' isoelectric

point. Despite their persistence under certain conditions, Cry proteins from Bt crops do not show consistent, significant or long-term effects on soil microbial communities or their activities. Additionally, Cry proteins have minimal to no adverse effects on soil-dwelling invertebrates such as earthworm and Collembola (APVMA, 2011). These findings are also supported by the toxicity assessment of mCry51Aa2 (Chapter 1, Section 4.4.2).

Conclusion

177. Risk scenario 1 is not identified as a substantive risk, due to limited exposure of humans and other desirable animals to the expressed mCry51Aa2 protein, the lack of toxicity or allergenicity of the mCry51Aa2 protein to humans or toxicity to other desirable organisms including non-target invertebrates and the modifications in the GMO are not expected to affect the levels of endogenous toxins present in non-GM cotton. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk Source	Introduced insect resistance <i>mCry51Aa2</i> gene
Causal pathway	Commercial cultivation of GMO expressing the <i>mCry51Aa2</i> gene ↓ Persistence of the GMO seeds in agricultural areas or dispersal of the GMO to nature reserves or intensive use areas. ↓ Establishment of population of volunteer GMO in agricultural areas, nature reserves or intensive use areas. ↓
Potential harm	Increased toxicity or allergenicity to people OR Increased toxicity to other desirable organisms including non-target invertebrates OR Reduced establishment or yield of desirable agricultural crops OR Reduced establishment or yield of desirable plants in the environment OR Reduced utility or quality of the environment OR Increased reservoir for pest or pathogens

Risk source

178. The source of potential harm for this postulated risk scenario is the introduced insect resistance *mCry51Aa2* gene.

Causal pathway

179. The applicant proposes that the GMO would be cultivated on a commercial scale in all Australian cotton producing areas. Following harvest, the GMO seeds could remain in agricultural areas, potentially establishing populations of volunteer GM cotton plants in these areas.

180. Seeds from the GMO may be spread from farms into nature reserves by humans, animals, water or extreme weather. Cottonseed is primarily spread off-farm within a localised area during transport of modules to gins, and through irrigation and stormwater runoff. Cottonseed may also be dispersed during extreme weather events, i.e. via wind during wind storms and water during flooding, to adjacent agricultural areas and natural environments (OGTR, 2024).

181. The GMO may be introduced into regions that do not grow the crop through the use of whole cottonseed for supplementation feeding of cattle and sheep, particularly during drought when large piles of

cottonseed are placed into a paddock for stock to feed on over the course of several days ([QDAF website & Business Qld website](#), accessed April 2025). Cottonseed may also be introduced into environments around cattle feed lots and dairy farms, where it is used as stockfeed (OGTR, 2024). Cotton harvest equipment can transfer seeds between fields and properties if they are not cleaned prior to transport (CRDC, 2024).

182. Cotton volunteers are most likely to germinate in disturbed habitats, such as stockyards, roadsides along transport routes, and adjacent to waterways (OGTR, 2024). The geographic range of non-GM cotton in Australia is limited by a number of abiotic factors including climate (especially cold stress) and soil compatibility, as well as water and nutrient availability (OGTR, 2024). Feral cotton populations are sparse and ephemeral in all current cotton growing regions of Australia (OGTR, 2024).

183. 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 and Hearnden, 2006). Modelling of climatic factors limiting cotton persistence indicate that cotton has naturalisation potential only in the coastal regions of north-east Australia. A few small populations of naturalised cotton are reported in northern Australia, but these are not derived from modern cultivars (OGTR, 2024) and these tufted cottons may have a greater ability to survive outside agricultural settings than modern cotton cultivars.

184. Expression of the introduced *mCry51Aa2* insect resistance gene could reduce herbivory by certain hemipteran and thysanopteran species. This could in turn enhance the possibility of survival and establishment of the GMO, leading to increased persistence of the GMO in nature reserves. However, as discussed in Chapter 1, Section 5.2.3, Australian phenotypic characterisations showed no observed changes in the agronomic performance or phenotypic measures of the GMO compared to BG3 XF control. In addition, the germination and dormancy data for MON-88702-4 (Chapter 1, Section 4.4.4) showed that there were no differences in the germination and dormancy characteristics indicative of increased weediness potential of MON-88702-4 cotton compared to non-GM cotton. Additionally, factors other than insect herbivory, in particular abiotic stressors, are the key limiting factors for the survival of cotton outside cultivation, thus the presence of the *mCry51Aa2* gene is unlikely to provide a major advantage to GM cotton volunteers present after or outside cultivation. Taken together, these considerations indicate that the introduced gene *mCry51Aa2* is not expected to increase the spread and persistence of the GM seed.

Potential Harm

185. If the GMO expressing *mCry51Aa2* gene remained as a volunteer population in agricultural areas after cultivation, or was able to spread and persist outside in the environment, the postulated harms are increased toxicity or allergenicity to people, increased toxicity to desirable animals, reduced establishment or yield of desirable plants and other organisms that rely on those plants, or increased reservoirs for pathogens or pests.

186. As discussed in Risk scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GMO expressing *mCry51Aa2* for humans or increased toxicity to desirable organisms other than certain target hemipteran and thysanopteran species.

187. The GMO could reduce establishment or yield of desirable plants, provide a reservoir for pests and pathogens in agricultural settings, or to provide an ongoing source of GM seed for spread into the environment.

188. If GM cotton seed persisted in agricultural areas after harvest and volunteer GM cotton plants emerged, it is not expected that expression of the *mCry51Aa2* gene would result in increased persistence of GM volunteers or reduced ability to control volunteer cotton plants. Thus, it is expected that the GM volunteers would be controlled by standard management practice for control of cotton volunteers, such as cultivation or use of herbicides (Chapter 1, Section 6.1). This would limit their ability to persist in agricultural areas, to provide a reservoir for pests or pathogens, or to provide a source of seed for spread outside agricultural areas following harvest.

189. If GM cotton seed was spread and persisted outside agricultural areas, it could reduce the establishment and yield of desirable plants in the environment, thereby reducing plant biodiversity and potentially adversely affecting populations of other organisms that rely on those plants in the environment, or it could act as a reservoir for pests and pathogens. These effects could occur in nature reserves or intensive use areas such as roadsides and waterways. As discussed in Chapter 1 (Section 5.2.3), there was no significant difference between the GMO and non-GM cotton varieties in their responses to a number of abiotic stressors. Therefore, the introduced genes are unlikely to make the GMO more tolerant to abiotic stresses that are naturally encountered in the environment and are unlikely to alter the potential distribution of the GMO plant. Thus, the introduced gene *mCry51Aa2* is not expected to increase the limited potential for cotton to survive outside agricultural settings, or its ability to spread and persist in the broader environment.

Conclusion

190. Risk scenario 2 is not identified as a substantive risk because cotton has limited ability to establish outside cultivation, establishment of cotton populations outside intended cropping areas and competition with desirable vegetation is primarily limited by abiotic factors, rather than by the herbivory of certain hemipteran and thysanopteran insects, and the introduced *mCry51Aa2* gene for insect resistance is not expected to affect the GMO's ability to respond to these limiting factors. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk Source	Introduced insect resistance <i>mCry51Aa2</i> gene
Causal pathway	Commercial cultivation of GM cottons expressing the <i>mCry51Aa2</i> gene ↓ Pollen from the GM plants dispersed outside the cropping areas ↓ Outcrossing with sexually compatible plants ↓ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment ↓
Potential harm	Increased toxicity or allergenicity in people or toxicity to desirable organisms OR Reduced establishment or yield of desirable agricultural crops OR Reduced utility or quality of the environment OR Increased reservoir for pest or pathogens

Risk source

191. The source of potential harm for this postulated risk scenario is the introduced insect resistance *mCry51Aa2* gene.

