



**Australian Government**

**Department of Health and Ageing**

**Office of the Gene Technology Regulator**

**Risk Assessment and  
Risk Management Plan for  
DIR 066/2006**

**Commercial release of GM  
herbicide tolerant and/or insect resistant  
cotton lines north of latitude 22°South**

**Applicant: Monsanto Australia Ltd**

**October 2006**

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# Executive Summary

## Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release (DIR) of five herbicide tolerant and/or insect resistant genetically modified (GM) cotton lines into the Australian environment, in respect of application DIR 066/2006 from Monsanto Australia Ltd (Monsanto).

The DIR 066/2006 licence permits the commercial release of the GM cotton lines on an unrestricted basis in northern Australia, north of latitude 22° South. It should be noted that cultivation of these GMOs may require additional approvals under State or Territory legislation that restrict the commercial release of GM crops on marketing grounds.

The *Gene Technology Act 2000* (the Act) and the Gene Technology Regulations 2001 (the Regulations) govern the process undertaken by the Regulator before a decision is made on the whether or not to issue a licence. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and in consultation with a wide range of experts, agencies and authorities, and the public.

More information on the process required for the comprehensive assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030) or at <<http://www.ogtr.gov.au/>>.

## The application

Monsanto applied for a licence to release the following GM cotton lines, without specific containment measures, north of latitude 22°South:

- insect resistant Bollgard II<sup>®</sup> cotton (also known as MON15985)
- herbicide tolerant Roundup Ready<sup>®</sup> cotton (also known as MON1445)
- herbicide tolerant Roundup Ready Flex<sup>®</sup> cotton (also known as MON88913)
- herbicide tolerant/insect resistant Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton (also known as MON1445/MON15985)
- herbicide tolerant/insect resistant Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (also known as MON88913/MON15985).

The Regulator comprehensively assessed the GM cotton lines proposed for release prior to issuing licences for their unrestricted commercial release south of latitude 22°S (under DIRs 012/2002, 023/2002 and 059/2005) and for field trials under limited and controlled conditions north of latitude 22° S (under DIRs 006/2001, 009/2001, 012/2002, 035/2003 and 055/2004).

Monsanto intends to conduct plant breeding, agronomic trials and seed production, and to cultivate the GM cotton lines in areas suitable for cotton growing in northern Australia. Monsanto indicates that commercial scale plantings are not planned at this stage as a range of industry, community and infrastructure issues would need to be resolved before commercial cotton production could take place in northern Australia.

Monsanto intends to use the GM cotton plants and their products in the same manner as non-GM cotton and GM cotton lines commercially approved north and south of latitude 22°S, including use in human food and stockfeed, transportation and sale of lint.

Under Australia's integrated framework for the regulation of genetically modified organisms, regulatory decisions by relevant agencies are coordinated as far as possible. Monsanto has received approval from Food Standards Australia New Zealand for the use of oil and linters derived from Bollgard II<sup>®</sup> cotton, Roundup Ready<sup>®</sup> cotton and Roundup Ready Flex<sup>®</sup> cotton in food (FSANZ reports A436, A355 and A553). No additional approvals are required from FSANZ for the stacked GM cotton lines.

The Agricultural Pesticides and Veterinary Medicines Authority (APVMA) has registered Roundup Ready<sup>®</sup> Herbicide by Monsanto for use on Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton varieties. The APVMA has also registered the use of the insecticidal proteins as produced by the insecticidal genes (*cry1Ac* and *cry2Ab*) in GM Bollgard II<sup>®</sup> cotton as insecticidal products for New South Wales (NSW) and Queensland (QLD) south of latitude 22°S. It is currently assessing an application from Monsanto to vary the label for Bollgard II<sup>®</sup> to remove the condition for restriction on planting Bollgard II<sup>®</sup> north of latitude 22°S.

## **Risk assessment**

### **Background**

The risk assessment first considered what harm to the health and safety of people or the environment could arise as a result of gene technology, and how it could happen, during the proposed release of the GM cotton lines into the environment (**hazard identification** refer to Chapter 2 for more information).

The risks to people and the environment from the proposed commercial release were assessed in comparison to non-GM cotton and GM Liberty Link<sup>®</sup> Cotton (previously approved for commercial release by the Regulator in northern Australia under DIR 062/2005), in the context of information gained from growing the GM cotton lines commercially in southern Australia, intended agronomic management practices, and the environmental conditions in the regions proposed for the release.

Hazards are particular sets of circumstances (**events**) that might give rise to adverse outcomes (ie cause harm). When an event was considered to have some chance of causing harm, it was identified as posing a risk that required further assessment.

Each event associated with an **identified risk** was then assessed to determine the seriousness of harm (**consequence** - ranging from marginal to major) and the chance of harm (**likelihood** - ranging from highly unlikely to highly likely). The level of risk (ranging from negligible to high) was then estimated using a Risk Estimate Matrix (refer to Chapter 2 for more information).

### **Hazard identification**

Of the 35 events compiled during the hazard identification process, six were selected for further assessment. The potential adverse outcomes to the environment associated with these events were increased toxicity to non-target invertebrates and enhanced spread and persistence (weediness). The remaining 29 events were not assessed further as they were considered not to give rise to an identified risk to human health and safety or the environment (refer to Chapter 2 for more information).

### **Risk of increased toxicity to non-target invertebrates**

One event was considered that might result in the insect resistant GM cotton lines exhibiting greater toxicity to non-target invertebrates than non-GM cotton.

- Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates (Identified Risk 1).

## Risk of weediness

Five events were considered that might result in the introduced genes causing greater weediness in the GM cotton lines or in related species following gene transfer, than non-GM cotton or GM Liberty Link<sup>®</sup> cotton.

- Tolerance to glyphosate due to expression of the *cp4 epsps* gene(s) in the GM cotton plants (Identified Risk 2)
- Reduced lepidopteran herbivory due to expression of the *cry1Ac* and *cry2Ab* genes in the GM cotton plants (Identified Risk 3)
- Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination in the GM cotton plants (Identified Risk 4)
- Expression of the *cp4 epsps*, and/or *cry1Ac* and *cry2Ab* genes in naturalised *G. hirsutum* or *G. barbadense* cotton plants providing glyphosate tolerance and/or reduced lepidopteran herbivory resulting from vertical gene transfer (Identified Risk 5)
- Expression of the *cp4 epsps*, and/or *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene (from Liberty Link<sup>®</sup> Cotton) providing dual herbicide tolerance and reduced lepidopteran herbivory resulting from vertical gene transfer (Identified Risk 6).

The risk assessment considered the consequence and likelihood of harm that might result from each of the above events. The estimate of risk for all six Identified Risks is **negligible**.

## Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. The level of risk to health and safety of people or the environment for the six Identified Risks that were assessed was estimated as **negligible**.

The *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation. Therefore, no risk treatment measures are required and no specific risk management conditions have been imposed. However, as part of the Regulator's oversight of licensed dealings involving the release of genetically modified organisms, the licence contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring provisions; and reporting requirements, including a compliance plan, annual report and other relevant information<sup>1</sup>.

## Conclusions of the RARMP

The risk assessment concludes that this commercial release of five herbicide tolerant and/or insect resistant GM cotton lines in northern Australia poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

The risk management plan concludes that the negligible risks do not require risk treatment measures and no specific risk management conditions have been imposed. The licence contains general conditions that enable the Regulator to maintain oversight of the licensed dealings in accordance with her statutory obligations.

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<sup>1</sup> The licence and conditions for DIR 066/2006 are available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>, following the path to DIR 066/2006).

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## Abbreviations

<i>aad</i>	Gene encoding the aminoglycoside adenytransferase from <i>Escherichia coli</i>
AAD	Aminoglycoside adenytransferase from <i>E. coli</i>
ACCRC	Australian Cotton Cooperative Research Centre
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine Inspection Service
Bt	<i>Bacillus thuringiensis</i>
Btk	<i>Bacillus thuringiensis</i> variety <i>kurstaki</i>
CaMV	Cauliflower mosaic virus
CFIA	Canadian Food Inspection Agency
<i>cp4 epsps</i>	<i>epsps</i> gene from <i>Agrobacterium</i> sp. strain CP4
CP4 EPSPS	EPSPS protein from <i>Agrobacterium</i> sp. strain CP4
<i>cry</i>	Gene encoding a Cry protein
Cry protein	Crystal insecticidal protein isolated from <i>Bacillus thuringiensis</i>
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTP	Chloroplast transit peptide
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ELISA	Enzyme-Linked ImmunoSorbent Assay
FMV	Figwort mosaic virus
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
GUS	The $\beta$ -glucuronidase protein from <i>E. coli</i>
ha	Hectare
kDa	Kilodalton
kg	Kilogram
km	Kilometre
LD <sub>50</sub>	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
mg	Milligram
mL	Millilitre
mm	Millimetre
ng	Nanogram
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
<i>nos</i>	Gene encoding nopaline synthase
<i>nptII</i>	Gene encoding the neomycin phosphotransferase type II protein from <i>E. coli</i>
NPTII	Neomycin phosphotransferase type II from <i>E. coli</i>
OGTR	Office of the Gene Technology Regulator
TIMS committee	Transgenic and Insect Management Strategy committee
TGA	Therapeutic Goods Administration

TGNARM	Technical Group for Northern Australia Resistance Management
<i>uidA</i>	Gene encoding the $\beta$ -glucuronidase protein from <i>E. coli</i>
USDA	United States Department of Agriculture
USDA-APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture
US FDA	United States Food and Drug Administration
X-gluc	5-bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide

# Technical summary

## Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 066/2006) to Monsanto Australia Ltd (Monsanto) for dealings involving the intentional release of five herbicide tolerant and/or insect resistant genetically modified (GM) cotton lines into the Australian environment.

The DIR 066/2006 licence permits the commercial release of the GM cotton lines on an unrestricted basis in northern Australia, north of latitude 22° South. It should be noted that cultivation of these GMOs may require additional approvals under State or Territory legislation that restrict the commercial release of GM crops on marketing grounds.

The Gene Technology Act 2000 (the Act), the Gene Technology Regulations 2001 (the Regulations) and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a GMO.

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions<sup>2</sup>.

The RARMP for DIR 066/2006 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the dealings proposed by the applicant were taken into account by the Regulator in deciding to issue a licence and the conditions that have been imposed.

Consistent with Australia's integrated regulatory framework for gene technology, the Regulator has also liaised closely with other regulatory agencies that have been considering applications relating to this release, namely Food Standard Australia New Zealand (FSANZ) and the Australian Pesticides and Veterinary Medicines Authority (APVMA), to avoid duplication and enable coordinated decision making.

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<sup>2</sup> More information on the assessment of licence applications and copies of the *Risk Analysis Framework* are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>> and <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>> respectively.

## Section 1 Application

1. .... <b>title:</b>	2. .... commercial release of GM herbicide tolerant and/or insect resistant cotton lines north of latitude 22°South*
3. .... <b>applicant:</b>	4. .... Monsanto Australia Ltd
5. .... <b>common name of the parent organism:</b>	6. .... cotton
7. .... <b>scientific name of the parent organism:</b>	8. .... <i>Gossypium hirsutum</i> L.
9. .... <b>modified trait(s):</b>	10. .... herbicide tolerance and/or insect resistance
11. .... <b>identity of the gene(s) responsible for the modified trait(s):</b>	12. .... <i>cp4 epsps</i> gene from <i>Agrobacterium</i> sp. strain CP4 (herbicide tolerance) 13. .... <i>cry1Ac</i> and <i>cry2Ab</i> genes from the bacterium <i>Bacillus thuringiensis</i> (insect resistance) 14. .... <i>nptII</i> gene from the bacterial Tn5 transposon (antibiotic resistance) 15. .... <i>uidA</i> gene from <i>Escherichia coli</i> (reporter gene)
16. .... <b>proposed location(s):</b>	17. .... north of latitude 22° South in areas suitable for cotton growing
18. .... <b>proposed release size:</b>	19. .... plant breeding, agronomic trials and seed production and if feasible, commercial scale planting in the future
20. .... <b>proposed time of release:</b>	21. .... ongoing from November 2006

22. ....  
23. .... \*

The title of the licence application submitted by Monsanto was Licence Application covering use of Bollgard II (MON 15985), Roundup Ready Flex (MON 88913) and Roundup Ready (MON 1445) technology in cotton in areas north of latitude 22° South.

Monsanto applied for a licence to release the following GM cotton lines, without specific containment measures, north of latitude 22°S:

- insect resistant Bollgard II<sup>®</sup> cotton (also known as MON15985)
- herbicide tolerant Roundup Ready<sup>®</sup> cotton (also known as MON1445)
- herbicide tolerant Roundup Ready Flex<sup>®</sup> cotton (also known as MON88913)
- herbicide tolerant/insect resistant Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton (also known as MON1445/MON15985)
- herbicide tolerant/insect resistant Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (also known as MON88913/MON15985).

Bollgard II<sup>®</sup> cotton has been developed from GM Ingard<sup>®</sup> cotton (containing a single insect resistance gene, *cry1Ac*) by the introduction of a second insect resistance gene, *cry2Ab*. Both of the insect resistance genes are derived from *Bacillus thuringiensis* variety *kurstaki*, a common soil bacterium. These genes produce insect resistant proteins (Cry1Ac and Cry2Ab) that are highly specific and toxic to caterpillars of some lepidopterans (butterflies and moths), including *Helicoverpa armigera* and *H. punctigera*, the two major insect pests of cultivated cotton in Australia.

Roundup Ready® cotton has been modified by the introduction of one copy of the herbicide tolerance *cp4 epsps* gene, derived from *Agrobacterium* sp. strain CP4. This gene produces a protein (CP4 EPSPS) that provides tolerance to glyphosate, the active constituent in Roundup Ready® Herbicide. The presence of the gene enables GM cotton plants to be sprayed with glyphosate prior to flower formation (approximately 3-5 weeks after planting) to kill weeds without damaging the cotton plants.

Roundup Ready Flex® cotton has been modified by the introduction of two copies of the same herbicide tolerance *cp4 epsps* gene and is tolerant to the herbicide throughout the growing season (approximately 24-28 weeks). This gives growers increased flexibility in the timing of herbicide application and for integrated weed management.

Roundup Ready®/Bollgard II® cotton and Roundup Ready Flex®/Bollgard II® cotton were produced by conventional crossing of the respective herbicide tolerant cotton with Bollgard II® cotton and contain all the genes introduced into each of the parent plants.

Some of the GM cotton lines also contain antibiotic resistance marker genes (*nptII* and *aad*) and a reporter gene (*uidA*) which helped identify and select modified bacteria, plants or plant tissues during the development of the GM plants in the laboratory.

More detailed information on the GMOs, the introduced genes and their products is provided in Chapter 1.

The GM cotton lines proposed for release have previously been comprehensively assessed prior to licences being issued for their unrestricted commercial release south of latitude 22°S (under DIRs 012/2002, 023/2002 and 059/2005) and for field trials north of latitude 22° S (under DIRs 006/2001, 009/2001, 012/2002, 035/2003 and 055/2004).

Monsanto intends to conduct plant breeding, agronomic trials and seed production, and to cultivate the GM cotton lines in areas suitable for cotton growing in northern Australia. Monsanto indicates that commercial scale plantings are not planned at this stage as a range of industry, community and infrastructure issues would need to be resolved before commercial cotton production could take place in northern Australia.

Monsanto intends to use the GM cotton plants and their products in the same manner as non-GM cotton and GM cotton lines commercially approved north and south of latitude 22°S, including use in human food and stockfeed, transportation and sale of lint.

## **Section 2 Risk assessment**

The risk assessment considered information contained in the application, previous GM cotton assessments, current scientific knowledge, and issues relating to risks to human health and safety or the environment raised in submissions received during consultation with a wide range of prescribed experts, agencies and authorities on the application (summarised in Appendix B), and on the RARMP (see Appendix D), including every Local Council north of latitude 22°S.

Similarly, advice received from the public on the application and from consultation on the RARMP, and how it was considered is summarised in Appendices C and E, respectively. A total of fifty-five public submissions were received. A variety of views were expressed regarding the release, ranging from strong opposition to substantial support.

The risk assessment first considered what harm to the health and safety of people or the environment could arise due to gene technology, and how it could happen during this release of GMOs into the environment (hazard identification).

A hazard (source of potential harm) may be an event, substance or organism. The hazard identification process resulted in the compilation of a list of 35 events that describe sets of circumstances by which the proposed release could potentially give rise to adverse outcomes.

A risk is identified when a hazard is considered to have some chance of causing harm to people and/or the environment. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process. The events that are considered to have the potential to lead to adverse outcomes are assessed further to determine the seriousness of harm (consequence) that could result and how likely it is that the harm would occur. The level of risk is then estimated using the *Risk Estimate Matrix* (see below and Chapter 2).

		RISK ESTIMATE			
		Low	Moderate	High	High
LIKELIHOOD	Highly likely	Negligible	Low	High	High
	Likely	Negligible	Low	High	High
	Unlikely	Negligible	Low	Moderate	High
	Highly unlikely	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
CONSEQUENCES					

**Risk Estimate Matrix:** A *negligible* risk is considered to be insubstantial with no present need to invoke actions for mitigation. A *low* risk is considered to be minimal but may invoke actions for mitigation beyond normal practices. A *moderate* risk is considered to be of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. A *high* risk is considered to be unacceptable unless actions for mitigation are highly feasible and effective.

Six of the 35 events characterised in the hazard identification process for the proposed release were identified as requiring further assessment. The potential adverse outcomes associated with these events were: toxicity to non-target invertebrates and increased spread and persistence (weediness). These identified risks were assessed in comparison to non-GM cotton and GM Liberty Link<sup>®</sup> Cotton (previously approved for commercial release by the Regulator in northern Australia under DIR 062/2005), in the context of information provided from growing the GM cotton lines commercially in southern Australia and field trials in northern Australia, intended agronomic management practices, and the environmental conditions in the regions proposed for the release.

The consequence and likelihood assessments used to derive risk estimates for these six Identified Risks are summarised in Table 1 (the detailed risk assessments are in Chapters 3 and 4). More information on the remaining 29 events that were considered not to give rise to an identified risk is provided in Chapter 2.

If a risk is estimated to be higher than negligible, risk treatment measures may be required to protect the health and safety of people or the environment.