Causal pathway

192. The applicant proposes that the GMO would be cultivated on a commercial scale in all Australian cotton producing areas. Outcrossing could occur when the GMO proposed for release and other cotton crops are grown in proximity, with synchronous flowering times.

193. Cotton is predominately 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. 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 (Chapter 1, Section 6.3.1 – 6.3.2). It is not

expected that the introduced insect resistance gene *mCry51Aa* would alter the pollen dispersal characteristics of the GMO proposed for release.

194. Gene transfer to non-GM cotton or other GM cottons, including the parental cottons of the GMO, could occur. However, the resulting progeny would not have an increased range of insect resistance compared to the GMO and as such would not pose any harm other than those that have already been considered for the GMO. As discussed in Chapter 1, Section 5.4, *mCry51Aa2* and other 3 insect resistance proteins expressed by the GMO do not show synergistic or antagonistic effects on toxicity to their respective target insect species.

195. The GM cottons Widestrike® (DIR 091) and GlyTol TwinLink Plus® (DIR 143) have been approved for commercial release, although none has been planted commercially. Widestrike® expresses *cry1Ac* and *cry1F*, and GlyTol TwinLink Plus® expresses *cry1Ab*, *cry1Ae* and *vip3Aa19* insect resistance genes. If they are produced commercially in the future, the GMO could cross with these cottons and such crosses could result in progeny with some or all of the *cry* gene(s) from those GM cottons and/or an extra copy of the *cry1Ac* or *vip3Aa19* gene, in addition to the insect resistance genes in the GMO.

196. The *cry1F*, *cry1Ab* and *cry1Ae* gene products share the same mode of action as the Cry proteins produced by the GMO. The 3 gene products show specificity to lepidopteran insects and *cry1Ab* is also toxic to hemipteran insects. In the event of hybridisation, no additional insect orders would be targeted compared to the GMO. There could be additive effects resulting in hybrids with increased resistance to lepidopteran and/or hemipteran insects. However, these additive effects are unlikely to improve the fitness of the hybrid cottons in response to the range of abiotic and biotic factors that limit establishment. Therefore, it is unlikely that any hybrids would be more likely to spread and persist in the environment than the GM parental lines or the GMO.

197. Liberty Link® cotton has also been approved for commercial cultivation, however this cotton does not contain any insect resistance genes, so even if it crossed with the GMO, no insect resistance traits would occur in any hybrids other than those present in the GMO.

Potential Harm

198. The potential harms from this risk scenario are increased toxicity or allergenicity to people, increased toxicity to desirable organisms, reduced establishment or yield of desirable plants in agricultural areas or in the natural environment, or an increased reservoir for pathogens or pests.

199. As discussed in Risk scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GMO for people or increased toxicity to other desirable organisms. Similarly, in hybrids between the GMO and sexually compatible plants, the same consideration would apply.

200. As discussed in Risk scenario 2, the GMO expressing *mCry51Aa2* is not expected to be more able to spread and persist in the environment than its GM parental cotton lines. Similarly, in the event of hybridisation with sexually compatible plants, the introduced genetic modifications are not expected to increase the ability of hybrid(s) to spread and persist, or to change their susceptibility to the abiotic factors (particularly frost and water availability) that limit the survival of cotton in the environment. It is also expected standard weed management practices for cotton volunteers in agricultural settings would control hybrids.

Conclusion

201. Risk scenario 3 is not identified as a substantive risk because cotton has limited ability to outcross and any hybrids between the GMO and other commercial cottons are not expected to show increased levels of toxicity or allergenicity, or increased ability to spread and persist in the environment. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

203. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk the Regulator will take this uncertainty into account in making decisions.

204. As mentioned in Chapter 1 (Section 5.1), the RARMP for DIR 147 identified information that may be required to assess an application for a large scale or commercial release of the GMO. This included:

- Molecular, biochemical and phenotypic characterisation of the GM cotton MON-88702-4 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 cotton and the potential for increased weediness.

Further information has been outlined in the relevant sections in Chapter 1 and considered in the risk scenarios in Chapter 2.

205. Uncertainty can arise from a lack of experience growing the GMO expressing the *mCry51Aa2* gene, encoding the mCry51Aa2 protein, which increases resistance to certain hemipteran and thysanopteran insect pests. The GMO has been approved for field trials in Australia under DIR 147 and 27 ha has been planted under this licence. In addition, the GMO has been commercially cultivated in the USA on over 172,800 ha following the approval by the USDA-APHIS in 2021. No adverse effects have been reported from these releases. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

206. Post release review will be used to address uncertainty regarding future changes to knowledge about the GMO (Chapter 3, Section 4). This is typically used for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

207. 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.

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

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

209. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible, considering both the short and long term. The principal reasons for these conclusions are summarised in Table 10.

210. The Risk Analysis Framework, which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation (OGTR 2013). Therefore, no controls are required to treat these negligible risks. 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

211. 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.

212. 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.

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

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

Section 2 Risk treatment measures for substantive risks

215. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of the GMO. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

216. 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
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

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

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

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

3.2 Testing methodology

219. The licence holder is required to provide a method to the Regulator for the reliable detection of all events included in the GMO. Bayer has supplied appropriate detection methods for all events included in the GMO as part of the application and as required with previous licences (DIR 145 and DIR 147). Therefore, a requirement to provide detection methods is not included in the licence conditions.

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

220. Any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

221. The licence obliges the licence holder to report without delay any of the following to the Regulator:

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

222. The licence holder is obliged to submit an Annual Report containing any information required by the licence.

223. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

224. 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 the Regulator, or a person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

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

Section 4 Post release review

226. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

227. The Regulator engages in ongoing oversight of licences to take account of future findings or changes in circumstances. This ongoing oversight is achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

228. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

229. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

230. Collection of additional specific information on an intentional release provides a mechanism for ‘closing the loop’ in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

231. The term ‘specific indicators of harm’ does not mean that it is expected that harm would necessarily occur. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

232. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

233. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 216. However, specific indicators of harm may also be identified during later stages, through either of the other components of PRR.

234. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

235. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR, or by relevant new scientific information or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s) or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

236. The risk assessment concludes that the proposed commercial release of the GMO poses negligible risks to the health and safety of people or the environment as a result of gene technology.

237. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions are imposed to ensure that there is ongoing oversight of the release.

References

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Appendix A: Additional Data tables

Table A1 Expression levels of insect resistance proteins in the GMO during Australian field trials