**Table 1** Summary table for the risk assessment

Event that may give rise to toxicity for non-target invertebrates	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<b>Identified Risk 1</b> Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates.	<b>Minor</b> <ul style="list-style-type: none"> <li>The Cry1Ac and Cry2Ab proteins are toxic only to lepidopteran insects.</li> <li>Field studies indicated that growing Bollgard II® cotton plants has no significant effect on non-target invertebrate populations when compared to unsprayed non-GM cotton.</li> </ul>	<b>Highly Unlikely</b> <ul style="list-style-type: none"> <li>Exposure to the GM cotton lines and the Cry proteins would occur mostly to those non-target invertebrates consuming the GM cotton within the cotton field.</li> <li>Non-target invertebrates are insensitive to the levels of Cry1Ac and Cry2Ab proteins expressed in the Bollgard II® plants.</li> </ul>	<b>Negligible</b>	<b>No</b>
Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<b>Identified Risk 2</b> Tolerance to glyphosate due to expression of the <i>cp4 epsps</i> gene(s) in the GM cotton plants	<b>Minor</b> <ul style="list-style-type: none"> <li>Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>In the presence of glyphosate, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> </ul>	<b>Highly unlikely</b> <ul style="list-style-type: none"> <li>Glyphosate tolerant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> <li>Glyphosate tolerant cotton is not likely to be cultivated as extensively as lepidopteran resistant cotton in northern Australia (unless stacked with lepidopteran resistant cotton) due to the requirement for multiple insecticide applications.</li> </ul>	<b>Negligible</b>	<b>No</b>



Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 3</b> Reduced lepidopteran herbivory due to expression of the <i>cry1Ac</i> and <i>cry2Ab</i> genes in the GM cotton plants</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of herbicides.</li> <li>• The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> </ul>	Negligible	No
<p><b>Identified Risk 4</b> Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the <i>cp4 epsps</i>, <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination in the GM cotton plants</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of both glyphosate and lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interaction.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Glyphosate tolerant and lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>• The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> </ul>	Negligible	No

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 5</b> Expression of the <i>cp4 epsps</i>, and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in naturalised <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants providing glyphosate tolerance and/or reduced lepidopteran herbivory</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of glyphosate and/or lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interaction.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies.</li> <li>• Glyphosate tolerant and/or lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>• The chance of volunteer GM plants finding suitable ecological niches and establishing as weeds would be no greater than for the non-GM parent.</li> </ul>	Negligible	No

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 6</b> Expression of the <i>cp4 epsps</i>, and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination with the <i>bar</i> gene (from Liberty Link<sup>®</sup> Cotton) providing dual herbicide tolerance and reduced lepidopteran herbivory</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Neither glyphosate nor glufosinate ammonium are effective in controlling established cotton plants.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of glufosinate ammonium, and glyphosate and/or lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interactions.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies.</li> <li>• If Liberty Link<sup>®</sup>, Roundup Ready<sup>®</sup> or Roundup Ready<sup>®</sup> Flex cotton lines were to be cultivated in northern Australia, they will require multiple insecticide applications resulting in limited gene flow because of the reduced numbers of insect pollinators.</li> <li>• Cotton volunteers with glufosinate ammonium tolerance in combination with glyphosate tolerance and/or lepidopteran resistance would be effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> </ul>	<p><b>Negligible</b></p>	<p><b>No</b></p>

## Section 3 Risk management

A risk management plan builds upon the risk assessment to consider whether any action is required to mitigate the identified risks, and what can be done to protect the health and safety of people and the environment.

The risk assessment considered six events that might lead to risks to the environment. The risk estimates for the adverse outcomes associated with all six Identified Risks are **negligible** (ie insubstantial with no present need to invoke actions for their mitigation). Therefore, no risk treatment measures for identified risks were required and no specific risk management conditions have been imposed. However, as part of the Regulator's oversight of licensed dealings involving the release of genetically modified organisms, the licence contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring provisions; and reporting requirements, including a compliance plan, annual report and other relevant information<sup>3</sup>.

### 3.2 Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Health and Medical Research Council (NHMRC) and Australian Quarantine Inspection Service (AQIS). Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies<sup>4</sup>.

FSANZ is responsible for human food safety assessment, including GM food. FSANZ has approved the use of food (oil and linters) derived from Bollgard II<sup>®</sup> cotton, Roundup Ready<sup>®</sup> cotton and Roundup Ready Flex<sup>®</sup> cotton (FSANZ reports A436, A355 and A553). No additional approvals are required by FSANZ for the stacked GM cotton lines.

The APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. Roundup Ready<sup>®</sup> Herbicide by Monsanto is currently registered for use on Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton varieties. The APVMA registered the use of the insecticidal proteins as produced by the insect resistance genes (*cry1Ac* and *cry2Ab*) in GM Bollgard II<sup>®</sup> cotton as insecticidal products for New South Wales (NSW) and Queensland (QLD) south of latitude 22°S in 2003. It is currently assessing an application from Monsanto to vary the label for Bollgard II<sup>®</sup> to remove the condition for restriction on planting Bollgard II<sup>®</sup> north of latitude 22°S.

## Section 4 Conclusions of the RARMP

The risk assessment concludes that this commercial release of five herbicide tolerant and/or insect resistant GM cotton lines in northern Australia poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

The risk management plan concludes that the negligible risks do not require risk treatment measures and no specific risk management conditions have been imposed. The licence

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<sup>3</sup> The licence and conditions for DIR 066/2006 are available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>, following the path to DIR 066/2006).

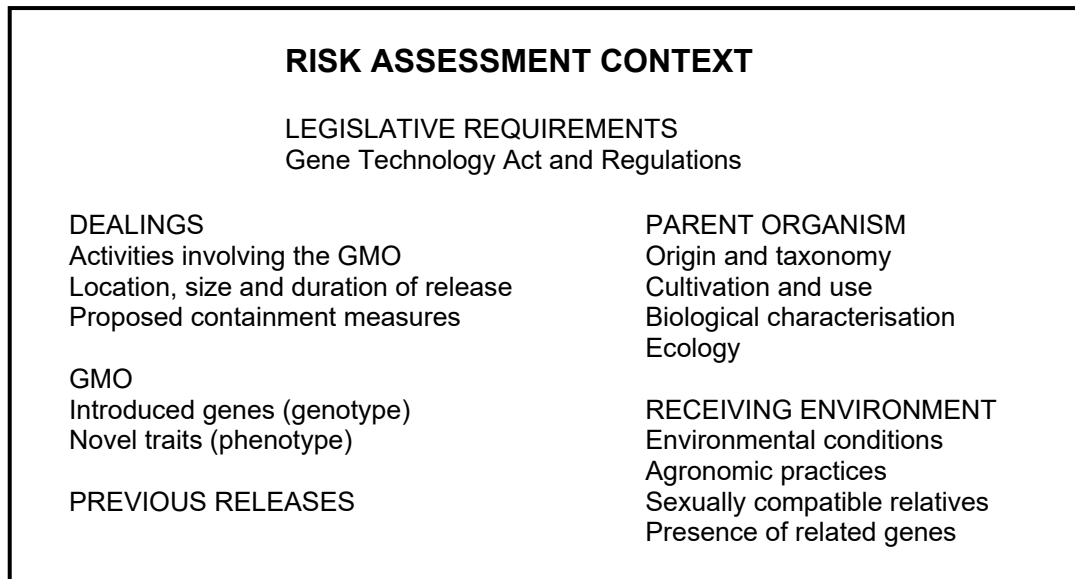
<sup>4</sup> More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

contains general conditions that enable the Regulator to maintain oversight of the licensed dealings in accordance with her statutory obligations.

# Chapter 1 Risk assessment context

## Section 1 Background

1. This Chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation<sup>5</sup>, details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.1.



**Figure 1.1 Components of the risk context considered during the preparation of the risk assessment**

2. Sections 49 to 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decision on licence applications.

3. For this application, establishing the risk assessment context includes consideration of:

- the proposed size, duration and regions requested by the applicant
- characteristics of the parent organism and the commercially released GM Liberty Link<sup>®</sup> Cotton
- the nature and effect of the genetic modification
- the environmental conditions in the regions where the release would occur
- relevant agricultural practices
- the presence of the same or related GM and non-GM cotton in the environment

<sup>5</sup> The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/ir/process.htm>> and in the *Risk Analysis Framework* (OGTR 2005) <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

- the presence of the introduced or similar genes and their encoded proteins in the environment
  - previous approvals for release of these GMOs in Australia and overseas.
4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. Even though public comment was not sought on the preparation of the consultation version of the RARMP, five submissions from the public were received (summarised in Appendix C).
5. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and all local councils in northern Australia were consulted on matters relevant to the preparation of the RARMP. This advice, and where it was taken into account in the RARMP, is summarised in Appendix B.
6. In accordance with section 52 of the Act, the Regulator notified the public that a RARMP had been prepared and invited written submissions. Advice on the RARMP was also sought from the same experts, agencies and authorities as mentioned above. The issues raised, and how they were addressed in the RARMP, are summarised in Appendices D and E, respectively.

## **Section 2    *The application***

7. Monsanto proposes to release the following GM cotton lines into the Australian environment, north of latitude 22°South (22°S):
- insect resistant Bollgard II<sup>®</sup> (also known as MON 15985)
  - herbicide tolerant Roundup Ready<sup>®</sup> (also known as MON 1445)
  - herbicide tolerant Roundup Ready Flex<sup>®</sup> (also known as MON 88913)
  - herbicide tolerant/insect resistant Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> (also known as MON 1445/MON 15985)
  - herbicide tolerant/insect resistant Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> (also known as MON 88913/MON15985).
8. Monsanto is seeking approval to conduct plant breeding, agronomic trials and seed production, and potentially, commercial scale planting of the five GM cotton lines in areas north of latitude 22°S where environmental conditions are suitable for growing cotton.
9. The applicant indicates that commercial scale plantings are not planned at this stage as a range of industry, community and infrastructure issues would need to be resolved before commercial cotton production in northern Australia could take place.
10. No specific containment measures have been proposed and Monsanto intends to use the GM cotton plants and their products in the same manner as both non-GM and GM cotton currently grown commercially in Australia south of latitude 22°S. Hence the dealings would include:
- sale of seed for planting
  - planting and growing of GM cotton lines
  - conventional crossing with elite non-GM cotton varieties suitable for use in Australian conditions

- transportation of seed for planting and cotton seed after harvest to cotton gins for ginning
- sale of lint
- export of seed.

11. In addition, Monsanto proposes the transport and use of the GM cotton seed as stockfeed.

12. The cotton lint (long cellulose fibres) removed from seed cotton during ginning are used to produce cotton fabrics for clothing, upholstery, towels and other household products. Processed lint does not contain detectable protein or genetic materials (Sims et al. 1996; USDA 2004).

13. Delinted cotton seed is processed into four major products: oil, meal, hulls and linters (a type of short cellulose fibre) (Cherry & Leffler 1984). Whole cotton seed, meal and hulls are used in stockfeed. The oil is used in a variety of food products (including frying oil, salad dressing and margarine) and the linters are used as a cellulose base for several consumer food and hygiene products. Protein or genetic materials are not detectable in processed cotton seed oil and linters (Sims et al. 1996; USDA 2004).

14. The use of oil and linters from the GM cotton lines in human food has previously been approved by Food Standards Australia and New Zealand (FSANZ). Approval for use of oil and linters derived from Roundup Ready<sup>®</sup> and INGARD<sup>®</sup> cotton occurred in 2000 (application A355 and A341 respectively), for Bollgard II<sup>®</sup> cotton in 2002 (application A436) and for Roundup Ready Flex<sup>®</sup> cotton in 2006 (application A553). No additional approvals are required by FSANZ for the stacked GM cotton lines.

### **Section 3 The parent organism**

15. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in NSW, southern and central QLD. More detailed information on cotton can be found in the document, *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002), which was produced to inform the risk assessment process for licence applications involving GM cotton plants. This document is available at <<http://www.ogtr.gov.au>> under 'Publications & Forms'.

16. Previous attempts to cultivate cotton in northern Australia over the last 100 years ended in failure due to a combination of climatic and agronomic factors that are discussed further in Section 5.3.

### **Section 4 GMOs, nature and effect of the genetic modification**

#### **4.1 Introduction to the GMOs**

17. Five GM cotton lines are proposed for release. Three GM cotton lines have a single introduced trait, either insect resistance (Bollgard II<sup>®</sup>) or herbicide tolerance (Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup>). Two GM cotton lines have both traits and were produced by conventional crossing of the single trait lines (Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>).

18. The GM cotton lines with the insect resistance trait contain two genes (*cry1Ac* and *cry2Ab*) derived from the common soil bacterium *Bacillus thuringiensis* (Bt). The insecticidal Cry1Ac and Cry2Ab proteins (Bt toxins) encoded by the introduced genes are specifically toxic to lepidopteran caterpillars, including *Helicoverpa armigera* (cotton bollworm) and *H. punctigera* (native budworm), which are major insect pests of cultivated cotton in Australia (Dankocsik et al. 1990; Macintosh et al. 1990; Widner & Whiteley 1990).



19. Bollgard II<sup>®</sup> cotton was approved for commercial release in 2002 and is widely grown in the cotton growing areas south of latitude 22°S. Approximately 81% of GM cotton grown in the 2005/2006 season consisted of Bollgard II<sup>®</sup> or stacks with Bollgard II<sup>®</sup>. It has replaced an earlier product, INGARD<sup>®</sup> cotton (grown since 1996), which contained only the *cry1Ac* gene. As the two insecticidal proteins in Bollgard II<sup>®</sup> cotton differ in their mechanisms of action, and increase the overall insecticidal activity relative to INGARD<sup>®</sup> cotton (Stewart et al. 2001), this is expected to delay the emergence of resistant insects (APVMA 2003).

20. The GM cotton lines with the herbicide tolerant trait contain either one or two copies of the *cp4 epsps* (*5-enolpyruvylshikimate-3-phosphate synthase*) gene derived from *Agrobacterium* sp. strain CP4, a common soil bacterium. This gene encodes the enzyme CP4 EPSPS, which confers tolerance to glyphosate, the active constituent of a number of herbicides including Roundup Ready<sup>®</sup> Herbicide by Monsanto.

21. Roundup Ready<sup>®</sup> cotton, which was approved for commercial release south of latitude 22°S in 2000, is widely grown. It contains only one copy of the *cp4 epsps* gene and has little tolerance to glyphosate in reproductive tissues, which means that glyphosate can only be applied up to the four-leaf stage of growth (i.e. prior to flower formation, approximately 3 to 5 weeks after planting) to control weeds. After this stage, application of the herbicide can lead to yield loss.

22. Roundup Ready Flex<sup>®</sup> cotton was approved for commercial release south of latitude 22°S in 2006 (see DIR 059/2005) and is expected to replace Roundup Ready<sup>®</sup> cotton varieties. It has two copies of the *cp4 epsps* gene and has increased and prolonged expression of the CP4 EPSPS protein, which results in tolerance to glyphosate throughout the growing season (approximately 24 to 28 weeks). Hence, the window in which glyphosate can be applied for weed control is longer, giving growers increased flexibility in timing herbicide applications for integrated weed management.

23. Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton (approved in 2002) is currently widely grown on a commercial scale in the cotton growing regions south of latitude 22°S, but is expected to be replaced by Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (approved in 2006).

24. Some of the GM cotton lines also contain antibiotic resistance genes (*nptII* and *aad*) and a reporter gene (*uidA*) that were used to identify and select modified bacteria, plants or plant tissue during the development of the GM plant in the laboratory (see Table 1.1 for details).

- **Table 1.1 Introduced genes in the GM cotton lines proposed for commercial release.**

GM Cotton Line	Introduced Genes	Protein Function
<b>Bollgard II<sup>®</sup></b> (MON 15985)	<i>cry1Ac</i> <i>cry2Ab</i> <i>uidA</i> <i>nptII</i> <i>aad</i>	Insect resistance Insect resistance Reporter Antibiotic resistance selectable marker <sup>a</sup> Antibiotic resistance selectable marker <sup>b</sup>
<b>Roundup Ready<sup>®</sup></b> (MON 1445)	<i>cp4 epsps</i> <i>nptII</i> <i>aad</i>	Herbicide tolerance Antibiotic resistance selectable marker <sup>a</sup> Antibiotic resistance selectable marker <sup>b</sup>
<b>Roundup Ready Flex<sup>®</sup></b> (MON 88913)	<i>cp4 epsps</i>	Herbicide tolerance

GM Cotton Line	Introduced Genes	Protein Function
<b>Roundup Ready® / Bollgard II®</b> (MON 1445 / MON 15985)	<i>cry1Ac</i> <i>cry2Ab</i> <i>cp4 epsps</i> <i>uidA</i> <i>nptII</i> <i>aad</i>	Insect resistance Insect resistance Herbicide tolerance Reporter Antibiotic resistance selectable marker <sup>a</sup> Antibiotic resistance selectable marker <sup>b</sup>
<b>Roundup Ready Flex® / Bollgard II®</b> (MON 88913 / MON15985)	<i>cry1Ac</i> <i>cry2Ab</i> <i>cp4 epsps</i> <i>uidA</i> <i>nptII</i> <i>aad</i>	Insect resistance Insect resistance Herbicide tolerance Reporter Antibiotic resistance selectable marker <sup>a</sup> Antibiotic resistance selectable marker <sup>b</sup>

<sup>a</sup> used for selection of GM plants in laboratory

<sup>b</sup> used for selection of GM bacteria in laboratory

25. Short regulatory sequences that control expression of the introduced genes are also present in the GM cotton lines (Table 1.2). Details of these genetic elements are given in Section 4.2. These sequences are derived from the Cauliflower mosaic virus (CaMV), Figwort mosaic virus (FMV), *Agrobacterium tumefaciens*, *Escherichia coli* and four plant species, *Petunia x hybrida* (petunia), *Arabidopsis thaliana* (thale cress), *Glycine max* (soybean) and *Pisum sativum* (pea). Although the first three of these organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and do not in themselves cause disease.

- **Table 1.2 Regulatory sequences<sup>1</sup> of the introduced genes in each of the GM cotton lines.**

Introduced gene	Promoter	Terminator	Other regulatory sequences
<b>Bollgard II®</b> <i>cry1Ac</i> <i>cry2Ab</i> <i>uidA</i> <i>nptII</i> <i>aad</i>	<i>CaMV 35S</i> <i>CaMV 35S</i> <i>CaMV 35S</i> <i>CaMV 35S</i> <i>Tn7</i>	<i>β-conglycinin</i> <i>nos</i> <i>nos</i> <i>nos</i> —	— <i>PetHSP70</i> ; <i>ctp2</i> targeting sequence — — —
<b>Roundup Ready®</b> <i>cp4 epsps</i> <i>nptII</i> <i>aad</i>	<i>FMV 34S</i> <i>CaMV 35S</i> <i>Tn7</i>	<i>nos</i> <i>nos</i> —	<i>ctp2</i> targeting sequence — —
<b>Roundup Ready Flex®</b> <i>cp4 epsps</i> (gene 1) <i>cp4 epsps</i> (gene 2)	<i>P-FMV/TSF1</i> <i>P-35S/ACT8</i>	<i>E9</i> <i>E9</i>	<i>L-TSF1</i> and <i>I-TSF</i> ; <i>ctp2</i> targeting sequence <i>L-ACT8</i> and <i>I-ACT8</i> ; <i>ctp2</i> targeting sequence

<sup>1</sup> further detail is given in Section 4.2

## 4.2 The introduced genes, regulatory sequences and the gene products

26. The genetic materials (genes and regulatory sequences) introduced into the GM cotton lines are briefly discussed in this section, including their sources and inherent toxicity and allergenicity. For more detailed information on the genetic material and inherent toxicity or allergenicity refer to previous RARMPs prepared for DIRs 012/2002 (Bollgard II® and Roundup Ready®/Bollgard II®), 023/2002 (Roundup Ready®) and 059/2005 (Roundup Ready Flex® and Roundup Ready Flex®/Bollgard II®), available at <<http://www.ogtr.gov.au>>.

#### 4.2.1 *Insect resistance genes (cry1Ac and cry2Ab) and the encoded proteins*

27. The *cry1Ac* and *cry2Ab* genes are derived from *B. thuringiensis*, a common soil bacterium (Martin & Travers 1989) readily isolated from soil samples, from leaf surfaces and in association with insects (Meadows 1993). The Cry (crystalline) proteins (also called Bt proteins or Bt toxins) encoded by these genes belong to a diverse family of insecticidal proteins, each with specific toxicity to certain groups of insects and are produced by various subspecies of *B. thuringiensis*. The *cry1Ac* and *cry2Ab* genes are derived from *B. thuringiensis* variety *kurstaki* (Btk) and encode Bt toxins that are highly specific to a subset of lepidopteran insects (moths and butterflies), including *H. armigera* and *H. punctigera*, which are major pests of cultivated cotton in Australia (Dankocsik et al. 1990; Macintosh et al. 1990; Widner & Whiteley 1990).