Protein	Year	Boll-1 ^{a,b} (µg/g dw)	Boll-2 (µg/g dw)	Seed (µg/g dw)	OSL1 (µg/g dw)	OSL2 (µg/g dw)	OSL3 (µg/g dw)	OSL4 (µg/g dw)	Pollen (µg/g dw)	Square-1 (µg/g dw)	Square-2 (µg/g dw)	Square-3 (µg/g dw)	Square-4 (µg/g dw)
mCry51Aa2	2018 ^c	220 ± 10 190 - 230	330 ± 150 180 - 770	130 ± NA ^d NA - NA	460 ± 100 330 - 770	460 ± 83 350 - 710	530 ± 99 350 - 710	480 ± 93 360 - 750	3.3 ± NA NA - NA	2700 ± 300 1900 - 3200	2300 ± 97 2000 - 2400	2100 ± 200 1500 - 2500	2200 ± 160 1700 - 2500
	2018- 2019	540 ± 28 350 - 740	460 ± 44 250 - 940	280 ± 42 200 - 370	830 ± 99 27 - 1200	870 ± 40 530 - 1100	1200 ± 100 830 - 1700	1100 ± 56 670 - 1400	42 ± 22 7.2 - 100	1300 ± 88 630 - 1800	1200 ± 57 770 - 1700	1300 ± 68 990 - 1500	1100 ± 54 820 - 1500
	2019	1200 ± 130 830 - 1400	1200 ± 260 400 - 1500	180 ± 19 130 - 210	1400 ± 9.9 1400 - 1400	1400 ± 82 1200 - 1600	1400 ± 52 1300 - 1500	1800 ± 40 1700 - 1800	26 ± NA ^d NA - NA	2700 ± 190 2500 - 3000	2700 ± 230 2200 - 3300	2200 ± 89 1900 - 2400	2400 ± 83 2200 - 2500
	2019- 2020	1000 ± 80 630 - 1500	660 ± 46 410 - 1000	530 ± 23 350 - 700	1900 ± 85 1100 - 2400	1800 ± 110 1100 - 2700	1700 ± 150 890 - 2800	990 ± 50 740 - 1500	26 ± 14 1.8 - 54	3800 ± 160 2500 - 4800	4300 ± 95 3400 - 5000	3900 ± 220 2400 - 5900	3700 ± 130 2800 - 4700
Cry1Ac	2018	16 ± 1.3 14 - 20	21 ± 1.3 17 - 23	17 ± NA NA - NA	45 ± 3.4 39 - 53	29 ± 1.5 27 - 33	41 ± 1.8 36 - 44	25 ± 0.61 24 - 27	0.63 ± NA NA - NA	47 ± 2.5 42 - 52	19 ± 0.62 17 - 20	17 ± 1.2 14 - 20	14 ± 0.19 13 - 14
	2018- 2019	15 ± 0.42 12 - 18	14 ± 0.96 9.0 - 19	16 ± 2.8 9.4 - 22	26 ± 3.4 0.47 - 45	28 ± 2.9 12 - 43	23 ± 0.99 19 - 27	16 ± 0.82 12 - 22	1.3 ± 0.54 0.41 - 2.6	26 ± 1.3 19 - 33	18 ± 1.5 9.6 - 30	16 ± 0.73 12 - 19	13 ± 0.48 11 - 16
	2019	14 ± 0.40 14 - 16	11 ± 2.3 4.4 - 15	26 ± 0.72 24 - 28	40 ± 3.5 34 - 50	55 ± 6.4 38 - 69	31 ± 2.0 28 - 37	39 ± 0.85 37 - 41	1.7 ± NA NA - NA	48 ± 3.8 44 - 55	38 ± 3.0 33 - 47	25 ± 0.77 23 - 27	22 ± 0.51 21 - 24
	2019- 2020	16 ± 1.4 7.0 - 28	20 ± 1.0 15 - 29	23 ± 1.8 14 - 41	50 ± 2.2 35 - 63	41 ± 3.2 20 - 60	30 ± 5.7 3.2 - 63	39 ± 3.3 20 - 74	0.81 ± 0.32 0.44 - 1.8	52 ± 3.0 35 - 76	27 ± 0.89 20 - 34	22 ± 1.0 16 - 31	24 ± 0.96 17 - 32
Cry2Ab	2018	230 ± 15 200 - 270	780 ± 120 520 - 1100	580 ± NA NA - NA	500 ± 31 440 - 570	240 ± 16 210 - 290	240 ± 16 210 - 270	210 ± 19 170 - 260	1.9 ± NA NA - NA	1100 ± 83 850 - 1200	490 ± 12 460 - 520	350 ± 11 330 - 380	380 ± 5.7 370 - 400
	2018- 2019	320 ± 26 220 - 570	390 ± 30 220 - 600	770 ± 110 530 - 1000	550 ± 73 9.9 - 930	470 ± 46 170 - 720	420 ± 24 310 - 490	250 ± 18 100 - 360	43 ± 31 2.2 - 100	500 ± 26 320 - 670	390 ± 24 220 - 500	370 ± 20 270 - 460	280 ± 8.3 250 - 340
	2019	130 ± 19 89 - 180	120 ± 23 55 - 160	720 ± 96 520 - 940	390 ± 66 250 - 570	260 ± 53 110 - 350	90 ± 8.4 75 - 110	390 ± 44 310 - 490	54 ± NA NA - NA	620 ± 19 580 - 640	470 ± 32 420 - 560	280 ± 12 250 - 310	310 ± 14 290 - 350
	2019- 2020	300 ± 24 180 - 500	360 ± 42 82 - 580	1100 ± 77 810 - 1900	1400 ± 82 930 - 1900	920 ± 110 98 - 1700	550 ± 110 110 - 1200	510 ± 52 280 - 870	19 ± 11 0.50 - 44	1000 ± 55 630 - 1500	800 ± 28 620 - 1000	630 ± 30 450 - 880	540 ± 25 420 - 740
Vip 3Aa19	2018	5.7 ± 0.57 4.4 - 7.2	<LOQ ^e ± NA NA - NA	<LOQ ± NA NA - NA	58 ± 4 50 - 68	59 ± 3.8 52 - 70	48 ± 5.4 35 - 61	<LOQ ± NA NA - NA	<LOQ ± NA NA - NA	130 ± 13 100 - 160	71 ± 2 66 - 74	38 ± 3.9 30 - 47	40 ± 2.4 36 - 47
	2018- 2019	5.9 ± 0.77 4.2 - 12	<LOQ ± NA NA - NA	<LOQ ± NA NA - NA	44 ± 1.9 31 - 56	48 ± 3.0 31 - 66	32 ± 1.0 28 - 36	30 ± 1.4 25 - 36	<LOQ ± NA NA - NA	45 ± 6.3 16 - 110	40 ± 3.8 18 - 71	34 ± 3.0 26 - 53	24 ± 2.2 14 - 39
	2019	6.2 ± 1 4.5 - 8.0	6.2 ± 0.89 4.4 - 8.4	<LOQ ± NA NA - NA	170 ± 11 140 - 190	110 ± 9.4 83 - 120	54 ± 7.7 32 - 66	110 ± 4.5 100 - 120	<LOQ ± NA NA - NA	130 ± 11 110 - 150	65 ± 6.1 58 - 83	57 ± 3.1 39 - 97	53 ± 4 44 - 62
	2019- 2020	<LOQ ± NA NA - NA	<LOQ ± NA NA - NA	<LOQ ± NA NA - NA	110 ± 5.1 69 - 140	110 ± 7.9 58 - 160	110 ± 6.8 83 - 150	110 ± 12 45 - 240	<LOQ ± NA NA - NA	91 ± 3.5 63 - 120	72 ± 3.6 48 - 93	57 ± 3.8 39 - 97	66 ± 2.1 50 - 77

^a Boll-1 and Boll-2, collected at 14 days after first flower and cutout stages; seed, collected at maturity; Leaf – OSL1, OSL2, OSL3 and OSL4, collected at Match head square, Pre flower, Peak flower and Cutout stages, respectively; pollen, collected at peak bloom stage; Square 1, 2, 3 and 4 collected at Match head square, Pre flower, Peak flower and Cutout stages, respectively.