28. The Cry proteins diffuse through the midgut membrane of feeding lepidopteran insects and bind to specific high affinity receptors on the midgut epithelium surface (Hofmann et al. 1988; Karim et al. 2000; Van Rie et al. 1989; Van Rie et al. 1990). Non-target insects, mammals, birds and fish do not possess these receptors and therefore are not susceptible to the toxic effects of these insecticidal proteins.

29. The toxic effect of Cry proteins requires alkaline conditions (as provided in the larval insect gut) to dissolve the crystals, partial digestion by specific proteases to release the active core toxin, and binding to specific receptors found on the insect midgut epithelium surface. Binding leads to formation of pores in the cell membrane which leads to leakage of intracellular contents into the gut lumen and water into the cell, resulting in cell death, gut paralysis and starvation. It is these steps that provide the high degree of target specificity of each Cry protein (English & Slatin 1992; Hofmann et al. 1988; Knowles & Dow 1993; Van Rie et al. 1989).

30. The Cry1Ac and Cry2Ab proteins belong to two very divergent families of Cry proteins (Crickmore et al. 1998). They share low sequence homology (only 27% identical) and interact with different receptor sites in the target insects (Estela et al. 2004).

#### *Regulatory sequences controlling the expression of the cry1Ac and cry2Ab genes*

31. Expression of the *cry1Ac* and *cry2Ab* genes in the GM cotton lines are controlled by an enhanced 35S promoter from CaMV (Kay et al. 1987; Odell et al. 1985), which is a retrovirus that can infect cruciferous plants.

32. The *cry2Ab* gene is linked to the *PetHSP70* 5' untranslated leader sequence, from the petunia *heat shock protein 70* gene, and *ctp2*, which encodes the chloroplast transit peptide (CTP) from the *Arabidopsis thaliana epsps* gene (Klee et al. 1987). The CTP allows transport of the Cry2Ab protein in the GM cotton cells to the chloroplast. Once transported into the chloroplast stroma the CTP is proteolytically removed from the protein and rapidly degraded (Bartlett et al. 1982; della-Cioppa et al. 1986).

33. The termination sequences are derived from the alpha subunit of the *beta-conglycinin* gene of soybean (Schuler et al. 1982) for the *cry1Ac* gene, and from the *nopaline synthase (nos)* gene of *A. tumefaciens* (Depicker et al. 1982) for the *cry2Ab* gene. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants.

34. Although *A. tumefaciens* and the CaMV are plant pathogens, the regulatory sequences introduced into the GM cotton lines from these organisms are not capable of causing disease.

#### *Toxicity of Cry1Ac and Cry2Ab*

35. A large number of insect species from a range of orders have been tested for sensitivity to the Cry1A family of proteins (including the Cry1Ac protein) (Macintosh et al. 1990; Sims 1994b; Sims 1995; van Frankenhuyzen & Nystrom 2002). This class of Cry proteins is predominantly active against lepidopteran species, although there is some evidence that some dipteran species (eg mosquitoes) may also be affected when very high levels of protein are ingested. Where concentrations have been reported, these range from 50,000 to 100,000 ng of protein/mL (Liang & Dean 1994; Widner & Whiteley 1989). These levels are approximately 200 fold higher than the levels toxic for the target lepidopteran insects. Levels of Cry1Ac protein in Bollgard II<sup>®</sup> leaves have been measured at 2400 ng per g fresh weight (refer to RARMP prepared for DIR 012/2002).

36. The toxicity of the Cry2A family of Cry proteins, to which Cry2Ab belongs, has been tested against a large number of insect species from a range of orders (van Frankenhuyzen & Nystrom 2002). Cry proteins from this family are mainly toxic to lepidopteran insects but in some cases, dipteran insects can also be affected. The Cry2Ab protein present in Bollgard II<sup>®</sup> cotton is generally considered to have specific toxicity for lepidopterans (Dankocsik et al. 1990; Widner & Whiteley 1990; Widner & Whiteley 1989). The potential for toxicity of the Cry2Ab protein for non-target invertebrates is discussed further in Chapter 3.

37. Toxicity studies have also been performed on key beneficial, non-target species using the Cry1Ac or the Cry2Ab protein (Maggi 1993a; Maggi 1993b; Palmer & Beavers 1993a; Palmer & Beavers 1993b; Palmer & Beavers 1993c; Sims & Martin 1996; Sims 1994b). There were no adverse effects from the Cry proteins observed, even at concentrations well above the expression levels found in INGARD<sup>®</sup> and Bollgard II<sup>®</sup> cotton, for any of the beneficial, non-target species tested in these studies.

38. Purified Cry1Ac protein, at acute oral doses of up to 4300 mg/kg body weight, produced no adverse effects in mice (Naylor 1993a; Naylor 1993b). Likewise, acute oral toxicity studies in mice with purified Cry2Ab protein at doses of up to 1450 mg/kg have not shown any adverse effects (Bechtel 1999).

39. Multiple studies on the acute oral toxicity of Bt microbial preparations, containing Cry1Ac and Cry2Aa (to which Cry2Ab is 88% identical), in mammals such as rats and rabbits have revealed no adverse effects at very high doses (Barbera 1995; Carter & Liggett 1994; McClintock et al. 1995; Spencer et al. 1996). Two human studies found no observable health effect of an oral dose of 1000 mg of Bt microbial spores per day for 3 or 5 days (Betz et al. 2000; McClintock et al. 1995).

40. A study that investigated the effects of the Cry1Ab protein (86% identical to the Cry1Ac protein) on a bovine hepatocyte culture concluded that there were no significant changes to cell morphology or the secretion of albumin or the enzyme lactate dehydrogenase. These results indicate that Cry1Ab has little acute toxicity on mammalian cells even when applied directly (Shimada et al. 2003).

#### ***Allergenicity of Cry1Ac and Cry2Ab***

41. The Cry1Ac protein is approximately 133 kDa in size, which is considerably larger than typical allergenic proteins. It is heat labile and rapidly degraded (under 30 seconds) under simulated mammalian gastrointestinal conditions (Fuchs et al. 1993a). The Cry2Ab protein is approximately 71 kDa in size, which is at the upper end of the typical size range for allergenic proteins, and is also easily digested (Leach et al. 2000). The Cry1Ac and Cry2Ab proteins are not detectable in oil and linters derived from the insecticidal GM cotton lines and neither display characteristics common to known food allergen proteins such as glycosylation; resistance to degradation by heat, acid and proteases of the digestive system; or derivation

from a known allergenic source (Astwood et al. 1996; Kimber et al. 1999; Metcalfe et al. 1996; Taylor & Lehrer 1996). Searches of protein sequence databases have shown no significant matches of the Cry1Ac or Cry2Ab proteins to known allergens (Metcalfe et al. 1996).

#### **4.2.2 Herbicide tolerance gene (*cp4 epsps*) and the encoded protein**

42. The *cp4 epsps* gene, which confers tolerance to glyphosate (N-phosphonomethyl glycine), the active ingredient of a number of herbicides including Roundup Ready® Herbicide, was isolated from *A. tumefaciens* species strain CP4. This *cp4 epsps* gene encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgett et al. 1996).

43. In plants, the native *epsps* gene encodes an enzyme (EPSPS) critical for the biosynthesis of aromatic amino acids (tryptophan, tyrosine and phenylalanine), which are essential building blocks for cellular proteins. The EPSPS enzyme catalyses the addition of the enolpyruvyl moiety of phosphoenolpyruvate to shikimate-3-phosphate. EPSPS performs this function in plants, bacteria, algae and fungi but is absent from mammals, which are not able to synthesise these aromatic amino acids (Bentley 1990; Padgett et al. 1993).

44. Glyphosate herbicide inhibits the activity of the naturally occurring EPSPS enzyme in plants, thus blocking the biosynthesis of aromatic amino acids and eventually leading to cell death (Steinrücken & Amrhein 1980). The *cp4 epsps* gene from *Agrobacterium* is naturally insensitive to the effects of glyphosate (Padgett et al. 1993), as are a number of other microbial EPSPS enzymes (Eschenburg et al. 2002; Schulz et al. 1985). Consequently, in GM plant cells expressing the *Agrobacterium cp4 epsps* gene, biosynthesis of aromatic amino acids is not inhibited in the presence of glyphosate.

#### **Regulatory sequences for expression of the *cp4 epsps* genes**

45. Expression of the *cp4 epsps* gene in Roundup Ready® and Roundup Ready®/Bollgard II® cotton is controlled by the 34S promoter from FMV which can infect plants within the Scrophulariaceae (figwort) family.

46. Two copies of the *cp4 epsps* gene are present in Roundup Ready Flex® and Roundup Ready Flex®/Bollgard II® cotton lines and each copy is under the control of a different chimeric promoter. One *cp4 epsps* gene is controlled by the chimeric *P-FMV/TSF1* promoter consisting of the elongation factor *EF-1 alpha* promoter from the plant *Arabidopsis thaliana* (Axelos et al. 1989) and an enhancer sequence from the FMV 35S gene (Richins et al. 1987). The other *cp4 epsps* gene is controlled by chimeric *P-35S/ACT8* promoter consisting of the *act8* actin promoter from *A. thaliana* (An et al. 1996) and an enhancer sequence from the CaMV 35S (Kay et al. 1987).

47. Roundup Ready Flex® and Roundup Ready Flex®/Bollgard II® cotton lines also contain additional non-coding sequences from other plant species for improved gene expression. These include the non-translated leader (exon 1) and intron sequences (*L-TSF1* and *I-TSF1*, respectively) from the *A. thaliana EF-1 alpha A1* gene (Axelos et al. 1989), and the non-translated leader and intron/exon sequences (*L-ACT8* and *I-ACT8*, respectively) from the *act8* actin gene of *A. thaliana* (An et al. 1996).