^b Data are shown as the mean $\pm$ standard deviation, followed by the range of values recorded across all samples

^c 2018-2019: summer; 2019: winter; 2019-2020: summer

^d NA=not applicable

^e LOQ=limit of quantitation (lowest value of concentration that can be quantified with acceptable precision and accuracy)

Table A2 Expression levels of herbicide tolerance proteins in the GMO during Australian field trials

Protein	Year	Boll-1 ^{a,b} ($\mu\text{g/g dw}$)	Boll-2 ($\mu\text{g/g dw}$)	Seed ($\mu\text{g/g dw}$)	OSL1 ($\mu\text{g/g dw}$)	OSL2 ($\mu\text{g/g dw}$)	OSL3 ($\mu\text{g/g dw}$)	OSL4 ($\mu\text{g/g dw}$)	Pollen ($\mu\text{g/g dw}$)	Square-1 ($\mu\text{g/g dw}$)	Square-2 ($\mu\text{g/g dw}$)	Square-3 ($\mu\text{g/g dw}$)	Square-4 ($\mu\text{g/g dw}$)
DMO	2018 ^c	38 $\pm$ 4.4 28 - 48	56 $\pm$ 4.7 46 - 69	17 $\pm$ NA ^d NA - NA	170 $\pm$ 6.8 150 - 180	120 $\pm$ 6.9 100 - 130	130 $\pm$ 11 100 - 150	120 $\pm$ 3.4 110 - 120	<LOQ ^e $\pm$ NA NA - NA	84 $\pm$ 4.5 71 - 90	91 $\pm$ 2 87 - 96	88 $\pm$ 6.1 76 - 99	93 $\pm$ 3.3 89 - 100
	2018- 2019	38 $\pm$ 2.1 30 - 58	34 $\pm$ 1.1 27 - 43	16 $\pm$ 3.0 8.7 - 21	88 $\pm$ 11 2.1 - 120	110 $\pm$ 5.0 86 - 150	67 $\pm$ 3.6 52 - 85	110 $\pm$ 4.1 82 - 130	15 $\pm$ 3.3 12 - 19	53 $\pm$ 5.4 16 - 83	57 $\pm$ 4.5 26 - 99	100 $\pm$ 3.9 84 - 120	97 $\pm$ 6.6 71 - 160
	2019	86 $\pm$ 1.3 83 - 89	100 $\pm$ 23 35 - 140	18 $\pm$ 0.67 17 - 20	280 $\pm$ 13 250 - 300	250 $\pm$ 11 220 - 270	170 $\pm$ 14 130 - 200	240 $\pm$ 8.8 230 - 270	14 $\pm$ NA ^d NA - NA	450 $\pm$ 13 420 - 460	410 $\pm$ 17 380 - 460	410 $\pm$ 13 380 - 440	410 $\pm$ 13 380 - 440
	2019- 2020	89 $\pm$ 4.6 54 - 120	140 $\pm$ 6.9 84 - 190	21 $\pm$ 1.8 9.8 - 38	250 $\pm$ 11 180 - 320	370 $\pm$ 13 290 - 480	200 $\pm$ 28 39 - 390	360 $\pm$ 24 260 - 580	<LOQ $\pm$ NA NA - NA	420 $\pm$ 6.9 370 - 490	480 $\pm$ 9.8 410 - 560	450 $\pm$ 10 400 - 540	490 $\pm$ 12 360 - 550
PAT	2018	1.6 $\pm$ 0.22 0.99 - 2	1.3 $\pm$ 0.1 1.1 - 1.5	4.4 $\pm$ NA NA - NA	6.7 $\pm$ 0.47 5.9 - 7.8	4.4 $\pm$ 0.13 4 - 4.6	4.7 $\pm$ 0.26 4.1 - 5.3	2.9 $\pm$ 0.14 2.7 - 3.3	<LOQ $\pm$ NA NA - NA	4.4 $\pm$ 0.39 3.4 - 5.2	3.8 $\pm$ 0.11 3.5 - 5.2	3 $\pm$ 0.29 2.3 - 3.7	3.2 $\pm$ 0.14 2.8 - 3.4
	2018- 2019	1.2 $\pm$ 0.071 0.81 - 1.6	0.65 $\pm$ 0.09 0.26 - 1.2	3.9 $\pm$ 0.97 1.7 - 5.7	3.3 $\pm$ 0.43 0.19 - 5.2	3.1 $\pm$ 0.30 1.0 - 4.6	2.9 $\pm$ 0.10 2.5 - 3.3	2.4 $\pm$ 0.19 0.90 - 3.2	<LOQ $\pm$ NA NA - NA	2.7 $\pm$ 0.20 1.5 - 3.7	2.6 $\pm$ 0.20 1.4 - 4.3	3.2 $\pm$ 0.13 2.8 - 4.0	2.8 $\pm$ 0.11 2.2 - 3.7
	2019	0.88 $\pm$ 0.04 0.79 - 0.99	0.68 $\pm$ 0.11 0.44 - 0.87	5.9 $\pm$ 0.14 5.6 - 6.3	6.2 $\pm$ 0.49 5.1 - 7.5	5.1 $\pm$ 0.81 2.8 - 6.5	1.7 $\pm$ 0.16 1.4 - 2.2	3.5 $\pm$ 0.23 2.8 - 3.9	<LOQ $\pm$ NA NA - NA	5.7 $\pm$ 0.74 4.6 - 7.1	4.5 $\pm$ 0.28 4.0 - 5.3	4.1 $\pm$ 0.083 4.0 - 4.3	3.9 $\pm$ 0.13 3.6 - 4.2
	2019- 2020	1.0 $\pm$ 0.08 0.47 - 1.5	1.0 $\pm$ 0.085 0.57 - 1.7	13 $\pm$ 0.78 7.8 - 19	5.9 $\pm$ 0.20 4.4 - 7.2	5.9 $\pm$ 0.30 3.9 - 7.9	3.4 $\pm$ 0.65 0.55 - 6.4	4.9 $\pm$ 0.40 1.9 - 7.8	<LOQ $\pm$ NA NA - NA	4.5 $\pm$ 0.17 3.0 - 5.7	5.3 $\pm$ 0.19 4.5 - 7.0	4.8 $\pm$ 0.17 3.6 - 6.0	5.5 $\pm$ 0.23 4.2 - 7.3
CP4 EPSPS	2018	400 $\pm$ 27 340 - 470	250 $\pm$ 13 210 - 270	150 $\pm$ NA NA - NA	1200 $\pm$ 61 1100 - 1400	780 $\pm$ 43 670 - 880	780 $\pm$ 40 690 - 870	590 $\pm$ 20 550 - 650	7.8 $\pm$ NA NA - NA	840 $\pm$ 85 620 - 980	850 $\pm$ 27 770 - 880	740 $\pm$ 49 610 - 840	730 $\pm$ 29 670 - 810
	2018- 2019	550 $\pm$ 22 400 - 700	400 $\pm$ 44 200 - 800	280 $\pm$ 42 200 - 370	1100 $\pm$ 150 26 - 1800	1200 $\pm$ 96 600 - 1700	1100 $\pm$ 71 700 - 1300	840 $\pm$ 41 600 - 1200	41 $\pm$ 22 7.1 - 100	550 $\pm$ 29 400 - 700	560 $\pm$ 23 380 - 790	620 $\pm$ 26 480 - 710	530 $\pm$ 17 420 - 620
	2019	660 $\pm$ 61 490 - 770	680 $\pm$ 170 190 - 960	220 $\pm$ 29 150 - 290	1800 $\pm$ 200 1300 - 2100	2000 $\pm$ 270 1200 - 2400	1500 $\pm$ 78 1300 - 1600	1700 $\pm$ 25 1600 - 1700	51 $\pm$ NA NA - NA	1100 $\pm$ 67 1100 - 1300	1100 $\pm$ 62 980 - 1300	870 $\pm$ 43 750 - 930	1000 $\pm$ 46 890 - 1100
	2019- 2020	790 $\pm$ 66 300 - 1200	890 $\pm$ 53 680 - 1300	440 $\pm$ 17 300 - 560	2400 $\pm$ 85 1600 - 2800	2200 $\pm$ 180 420 - 3100	1500 $\pm$ 140 560 - 2200	1900 $\pm$ 90 1400 - 2700	39 $\pm$ 18 7.4 - 76	1300 $\pm$ 43 940 - 1500	1400 $\pm$ 30 1200 - 1600	1200 $\pm$ 44 840 - 1500	1100 $\pm$ 34 880 - 1400

^a Boll-1 and Boll-2, collected at 14 days after first flower and cutout stages; seed, collected at maturity; Leaf – OSL1, OSL2, OSL3 and OSL4, collected at Match head square, Pre flower, Peak flower and Cutout stages, respectively; pollen, collected at peak bloom stage; Square 1, 2, 3 and 4 collected at Match head square, Pre flower, Peak flower and Cutout stages, respectively.