48. In plants, the EPSPS enzyme and the site of aromatic amino acid synthesis are located in the chloroplast. Thus, the *cp4 epsps* gene in the GM cotton lines was linked to *ctp2* from the *epsps* gene of *A. thaliana* (Klee et al. 1987) to allow transport of the CP4 EPSPS enzyme in the GM cotton to the chloroplast. Once transported into the chloroplast, the chloroplast transit peptide (CTP) is removed and degraded (Bartlett et al. 1982; della-Cioppa et al. 1986).

49. The termination sequences for the *cp4 epsps* genes are derived from the *nos* gene of *A. tumefaciens* in Roundup Ready® and Roundup Ready®/Bollgard II® cotton, and from the pea *ribulose-1,5-bisphosphate carboxylase small subunit E9* gene (Coruzzi et al. 1984) in Roundup Ready Flex® and Roundup Ready Flex®/Bollgard II® cotton. Although some of the regulatory sequences are derived from plant pathogens (FMV and *A. tumefaciens*) they are not capable of causing disease.

#### **Toxicity of CP4 EPSPS**

50. Purified CP4 EPSPS protein, at acute doses of up to 572 mg/kg body weight, produced no adverse effects in mice (Harrison et al. 1996). This is more than a thousand times the anticipated concentration of CP4 EPSPS in commercial food derived from all GM food crops expressing this enzyme under development by Monsanto at that time (soybean, potato, tomato, corn)(Harrison et al. 1996).

51. Sequence homology does not show any structurally relevant similarity between the CP4 EPSPS protein and any known toxic or pharmacologically active protein relevant to human health (SwissProt, GenPept, PDB, PIR and PRF).

#### **Allergenicity of CP4 EPSPS**

52. The CP4 EPSPS protein is 47.6 kDa and, although this falls within the typical MW range for allergenic proteins, it is unlikely to be an allergen because it does not display characteristics common to known food allergen proteins, such as those discussed in paragraph 41 (ANZFA 2001b; Canadian Food Inspection Agency 1997; Harrison et al. 1996), and shows no significant amino acid sequence homology to known allergens in protein databases (Mitsky 1993). A recent search of databases (SwissProt, GenPept, PDB, PIR, PRF and SDAP) continues to support this finding. The CP4 EPSPS protein is rapidly inactivated by heat, enzymatic digestion, and acid in simulated mammalian digestive or gastric fluid (ANZFA 2001b; Canadian Food Inspection Agency 1997; Harrison et al. 1996). Furthermore, Roundup Ready® soybean expressing the identical introduced CP4 EPSPS protein has been shown not to be allergenic to humans (Batista et al. 2005).

#### **4.2.3 Antibiotic resistance genes (*nptII* and *aad*) and the encoded proteins**

53. The *nptII* gene was isolated from the *E. coli* Tn5 transposon (Beck et al. 1982). It encodes the enzyme neomycin phosphotransferase type II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. NPTII uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII-producing cell. The *nptII* gene functioned as a selectable marker, which allowed modified cotton plant cells to grow in the presence of the kanamycin or neomycin, and therefore be selected, while inhibiting the growth of non-modified cells.

54. The *aad* gene was isolated from the *E. coli* Tn7 transposon and encodes the enzyme aminoglycoside adenylyltransferase (AAD), which confers resistance to the antibiotics streptomycin and spectinomycin. The *aad* gene is not expressed in the GM cotton lines because the bacterial regulatory sequence that controls its expression is not active in plants. Thus, its toxicity and allergenicity are not considered in this RARMP. This gene was used in the laboratory prior to the genetic modification of cotton plant cells to select for bacteria containing the modified DNA. The absence of *aad* gene expression in the Bollgard II® cotton was confirmed by the inability to detect the AAD enzyme in an Enzyme-Linked ImmunoSorbent Assay (ELISA) protein assay (refer to RARMP prepared for DIR 012/2002).

### **Regulatory sequences for expression of the *nptII* and *aad* genes**

55. Expression of the *nptII* gene in the GM cotton lines is controlled by the CaMV 35S promoter (Kay et al. 1987; Odell et al. 1985) and the termination sequence of this gene is the 3' non-translated region of the *nos* gene from *A. tumefaciens* (Rogers et al. 1985). Although these regulatory sequences are derived from plant pathogens they are not capable of causing disease.

56. The *aad* gene is not expressed in the GM cotton plants because it is under the control of a bacterial regulatory sequence, the Tn7 promoter, which is not active in plants.

### **Toxicity of NPTII**

57. The *nptII* gene introduced into mammalian cell lines had no effects on viability or growth. During gene therapy experiments, mammalian cells expressing the NPTII protein were infused into cancer patients with no adverse effects (Flavell et al. 1992).

58. The NPTII protein produced in GM tomatoes has been fed to rodents and reported to be rapidly inactivated and degraded (Calgene 1990). An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). A similar study in mice also reported no adverse effects when fed NPTII at 5000 mg/kg of body weight (Fuchs et al. 1993c). Furthermore, the product of the *nptII* gene is considered safe and is approved by the FDA as a food additive in GM cotton, canola and tomatoes (21 CFR 173:170)(FDA 1994).

59. Protein and DNA sequence comparisons using sequences from four separate databases (Genbank, EMBL, PIR29, Swiss-Prot) indicated that NPTII does not have significant homology to any proteins listed as food toxins in these databases (FDA 1994).

### **Allergenicity of NPTII**

60. The NPTII protein is approximately 29 kDa in size, which is within the typical size range of allergenic proteins. However, it does not possess glycosylation sites, is not stable in the mammalian digestive system and is heat labile, all of which decreases the probability that it is allergenic (ANZFA 2001a; ANZFA 2001b; FDA 1994; Fuchs et al. 1993b; US FDA 1998). Fuchs et al. (1993b) reported that no NPTII was detected 10 seconds after the addition of simulated gastric fluid as measured by both western blot and enzymatic activity (Fuchs et al. 1993c). Protein sequence comparisons with sequences from protein databases indicated that NPTII does not have significant sequence homology to any known protein food allergens (Fuchs & Astwood 1996).

61. The FDA has evaluated data submitted for deliberate releases of GMOs expressing the NPTII protein and concluded that NPTII does not have any of the characteristics associated with allergenic proteins (US FDA 1998).

#### **4.2.4 Reporter gene (*uidA*) and the encoded protein**

62. The *uidA* reporter gene, which encodes the enzyme  $\beta$ -glucuronidase (GUS), is derived from the common gut bacterium *Escherichia coli*. It is the most widely used reporter gene in GM plants (Miki & McHugh 2004) as it allows GM tissues to be identified using a simple assay.

63. The GUS protein is a monomer with a molecular mass of 68 kDa, and the GUS enzyme is active as a tetramer. GUS catalyses the hydrolysis of  $\beta$ -glucuronides and, less efficiently, some  $\beta$ -galacturonides. A large variety of  $\beta$ -glucuronides exist in nature and have been described as the detoxified excretion forms of xenobiotics (eg drugs) and endogenous

compounds (eg steroids) in vertebrates (Jefferson & Wilson 1991). *E. coli* lives in the digestive tract of vertebrates, including humans (Jefferson et al. 1986), and the GUS enzyme enables it to metabolise  $\beta$ -glucuronides as a main source of carbon and energy.

64. GUS cleaves the chromogenic substrate X-gluc (5-bromo-4-chloro-3-indolyl  $\beta$ -D-glucuronide) to produce an insoluble blue colour (Jefferson et al. 1987). Endogenous GUS enzyme activity is found in many other bacterial species, and also in vertebrates and invertebrates, but there is very little background activity in non-GM plants (Gilissen et al. 1998; Jefferson et al. 1987). Therefore, the production of a blue colour in particular plant cells after staining with X-gluc indicates that these cells express GUS from the introduced *uidA* gene, and have been successfully genetically modified.

65. The *uidA* gene was used as a marker in the laboratory for selecting successfully modified cotton cells after the genetic modification.

#### ***Regulatory sequences for expression of the uidA gene***

66. Expression of the *uidA* gene in the GM cotton plants is controlled by the CaMV 35S promoter (Kay et al. 1987; Odell et al. 1985) and the termination sequence of this gene is the 3' non-translated region of the *nos* gene from *A. tumefaciens* (Rogers et al. 1985). Although these regulatory sequences are derived from plant pathogens they are not capable of causing disease.

#### ***Toxicity of $\beta$ -glucuronidase***

67. Acute oral toxicity studies in mice with purified GUS protein at doses of up to 100 mg/kg did not show any adverse effects (Naylor 1992). Studies feeding humans and animals with  $10^{10}$  GUS-containing *E. coli* bacteria per ingestion also did not show any toxic or pathogenic reactions (Gilissen et al. 1998).

68. When the amino acid sequence of the GUS protein expressed in the GM cotton lines was compared to sequences from protein databases, it only shared sequence similarities to homologous *E. coli* and other glucuronidase proteins, as expected (information provided by the applicant). These proteins have not been described as toxic to people. Metabolites of the *E. coli* GUS enzyme are non-toxic (Gilissen et al. 1998).

#### ***Allergenicity of $\beta$ -glucuronidase***

69. The GUS protein is approximately 68 kDa in size, which is within the typical size range of allergenic proteins. However, the widespread occurrence of GUS and the constant exposure of humans to the protein without known ill effect indicates that the likelihood of GUS being an allergen is extremely low (Gilissen et al. 1998). In addition, the GUS protein does not possess glycosylation sites and is rapidly denatured in simulated mammalian digestive system (ANZFA 2002a; Fuchs & Astwood 1996). The GUS protein from *E. coli* is rapidly degraded (<15 seconds) in simulated gastric fluid and loses its activity by heating/cooking (Fuchs & Astwood 1996). Protein sequence analysis indicates that GUS does not have significant sequence homology to any known protein food allergens.