^b Data are shown as the mean $\pm$ standard deviation, followed by the range of values recorded across all samples

^c 2018-2019: summer; 2019: winter; 2019-2020: summer

^d NA=not applicable

^e LOQ=limit of quantitation (lowest value of concentration that can be quantified with acceptable precision and accuracy)

Table A3 Phenotypic characterisation of the GMO (Australia)

Parameter	Season	Mean (SE) GMO	Control ^a
Early Stand Count ^b	2018-19	9.5 (0.77)	11.3 (0.97)
	2019-20	15.8 (1.22)	13.5 (0.70)
Late Stand Count	2018-19	10.3 (0.7)	11.4 (0.97)
	2019-20	15.0 (1.27)	13.1 (0.51)
Vigour ^c	2018-19	4.2 (0.46)	4.8 (0.28)
	2019-20	6.4 (0.45)	6.5 (0.51)
Plant Height ^d 2	2018-19	15.9 (1.29)	16.8 (1.52)
	2019-20	16.5 (1.90)	17.5 (2.06)
Plant Height 3	2018-19	39.8 (2.59)	41.3 (3.20)
	2019-20	35.8 (4.49)	38.4 (4.72)
Plant Height 4	2018-19	66.7 (2.24)	70.9 (2.88)
	2019-20	63.1 (3.29)	65.4 (3.53)
Plant Height 5	2018-19	70.1 (2.12)	71.2 (3.36)
	2019-20	77.7 (1.99)	81.1 (2.03)*
Plant Height 6	2018-19	75.8 (3.39)	77.3 (3.04)
	2019-20	No Data	No Data
Plant Height 7	2018-19	77.9 (3.59)	79.2 (2.87)
	2019-20	No Data	No Data
Nodes ^d 2	2018-19	6.9 (0.51)	7.3 (0.58)
	2019-20	6.2 (0.47)	6.6 (0.50)*
Nodes 3	2018-19	12.2 (0.41)	12.9 (0.34)
	2019-20	11.1 (0.67)	11.9 (0.67)*
Nodes 4	2018-19	18.0 (0.34)	18.6 (0.36)
	2019-20	15.9 (0.53)	17.0 (0.44)*
Nodes 5	2018-19	18.7 (0.62)	19.5 (0.50)
	2019-20	19.1 (0.43)	20.1 (0.36)*
Nodes 6	2018-19	17.3 (0.39)	18.9 (0.30)*
	2019-20	No Data	No Data
Nodes 7	2018-19	17.7 (0.46)	19.6 (0.32)*
	2019-20	No Data	No Data
NAWF ^e at 7 DAF	2018-19	7.2 (0.31)	7.3 (0.23)
	2019-20	7.0 (0.19)	7.1 (0.24)
NAWF at 14 DAF	2018-19	6.4 (0.40)	6.3 (0.41)
	2019-20	6.1 (0.19)	6.0 (0.19)
NAWF at 21 DAF	2018-19	4.4 (0.54)	4.4 (0.51)
	2019-20	4.4 (0.26)	4.4 (0.28)
Yield (kg lint/ha)	2018-19	1805.0 (121.24)	1956.0 (139.98)
	2019-20	2311.0 (137.20)	2571.0 (142.13)
Length (cm)	2018-19	1.14 (0.01)	1.16 (0.01)
Micronaire	2018-19	4.5 (0.12)	4.4 (0.08)
Strength	2018-19	29.1 (0.61)	30.9 (0.66)

^a Control is MON 15985 × COT102 × MON 88701 × MON 88913

^b 21 days after planting (DAP) the number of emerged plants was counted across 2 × 1 m² areas in each plot.

^c 21 DAP, plant vigour was rated across 2 × 1 m² areas of each plot using a rating scale of 1-9 where: 1 is excellent vigour and 9 is poor vigour.

^d At 21 DAP, ten plants were non-systematically selected and tagged. From this date, every 21 days the plant height (cm) was measured from the soil to the growing tip, and the number of nodes counted from the cotyledons (cotyledon position being zero) to the last fully unfurled leaf.

^e NAWF (Nodes Above White Flower): The approximate date of first flower of each plot was recorded. The number of nodes from the upper most first position white flower to the terminal bud was recorded on ten non-systematically selected plants from each plot once a week for 3 consecutive weeks, beginning 7 days after flowering (DAF).

*Indicates a statistically significant difference between the test and the control

Table A4 Compositional analysis for cotton seed from the GMO and non-GM cotton control

Component	Sample	Mean (SE) ^a	Mean Range (Min. - Max.)	p-value	Comparative Range ^b
Protein and Amino Acids					
Protein	GMO	24.24 (0.42)	22.19- 26.57	<0.001	19.19-32.97
	Control	25.66 (0.42)	23.93 - 27.93		
Alanine	GMO	0.91 (0.015)	0.79 - 1.03	<0.001	0.69-1.29
	Control	0.96 (0.015)	0.87 - 1.02		
Arginine	GMO	2.19 (0.058)	1.80 - 2.57	0.002	1.76-3.93
	Control	2.43 (0.058)	2.08 - 2.69		
Aspartic acid	GMO	2.07 (0.048)	1.73 - 2.42	<0.001	1.51-3.21
	Control	2.24 (0.048)	2.04 - 2.46		
Cystine	GMO	0.45 (0.018)	0.35 - 0.65	0.917	0.288-0.557
	Control	0.45 (0.018)	0.38 - 0.69		
Glutamic acid	GMO	4.59 (0.086)	3.92 - 5.44	<0.001	3.04-6.72
	Control	5.05 (0.086)	4.59 - 5.46		
Glycine	GMO	0.90 (0.015)	0.78 - 1.04	0.014	0.73-1.32
	Control	0.96 (0.015)	0.87 - 1.05		
Histidine	GMO	0.60 (0.012)	0.52 - 0.67	0.004	0.452-0.985
	Control	0.64 (0.012)	0.58 - 0.70		
Isoleucine	GMO	0.70 (0.011)	0.62 - 0.78	0.002	0.58-1.05
	Control	0.73 (0.011)	0.67 - 0.77		
Leucine	GMO	1.28 (0.021)	1.13 - 1.45	0.004	1.01-1.86
	Control	1.34 (0.021)	1.22 - 1.43		
Lysine	GMO	1.10 (0.019)	0.94 - 1.23	0.015	0.84-1.46
	Control	1.16 (0.019)	1.01 - 1.29		
Methionine	GMO	0.30 (0.014)	0.14 - 0.41	0.064	0.29-0.49
	Control	0.33 (0.014)	0.24 - 0.40		
Phenylalanine	GMO	1.09 (0.026)	0.92 - 1.27	0.028	0.88-1.76
	Control	1.17 (0.026)	1.02 - 1.31		
Proline	GMO	0.81 (0.014)	0.72 - 0.94	<0.001	0.60-1.37
	Control	0.87 (0.014)	0.80 - 0.93		
Serine	GMO	0.97(0.018)	0.83 - 1.12	0.013	0.74-1.39
	Control	1.03 (0.018)	0.91 - 1.10		
Threonine	GMO	0.73 (0.011)	0.65 - 0.82	0.047	0.55-1.06
	Control	0.76 (0.011)	0.70 - 0.81		
Tryptophan	GMO	0.28 (0.0083)	0.24 - 0.34	0.032	0.162-0.519
	Control	0.31 (0.0083)	0.28 - 0.37		
Tyrosine	GMO	0.47 (0.011)	0.42 - 0.55	0.030	0.47-1.00
	Control	0.50 (0.011)	0.44 - 0.56		
Valine	GMO	0.94 (0.015)	0.83 - 1.05	0.004	0.76-1.49
	Control	0.99 (0.015)	0.91 - 1.05		
Total Fat and Fatty Acids (FA)					
Total fat	GMO	19.37 (0.30)	16.38 - 20.98	0.521	15.05-27.90
	Control	19.59 (0.30)	17.17-21.14		
Myristic acid (% Total FA)	GMO	0.66 (0.014)	0.61 - 0.71	0.197	0.426-2.400
	Control	0.67 (0.014)	0.61 - 0.77		
Palmitic acid (% Total FA)	GMO	19.66 (0.34)	18.30 - 21.46	<0.001	15.11-27.90
	Control	21.02 (0.34)	19.84 - 22.18		
Palmitoleic acid (% Total FA)	GMO	0.64 (0.021)	0.53 - 0.76	<0.001	0.375-1.190
	Control	0.55 (0.021)	0.48- 0.65		
Stearic acid (% Total FA)	GMO	2.46 (0.079)	2.09 - 2.66	<0.001	0.20-3.54
	Control	2.30 (0.079)	2.01 - 2.56		
Oleic acid (% Total FA)	GMO	18.06 (0.71)	16.34 - 20.60	<0.001	12.8-25.4
	Control	17.00 (0.71)	15.51 - 19.24		

Component	Sample	Mean (SE) ^a	Mean Range (Min. - Max.)	p-value	Comparative Range ^b
Linoleic acid (% Total FA)	GMO Control	57.00 (0.92) 57.09 (0.92)	53.29 - 60.39 53.69 - 59.54	0.668	42.5-63.0
Linolenic acid (% Total FA)	GMO Control	0.17 (0.014) 0.16 (0.014)	0.13 - 0.28 0.13 - 0.24	0.147	0.10-0.64
Carbohydrates					
Carbohydrates by calculation	GMO Control	52.49 (0.43) 50.88 (0.43)	49.20 - 54.73 48.75 - 53.22	<0.001	39.04-59.25
ADF	GMO Control	35.09 (0.52) 34.34 (0.52)	32.45 - 37.72 31.27 - 36.28	0.062	19.74-38.95
NDF	GMO Control	43.67 (0.53) 43.51 (0.53)	40.60 - 46.93 40.93 - 45.56	0.655	25.56-51.87
TDF	GMO Control	44.50 (0.49) 43.56 (0.49)	42.14- 48.15 41.65 - 46.07	0.044	33.69-53.50
Ash and Minerals					
Ash	GMO Control	3.89 (0.19) 3.88 (0.19)	3.21-4.43 2.92 - 4.35	0.734	3.006-5.476
Calcium	GMO Control	0.14 (0.0096) 0.12 (0.0096)	0.11-0.17 0.083 - 0.15	0.005	0.070-0.326
Phosphorus	GMO Control	0.65 (0.060) 0.66 (0.060)	0.44 - 0.84 0.42 - 0.85	0.416	0.384-0.992
Vitamins					
Vitamin E (mg/kg dw)	GMO Control	106.50 (7.02) 105.21 (7.02)	81.09 - 135.09 77.77 - 125.51	0.544	26.57-197.24
Anti-nutrients					
Total gossypol	GMO Control	0.91 (0.039) 0.95 (0.039)	0.75-1.12 0.75 - 1.19	0.295	0.350-1.613
Free gossypol	GMO Control	0.72 (0.022) 0.73 (0.022)	0.54 - 0.84 0.52 - 0.85	0.545	0.384-1.418
Malvalic acid (% Total FA)	GMO Control	0.68 (0.030) 0.63 (0.030)	0.59 - 0.91 0.56 - 0.73	0.032	0.112-0.854
Sterculic acid (% Total FA)	GMO Control	0.25 (0.0085) 0.23 (0.0085)	0.22 - 0.30 0.20 - 0.26	0.062	0.061-0.556
Dihydrosterculic acid (% Total FA)	GMO Control	0.44 (0.015) 0.35 (0.015)	0.36 - 0.51 0.30 - 0.39	<0.001	0.031-0.325

^a All measures expressed as % dw unless stated

^b ILSI range is from ILSI Crop Composition Database, 2016 (Accessed February 24, 2017).

Appendix B: Summary of submissions from prescribed agencies on preparation of the RARMP

The Regulator received several submissions from prescribed experts, agencies and authorities³ on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees that the following should be included in the RARMP: - the potential for the GM cotton to be harmful to the environment - the potential for the GM cotton to be harmful to people through toxicity or allergenicity - the potential for the GM cotton to be harmful to other organisms, particularly beneficial invertebrates, through toxicity - the potential for gene flow to other cottons - whether commercial release is likely to result in changes to agricultural practices that may have an environmental impact. Advises that the Regulator should further consider risks associated with the potential for antibiotic resistance gene transfer.	These issues have been considered in Chapters 1 and 2 of the Risk Assessment and Risk Management Plan (RARMP). The GMO contains 3 antibiotic resistance genes, which originated from one of the GM parental lines that has been already approved for commercial release. No additional antibiotic resistance genes are included in the GMO. The genes from the GM parental lines were previously assessed by the Regulator and found to pose negligible risk to human health or to the environment. Literature searches conducted to address this concern did not find any new information to change this conclusion. For further information, please refer to Chapter 2, Section 2.1 and 2.2.3, of the RARMP.
2	No advice or comments on the RARMP.	Noted.
3	No specialist scientist expertise available. Concerned about:	Noted.

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

Submission	Summary of issues raised	Comment
	The GM cotton is resistant to common herbicides such as glyphosate. Concerned that frequent and heavy use of these herbicides leading to the development of resistant weeds, spread beyond farms, difficulty and expense of control. The GM insecticidal traits and associated herbicide applications could indirectly affect non-target organisms, including beneficial insects and soil microbes.	Issues relating to herbicide use are outside the scope of the <i>Gene Technology Act 2000</i> (The Act). Herbicide resistance issues come under the regulatory oversight of the Australian Pesticides and Veterinary Medicines Authority (APVMA). A range of issues, including effects on human health, weed resistance management and environmental impacts are considered by the APVMA in assessing agricultural chemicals for registration. In Australia, herbicide resistance is an important matter of general agricultural industry concern and is not confined to herbicides used in GM crops. Discussion of Integrated Weed Management, which is designed to limit the development of herbicide resistance in weed populations, is included in Chapter 1, Section 6.1 of the RARMP. Three of the <i>Bacillus thuringiensis</i> (Bt) derived insect resistance proteins which confer resistance to lepidopteran insects (moths) have been extensively assessed previously for commercial release (DIR 145). This assessment concluded that these genes and their expressed proteins pose negligible risks to human health or the environment. No new information was found to change these conclusions. Cotton expressing the Bt-derived insect resistance gene that confers resistance to hemipteran and thysanopteran insects (aphids and thrips) has been assessed previously by the Regulator for limited and controlled release (DIR 147). Information provided and reviewed in the current application indicates this insect resistance gene is not toxic to desirable organisms, including non-target invertebrates and soil microbes (Chapter 1 section 4.4.2) and is considered in the risk scenarios (Chapter 2 of the RARMP). As noted above, the APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia.
4	Unable to reach OGTR via the phone number. Requests information about the process to provide formal advice. Asks whether the documents attached to the request for comment can be shared publicly.	Response provided by email to clarify the process. Confirmed that documents provided as part of the request for comment are public documents and can be shared.
5	Noted that this application is similar to previous GM cotton trials and approvals by the OGTR. No further comment was provided.	Noted.

Submission	Summary of issues raised	Comment
6	Considered the proposed risk management measures adequate. Noted that removing the myc tag from the proteins could reduce the risk of introducing new potential allergens. No additional concerns at this point, notes there will be another opportunity to provide feedback during the draft RARMP consultation.	Noted. There is no myc tag attached to any of the introduced proteins in the GM cotton. Noted.
7	Concerns about: - reliance on specific branded chemical pesticides linked to this GM crop. - potential contamination of nearby farms affecting organic certification - the need for resistance management with three chemicals listed in the application - Seeks clarification on how the crop would be managed if it became a weed.	Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. Consideration of economic or trade issues is outside the scope of the Act. APVMA requirements, State-specific requirements or industry protocols address these issues. As noted above, the APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. Discussion of Integrated Weed Management is also included in the RARMP (Chapter 1, Section 6.1). The potential for weediness of the GM cotton is considered in risk scenarios 2 and 3 (Chapter 2). It is not expected that expression of the introduced genes would result in increased spread and persistence of GM volunteers or reduced the ability to control volunteer cotton plants. Thus, the risk of weediness for the GM cotton is considered to be no greater than currently grown GM and non-GM cotton varieties and any GM volunteers would be controlled by standard weed management practices for cotton volunteers.

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

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

Submission	Summary of issues raised	Comment
1	Notes that the GMO intended for export must have approval from relevant authorities in the importing country. Past incidents of exporting under incorrect approvals have led to import suspensions, highlighting the importance of verifying strain-specific authorisations to avoid trade disruptions.	Noted. Marketing and trade issues are outside the scope of the Gene Technology Act 2000 (the Act). These issues are the responsibility of the State and Territory governments and industry. The Risk Assessment and Risk Management Plan (RARMP) prepared for this application notes that the requirements of any other relevant regulators and legislation would need to be addressed by the licence holder. The licence holder is informed that they are responsible for being aware of and complying all relevant requirements of other regulators and legislation.
2	No advice or comments on the RARMP.	Noted.
3	Agrees that the risk assessment identifies all plausible risk scenarios by which the proposed release could give rise to risks relating to the health and safety of people or the environment.	Noted.
	Agrees with the licence conditions are appropriate for the commercial release of the GMO.	Noted.
	Agrees with the overall conclusion of the RARMP.	Noted.
	Recommends clarification on the Tn7 promoter of the <i>aad</i> antibiotic resistance gene.	Additional details have been included in Table 2 of the RARMP, noting that the introduced <i>aad</i> gene was isolated from the <i>E. coli</i> Tn7 transposon and is under the control of the native bacterial Tn7 promoter. Information indicating that the introduced antibiotic resistance genes are naturally present in the Australian environment has been provided in Chapter 1 (section 6.3.4) and in Chapter 2, Section 2.1, paragraph 150 of the RARMP.
4	Supports the conclusion that proposed dealings poses negligible risk of harm to human health and safety, and the environment.	Noted.

Submission	Summary of issues raised	Comment
5	Satisfied that the application has been thoroughly screened for adverse events and made no further comments	Noted.
6	Agrees with the assessment that risks to the health and safety of people or the environment from the proposed dealings are currently negligible. Raised concerns about the following: - GM cotton's increasing insect resistance gives fitness and weediness potential. Previous assessments considered GM cotton's weediness potential to be not significant because it was not grown in Top End areas. Recent expansion of cotton cultivation in these regions and increased fitness of GM cottons compared to non-GM cottons have increased the weediness potential in GM cotton. - Issue of suboptimal management of ratoon and volunteer cotton plants - Regulators need to consider the weediness risk through increased and ongoing oversight of environmental risks, including adherence stewardship and resistance management	Noted. The potential for weediness of the GM cotton is considered in risk scenarios 2 and 3 (Chapter 2). It is not expected that expression of the introduced insect resistance genes would result in increased spread and persistence of GM volunteers or reduced the ability to control volunteer cotton plants using standard weed management practices for cotton volunteers. Thus, the risk of weediness for the GM cotton is considered to be no greater than currently grown GM and non-GM cotton varieties. The RARMP considers that volunteer GM cotton plants can be controlled using standard weed management practices for cotton volunteers. The cotton industry is responsible for managing volunteer and ratoon cotton through developing and reviewing a resistance management plan (RMP) that specifies the control of volunteer and ratoon cotton. For further information, refer to Cotton Pest Management Guide on CottonInfo website. The RARMP concludes that the GM cotton does not pose risks greater than the non-GM cotton. General risk management conditions, particularly conditions 13, 15 and 17, are included in the licence to ensure that there is ongoing oversight of the release. In addition, the RARMP outlines post-release review activities that contribute to ongoing oversight of this commercial release (Chapter 3, Section 4). As mentioned above, the industry is responsible for stewardship and resistance management through developing and ensuring growers adhere to the RMP.
7	Accepts that overall application has negligible risks to the health and safety to people and the environment. Satisfied that the measures taken to manage the short- and long-term risks are adequate.	Noted.

Appendix D: Summary of submissions from the public on the consultation RARMP

The Regulator received 6 submissions from the public on the consultation RARMP. The issues raised in the submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence, in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Comment
1	While acknowledging the individual safety assessments of component traits, this submission raises a number of concerns (detailed below). Resistance management - The combination of 4 insect resistance genes and 3 herbicide tolerance traits creates high selection pressure that may accelerate resistance development. A robust resistance monitoring protocol with defined action thresholds should be required before approval. - Recommends a mandatory 20% structured refuge areas based on global evidence of resistance evolution in other Bt crops. Non-target effects - Laboratory studies showed toxicity to beneficial <i>Orius</i> species, yet field studies were limited in scope and duration.	Noted. The concerns are addressed below. Issues relating to development of insect and herbicide resistance are outside the scope of the Gene Technology Act 2000 (the Act). These issues come under the regulatory oversight of the Australian Pesticides and Veterinary Medicines Authority (APVMA). The APVMA considers a range of issues, including effects on human health, resistance management and environmental impacts when assessing agricultural chemicals for registration. Discussion of Integrated Pest Management (IPM) and Integrated Weed Management (IWM) is also included in the Risk Assessment and Risk Management Plan (RARMP - Chapter 1, Section 6.1). The cotton industry is responsible for developing and reviewing a resistance management plan (RMP) to minimise the development of insect resistance. It specifies a range of measures including the use of refuge crops, that growers are required to adhere to. For further information, refer to Cotton Pest Management Guide on CottonInfo website. In laboratory studies in the USA, under high exposure to introduced mCry51Aa2 protein, where conditions are designed to represent worst-case scenarios, one species, <i>Orius insidiosus</i>, was adversely affected. Follow-up field studies, which reflect more realistic environmental conditions showed no adverse effects on <i>O. insidiosus</i> or on 3 other closely related species under field conditions. <i>O. insidiosus</i> is not present in Australia, and other <i>Orius</i> spp. are not listed as beneficial species in Australian cotton fields. Australian field studies showed no adverse effects on non-target arthropod abundance compared to the control, including one <i>Orius</i> spp. (<i>O. armatus</i>) and other beneficial species.

Submission	Summary of issues raised	Comment
		For more detail see Chapter 1, Section 4.4.2 of the RARMP.
	Australian field trials covered only 2-3 seasons at limited sites, insufficient to detect subtle but cumulative impacts on beneficial arthropod communities. Multi-year, multi-region studies specifically assessing impacts on native Australian beneficial insects is recommended before commercial approval.	The GM cotton has been produced by conventional breeding of 5 GM parental cotton lines, of which 4 have been previously approved for commercial release, individually or combined, for cultivation in Australia for over 10 years. The fifth GM parental cotton line and the GMO have been approved for field trials in Australia under DIR 147 since 2017. The risks associated with the GM parental cottons and combinations thereof, have been assessed previously as negligible and this RARMP has found no new information to change these conclusions. Therefore, the field studies, in combination with other data, were considered adequate to assess the impact on Australian non-target species.
	Increased herbicide use Triple herbicide tolerance (glyphosate, glufosinate, dicamba) could significantly increase chemical use and weed resistance to herbicides, particularly volatility and off-target risks of dicamba. Hence, mandatory Integrated Weed Management is recommended before approval.	As noted above, issues relating to herbicide use (including spray drift) and weed resistance management are outside the scope of the Act. These are considered by the APVMA. As noted above, IWM is discussed in the RARMP.
	Cumulative risk assessment The RARMP fails to adequately consider synergistic effects of the combined traits and systems-level environmental risk modelling is recommended	These issues have been addressed in the Chapter 1, Section 5.4, and Chapter 2, Section 2.1. The potential for synergistic effects of 3 herbicide tolerance proteins and 3 insect resistance proteins have been considered in the RARMPs for DIR 145 and DIR 124, respectively, which concluded that these genes and their proteins pose negligible risk to human health or the environment. No new information found to change these conclusions. Combined effects of the Bt derived mCry51Aa2 with the other 3 insect resistance proteins has been considered in this RARMP (Chapter 1 Section 5.4 and Chapter 2 Section 2.4.3). No synergistic effects were found between mCry51Aa2 and other insect resistance proteins in the GMO. There is no evidence that insect resistance, herbicide tolerance, and/or antibiotic resistance genes in GM crops show synergistic effects.
	Post-release monitoring Draft licence conditions lack specific monitoring requirements for resistance development and environmental impacts and rely primarily on voluntary reporting	The RARMP concludes that the GM cotton does not pose risks greater than non-GM cotton and therefore specific risk management conditions are not required. However, general risk management conditions, for example conditions 13, 15 and 17, have been included in the licence

Submission	Summary of issues raised	Comment
	rather than systematic surveillance. Mandatory annual resistance testing, beneficial arthropod monitoring, and herbicide use reporting should be included as licence conditions.	to ensure ongoing oversight of the release. The Regulator also has a post-release review framework that allows ongoing oversight of commercial releases in Australia. The licence requires the licence holder to promptly report any adverse impacts or new information relating to risks to the human health and safety or the environment caused by the GMO. The licence holder must also, upon request by the Regulator, collect and provide further information on the progress of the dealings. There are penalties under the Act for non-compliance with licence conditions. As noted above, issues relating to resistance development, herbicide use and environmental impacts from herbicides are outside the scope of the Act. These are considered by the APVMA.
2	Concerns regarding Chapter 4 (draft licence): - Section 13a allows licence holder to self-determine what should be reported, which creates a potential conflict of interest. The OGTR should stipulate the coverage of the reporting data and non-compliance should be subject to a significant fine - Claims that conditions do not adequately ensure protection of human health, safety and the environment due to insufficient regulatory rigor. The licence holder should be held accountable for uncertainties and potential risks acknowledged in the RARMP. Data related to hospital admissions, respiratory impacts, GMO contamination of non-GMO land should be monitored and reported. Accountability should include scaled financial penalties and remedy requirements. This is rigorous follow through.	Licence condition 13 (a) states that “additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence”. It is not prescriptive about what those risks may be as this may result a narrow interpretation of what must be reported. All licence conditions must be complied with, and there are penalties under the Act for non-compliance with licence conditions. The Regulator is required to assess GMO applications in accordance with the Act, the object of which is to protect the health and safety of people and the environment. The RARMP found no greater risk to human health and safety or the environment, from the GM cotton than non-GM cotton, and general licence conditions ensure ongoing oversight and compliance. As stated above, this includes requirements for the licence holder to report adverse effects. Monitoring is not currently required as no specific indicators of harms were identified and the level of uncertainty was considered to be low.
3	Urges governments to put human health first over profit-driven activities that have been shown to pose risks to the public.	The Regulator is required to assess GMO applications in accordance with the Act, the object of which is to protect the health and safety of people and the environment. The RARMP concludes that the GM cotton does not pose risks greater than non-GM cotton.
4	Opposes the genetically modified cotton as it is unnatural.	Noted. Matters related to consumer preferences are outside the Regulator’s legislative responsibility.

Submission	Summary of issues raised	Comment
5	Opposes the approval of the GM cotton application for a range of reasons. Those that are relevant to this application: - Concerns about horizontal gene transfer of the introduced antibiotic resistance genes from GM cotton to E. coli and other organisms - Claims glyphosate residues are present in cotton clothing and links the exposure of glyphosate to skin allergies, especially infants - Cottonseed oil and cotton meal fed to livestock may introduce GM proteins into the human food chain. - States GM crop use has led to documented ecological degradation in Africa.	The potential for horizontal gene transfer and possible adverse outcomes has been reviewed in the literature, assessed in previous RARMPs, and examined in detail by regulatory agencies and international bodies. No risk greater than negligible was identified due to the rarity of these events. Additionally, the introduced genes or similar sequences and regulatory elements are already present in the environment. See Chapter 1, Section 6.3.4 and Chapter 2, Section 2.1 and 2.2.3 in the RARMP. OGTR has a Risk Assessment References page on marker genes which provides more information. The APVMA are responsible for assessing use of herbicides, including glyphosate, on the GM cotton. There is no evidence that products of the GMOs fed to animals would result in the GM proteins being introduced into the human food chain. Food Standards Australia New Zealand (FSANZ) has approved foods derived from each of the parental cottons. These approvals cover food produced from any offspring resulting from conventional breeding, including food produced from the GMO. The Regulator has assessed the GMO application in accordance with the Act. As noted above, the risk assessment concludes that the proposed release poses negligible risks to the health and safety of people and the environment as a result of gene technology. This assessment is based on all credible information available about the GMO for which this application is made, including the parental GM cottons.
6	Concerns about approving the GM cotton with 4 Bt insect resistance genes and 3 herbicide tolerance traits result in serious collateral environmental harm in northern Australia, including water and soil pollution, herbicide spray drift onto natural environments, herbicide resistant weeds, and Bt resistant insects. It's arguable that OGTR should be responsible. Notes that the Regulator's statutory scope can only consider direct risks, but sees this as a regulatory system failure to assess or mitigate the collateral environmental and	Issues relating to herbicide use, development of insect resistance, herbicide resistance, and weed resistance management are outside the scope of the Act. These are considered by the APVMA in assessing agricultural chemicals for registration. Issues of insect resistance are also managed by cotton industry stewardship plans and requirements of end user agreements for growers. The Regulator assesses GMO applications in accordance with the Act, the object of which is to protect the health and safety of people and the environment, by considering risks posed by or as a result of gene technology and managing those

Submission	Summary of issues raised	Comment
	health risks of GM crops and the herbicides used in conjunction with them.	risks through regulating certain dealings with GMOs. For each licence application, the Regulator must prepare a RARMP prior to making a decision whether or not to issue a licence. For details of the OGTR approach to risk assessment, please refer to Risk Analysis Framework 2013. The Regulator commissioned a report to provide advice on crops containing multiple herbicide tolerant traits and impacts on herbicide use, herbicide tolerance and herbicide resistance management issues in Australia.
	Stresses that APVMA's role in practice is not sufficient due to fragmented regulation and lack of accountability and requests the OGTR to advocate to Ministers and Departments for a review to close the regulatory gap between principle and practice	Noted.