### **4.3 Method of genetic modification**

70. The Bollgard II<sup>®</sup> cotton line was produced by inserting the *cry2Ab* and *uidA* genes into the genomic DNA of INGARD<sup>®</sup> cotton line DP50B containing the *cry1Ac*, *nptII* and *aad* genes (transformation event MON15985). The genes were delivered into the cotton meristematic cells by microprojectile bombardment (McCabe & Martinell 1993). This technique is a well-established method of plant transformation that uses compressed gas to 'shoot' tiny tungsten or gold particles coated with the genes to be inserted into plant cells. Where the introduced genes



became incorporated into the genome of the bombarded plant cells, they were identified *in vitro* by histochemical staining for the GUS marker protein (conferred by the *uidA* gene). INGARD<sup>®</sup> cotton was produced by *Agrobacterium*-mediated transformation of a Coker cotton variety, introducing the *cry1Ac*, *nptII* and *aad* genes (transformation event 531). Further information about these introduced gene constructs can be found in previous RARMPs (DIR 012/2002 for Bollgard II<sup>®</sup>, and DIRs 022/2002 and 023/2002 for INGARD<sup>®</sup>).

71. The Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines were produced by *Agrobacterium*-mediated DNA transformation (Zambryski 1992). *Agrobacterium tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (located between specific border sequences on a plasmid) from *A. tumefaciens*. The transfer of DNA is mediated by genes from the virulence region of Ti plasmids.

72. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains are unable to cause crown gall disease as they do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are necessary for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmids used to transfer DNA into the plant cell genome contain well characterised DNA segments required for their replication and selection in bacteria (Bevan 1984; Wang et al. 1984). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing any biosafety concerns or any adverse reactions.

73. For Roundup Ready<sup>®</sup> cotton, a disarmed binary vector (PV-GHGT07) was used to introduce a gene construct containing the *cp4 epsps*, *nptII* and *aad* genes into cotton variety Coker using standard *Agrobacterium* transformation protocols (transformation event MON1445). Further information about this construct can be found in previous RARMPs (DIR 012/2002 and 023/2002). Genetically modified plants were selected by resistance to kanamycin (conferred by the *nptII* gene). Roundup Ready<sup>®</sup> cotton was derived from a single genetic modification (transformation event MON1445).

74. For Roundup Ready Flex<sup>®</sup>, a disarmed binary plasmid vector (PV-GHGT35) was used to introduce a gene construct containing two copies of the *cp4 epsps* gene into cotton variety Coker using standard *Agrobacterium* transformation protocols (transformation event MON88913). Further information about this construct can also be found in previous RARMPs (DIR 035/2003, DIR 055/2004 and 059/2005). Genetically modified plants were selected by tolerance to glyphosate (conferred by the *cp4 epsps* genes). Roundup Ready Flex<sup>®</sup> cotton was derived from a single genetic modification (transformation event MON88913).

75. Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton were produced by conventional crossing of GM Bollgard II<sup>®</sup> cotton with GM Roundup Ready<sup>®</sup> or Roundup Ready Flex<sup>®</sup> cotton, respectively.

76. As part of the proposed commercial release, the Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup> and, Roundup Ready Flex<sup>®</sup> traits would be introduced into a range of cotton cultivars suitable for various growing conditions in Australia by conventional crossing.

#### **4.4 Characterisation of the GM cotton lines proposed for release**

##### **4.4.1 Stability and molecular characterisation**

77. Detailed information on the characterisation of the inserted genetic materials and stability of the genetic modification in the GM cotton lines is provided in the RARMPs prepared for

DIR 012/2002 (Bollgard II®), DIR 023/2002 (Roundup Ready®), DIR 035/2003 and DIR 055/2004 (Roundup Ready Flex®).

78. Phenotypic segregation and southern blot analysis from several generations of backcrossing has confirmed the stability of the inserted DNA for each of the five GM cotton lines. In each case, the introduced DNA (genes and regulatory sequences) is present as a single insert at one location in the cotton genome and does not include any unnecessary vector DNA. Analysis of phenotypic segregation also demonstrated that the transgenes are inherited in a normal Mendelian manner for a single dominant trait.

79. Bollgard II® was generated from two genetic modification events and, as expected, the *cry1Ac* and *cry2Ab* genes have been inserted into different regions of the plant genome and therefore segregate independently of one another (Shappley 2002).

80. Confirmation of the stability of the introduced genetic material in each of the GM cotton lines is also provided by the continued expression of the desired traits over many generations of breeding and several years of trialling and commercial scale plantings.

#### **4.4.2 Characterisation of the phenotype of the GMOs**

81. Phenotypic characteristics and agronomic properties of the GM cotton lines have been studied extensively prior to their commercial release south of latitude 22°S in Australia (for details see RARMPs prepared for DIR 012/2002, DIR 023/2002 and DIR 059/2005). The GM cotton lines do not display different morphological or agronomic traits compared to the non-GM parents, other than insect resistance and/or herbicide tolerance.

##### ***Expression of the Cry1Ac and Cry2Ab proteins in the GM cotton lines***

82. The promoters controlling expression of the *cry1Ac* and *cry2Ab* genes in Bollgard II® cotton result in expression of the Cry proteins throughout the growing season and in most of the tissues. Expression levels of the Cry1Ac and Cry2Ab proteins in Bollgard II® cotton have been extensively studied. Detailed information on the expression levels of these proteins in leaves, seeds and pollen of Bollgard II® cotton is presented and discussed in the RARMP for DIR 012/2002.

83. Expression of the Cry1Ac and Cry2Ab proteins in Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton was concluded to be similar to that of the parental lines based on glasshouse, field trials and bioassays, which demonstrated an equivalent level of insect resistance (Dr D Llewellyn, CSIRO, pers. comm.; Burns 2004). Therefore, conventional breeding of the herbicide tolerant GM cotton lines with Bollgard II® cotton does not alter *cry1Ac* and *cry2Ab* gene expression.

##### ***Expression of the CP4 EPSPS protein in the GM cotton lines***

84. The promoters controlling expression of the *cp4 epsps* gene in Roundup Ready® and Roundup Ready Flex® cotton result in expression of the CP4 EPSPS proteins throughout most parts of the plant. However, Roundup Ready Flex® cotton has increased and prolonged expression of the *cp4 epsps* gene compared to Roundup Ready® cotton, including expression in the reproductive parts of the plant, such as stigmas, anthers and floral buds.

85. The reproductive structures of cotton plants are very sensitive to the effects of glyphosate (Pline et al. 2002a; Pline et al. 2002b) and Roundup Ready® cotton crops sprayed with glyphosate beyond the four-leaf stage of growth exhibit reduced pollination and increased boll abortion (Monsanto Australia Limited 2001). Therefore, application of glyphosate over the top of these GM cotton plants after the four-leaf stage leads to yield loss (Charles 2002). However, Roundup Ready Flex® cotton can be sprayed with glyphosate throughout the growing season with no adverse impact on reproductive structures and hence no yield loss.

Detailed information on the expression levels of these proteins in Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton is provided in the RARMPs for DIR 023/2002 and DIR 059/2005, respectively.

86. Expression of the CP4 EPSPS protein in Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton was concluded to be similar to that of the parental lines based on glasshouse, field trials and bioassays, which demonstrated an equivalent level of herbicide tolerance (Dr D Llewellyn, CSIRO, pers. comm.; Burns 2004). Therefore, conventional breeding of Bollgard II<sup>®</sup> cotton with Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton does not alter *cp4 epsps* gene expression.

### ***Toxicity of the GM cotton plant material***

87. The results of extensive compositional analyses of whole cotton seed and processed cotton seed fractions demonstrate that the levels of important nutritional and anti-nutritional components in Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines are comparable to the parental non-GM varieties and are within established ranges for commercial cotton varieties (George et al. 2005; Hamilton 2000; Nida et al. 1994; Nida et al. 1995; Nida et al. 1996; Obert et al. 2003a; Obert et al. 2003b; Tang et al. 2006).

88. Cotton seed, meal and hulls are not generally used for human consumption because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids, but may be used for animal feed, particularly cattle, which are less affected by gossypol (OGTR 2002). The Roundup Ready<sup>®</sup>, Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines have been approved for use in stockfeed since 2000, 2002 and 2006, respectively. The use of cotton seed products derived from Roundup Ready<sup>®</sup> and Bollgard II<sup>®</sup> has not shown any adverse impacts for livestock as compared to non-GM cotton since the commercial release of these GM cotton lines in southern Australia (Monsanto annual reports submitted to the OGTR).

89. A recent study investigated the effects of feeding Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and non-GM cotton seed to dairy cows (Castillo et al. 2004). There were no significant differences between cows fed the alternative forms of cotton seed, as reflected by their milk yield, milk composition, body weight and body condition.

90. In studies on rats, quail or catfish fed cotton seed meal at 5 to 20% of their diets, no significant differences were found in weight gain and feed conversion, nor during gross autopsy, for animals fed Roundup Ready<sup>®</sup> cotton seed meal compared to those fed non-GM cotton seed meal (Canadian Food Inspection Agency 1997). Similarly, there was no significant difference between catfish, quail or broiler chicken fed Bollgard II<sup>®</sup> cotton seed meal and animals fed non-GM cotton seed meal (Gallagher et al. 2000; Li & Robinson 2000; Mandal et al. 2004). A review of studies which fed livestock diets containing GM plants (including insect resistant and glyphosate tolerant plants) found no adverse impacts on the animals (Aumaitre 2004).

91. FSANZ has approved the use of oil and linters from Roundup Ready<sup>®</sup>, Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton for use in food (ANZFA 2001b; ANZFA 2002b; FSANZ 2005).

92. The amino acid sequence of the CP4 EPSPS protein expressed in the GM cotton lines is identical to, or shares greater than 99% amino acid sequence identity with, the CP4 EPSPS protein produced in a number of other Roundup Ready<sup>®</sup> crops that are produced commercially, including soybean, corn and canola, in a number of countries. People have consumed these crops and/or their processed products since 1996 (James 2004) without any adverse effects reported.

93. Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton plants are toxic to some lepidopteran species which are the insect pests targeted to be killed by these GM cotton lines. Various researchers have investigated the potential impact of the Bollgard II<sup>®</sup> cotton on non-target invertebrates, both overseas (Naranjo & Ellsworth 2002) and in Australia (Addison 2001a; Addison 2001b; Addison 2001c). With the exception of lepidopteran insects, there were no significant negative effects of cotton with the Bollgard II<sup>®</sup> trait on the abundance or diversity of non-target invertebrates. This is discussed further in Chapter 3.

## **Section 5 The receiving environment**

94. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, duration and regions of the dealings, any relevant biotic/abiotic properties of the regions where the release would occur; intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already approved for commercial release; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2005).

95. Monsanto is seeking approval to conduct plant breeding, agronomic trials and seed production, and commercial scale planting of the five GM cotton lines in any area where environmental conditions are suitable north of latitude 22°S, without specific containment measures. The applicant intends to use the GM cotton plants and their products in the same manner as both non-GM and GM cotton currently grown commercially in Australia, south of latitude 22°S.

### **5.1 Relevant biotic factors**

96. There are a large number of pests that attack commercial cotton crops, with the type and number of pests differing from season to season and between different regions. The main pests of cultivated cotton in Australia include: cotton bollworm (*H. armigera*), native budworm (*H. punctigera*), cotton aphid (*Aphis gossypii*), green mirid (*Creontiades dilutus*), two-spotted spider mite (*Tetranychus urticae*), silverleaf whitefly (*Bemisia tabaci* b-biotype), thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*) and the green vegetable bug (*Nezara viridula*) (Farrell & Johnson 2005). Experience from growing cotton previously in northern regions of Australia suggests that insect pressure is higher in tropical areas during the wet season compared to the current southern cotton growing regions. The four key lepidopteran pests of cotton in northern Australia are cotton bollworm (*H. armigera*), native budworm (*H. punctigera*), cluster caterpillar (*Spodoptera litura*) and pink bollworm (*Pectinophora gossypiella*) (Cotton Catchment communities CRC 2006; Strickland et al. 2000; Strickland et al. 2003).

97. Cotton is also affected by a range of diseases which can affect the quality of the fibre and seed, as well as the yield and cost of production of the cotton crop (OGTR 2002). Again, the type and severity of infection differs from season to season and between different regions. The most significant diseases of cotton in Australia include: black root rot (*Thielaviopsis basicola*), Verticillium wilt (*Verticillium dahliae*), Fusarium wilt (*Fusarium oxysporum* var. *vasinfectum*), alternaria leaf spot (*Alternaria macrospora* and *A. alternata*), and boll rot (*Phytophthora nicotianae* var. *parasitica*) (Farrell & Johnson 2005). There are also over 30 species of fungi that can cause cotton seedling death, but this is predominantly caused by *Rhizoctonia solani*, *Pythium* spp. or *Fusarium* spp. (not Fusarium wilt) (Farrell & Johnson 2005).

## 5.2 Relevant abiotic factors

### 5.2.1 General cotton growing information

98. Areas where cotton can be grown in Australia are mainly limited by water availability (ie the right amounts at optimal times of the growth cycle via irrigation or rainfall), the suitability of the soil (good water retention qualities are required), temperature and the length of the growing season. Climatic data for some of the current cotton growing areas in southern Australia are given in Table 1.3.

99. Temperature is the dominant environmental factor affecting cotton development and yield (Australian Cotton Cooperative Research Centre 2002b; Constable & Shaw 1988). Cotton is planted when the minimum soil temperature at 10 cm depth is 14°C for at least three successive days. Cotton seedlings may be killed by frost and a minimum of 180–200 frost-free days of uniformly high temperatures (averaging 21–22°C) is required (Duke 1983). Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields (Constable & Shaw 1988).

- **Table 1.3 Climatic data for some of the current cotton growing regions in Australia.**

	Emerald Post Office (central QLD)	Narrabri West Post Office (northern NSW)	Bourke Post Office (northern NSW)	Hillston Airport (southern NSW)
Average daily max/min temperature (summer <sup>a</sup> )	34.2°C/20.3°C	32.3°C/17.3°C	35.6°C/20.3°C	32.4°C/17.6°C
Average daily max/min temperature (winter <sup>b</sup> )	23.3°C/7.8°C	18.9°C/4.5°C	18.9°C/5.5°C	15.8°C/4.6°C
Average monthly rainfall (summer)	84.4 mm	72.5 mm	38.8 mm	28.7 mm
Average monthly rainfall (winter)	27.8 mm	45.7 mm	23.6 mm	32.1 mm

- <sup>a</sup> December, January, February
- <sup>b</sup> June, July, August
- Source: <<http://www.bom.gov.au>>.

### 5.2.2 Potential cotton growing areas north of latitude 22°S

100. As discussed in Section 5.2.1, 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 and this is equally applicable to potential cotton growing areas in northern Australia.

101. The majority of the arable soils of northern Australia are similar in that they are red and yellow earths and poorly drained cracking clays. The fertility of these soils is moderate to low. Soil type and fertility impact on crop nutrition, soil surface management and irrigation systems.

102. Northern Australia experiences a wet summer and dry winter. During the summer (Dec-Feb), temperatures are extremely high and the monsoon brings humid conditions with strong westerly winds, showers and thunderstorms. Rainfall amounts can vary substantially from year-to-year, and occasional tropical cyclones can bring abundant rainfall to northern coastal regions and possibly further inland. The highest annual rainfall occurs on the east-facing slopes of north-east Queensland in the area surrounding Cairns and Tully. The end of the monsoon results in clear skies and mild, dry conditions during winter (information from the Bureau of Meteorology).

103. The reasons why attempts over the last hundred years to establish commercial cotton cultivation in northern Australia were unsuccessful are discussed in Section 5.3. Essentially they failed as a result of inappropriate agricultural practice and the barrier of geographic isolation that were insuperable at the time.

104. The GM cotton lines proposed for release have the same water, soil type, nutrient and climatic requirements as non-GM cotton. Therefore, if expansion of cotton production occurs into northern Australia, it would only be into regions suitable for growing cotton, and would be limited in the same way as non-GM cotton. Besides the weather, the location where GM cotton lines would be grown would also be determined by other factors such as access to infrastructure, seed and variety availability, and ultimately grower's preferences. The main difference in cultivating the GM cotton lines in northern Australia as opposed to non-GM cotton is inherently an economic one, as it would enable a reduction in the intensity of on-farm insect and weed management practices.

105. A study by the Australian Cotton Cooperative Research Centre (ACCRC)(Australian Cotton Cooperative Research Centre 2004b), based on average temperatures during the growing season, timing of rainfall, and the suitability of the soil for cotton cultivation, indicates considerable potential for expansion into northern Australia in particular areas of WA, the NT and QLD.

106. The ACCRC study examined potential regions for cotton growing in northern Australia and suggested at least 200,000 ha of potential irrigation areas that could be developed over the next 10 years. Climatic conditions and proposed region specific production systems for five of these sites where the ACCRC is currently involved in cotton research are given in Table 1.4. These are the most likely regions for the introduction of commercial cotton production, and previous field trials with GM cotton indicated cotton could be grown successfully in these areas.

- **Table 1.4 Climatic data and proposed production systems for potential cotton growing areas in northern Australia.**

	Broome Post Office (northern WA)	Kununurra ORIA (northern WA)	Katherine Council (northern NT)	Richmond Post Office (northern QLD)	Lower Burdekin Ayr DPI RS (northern QLD)
Average daily max/min temperature (summer <sup>a</sup> )	33.6°C/26.1°C	36.7°C/25.2°C	35.3°C/24.0°C	36.9°C/22.6°C	31.7°C/22.5°C
Average daily max/min temperature (winter <sup>b</sup> )	28.6°C/14.9°C	31.4°C/16.1°C	30.9°C/14.3°C	26.8°C/9.4°C	25.6°C/12.3°C
Average monthly rainfall (summer <sup>a</sup> )	126.1 mm	171.6 mm	216.4 mm	98.7 mm	182.4 mm
Average monthly rainfall (winter <sup>b</sup> )	10.1 mm	1.8 mm	0.9 mm	9.3 mm	18.2 mm
Growing season	May–November	April–October	March–October	December–July	March–November
Arable soil type	Sandy loam	Cracking clay	Clay loam and sandy clay loam	Cracking clay, some inherent salinity	Cracking clay
Irrigation system	Sub surface drip	Furrow	Sub surface drip/overhead	Furrow	Furrow

Development status	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.	New area under development or evaluation	New area under development or evaluation	Existing (non-cotton) irrigated cropping and/or potential for expansion.
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- <sup>a</sup> December, January, February
- <sup>b</sup> June, July, August
- ORIA: Ord River Irrigation Area
- DPI RS: Department of Primary Industries Research Station
- Sources: [BOM](#) and ACCRC (2004)

107. While global climate change may result in alterations to areas that are potentially suitable for cotton cultivation, these changes would apply to both GM and non-GM cotton.

### 5.3 Relevant agricultural practices

108. The agronomic management of the GM cotton lines containing the *cp4 epsps* gene (herbicide tolerance trait) would differ from the management of non-GM cotton in that glyphosate herbicide could be applied over the top of the cotton crop to control weeds. Fewer applications of insecticide sprays are required for those cotton lines containing the *cry1Ac* and *cry2Ab* genes, since they are resistant to the major lepidopteran pests of cultivated cotton.

109. For Roundup Ready<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup>, which contains one copy of the *cp4 epsps* gene, glyphosate applications are restricted to before the four-leaf stage of growth (i.e. prior to flower formation, approximately 3 to 5 weeks after planting). For Roundup Ready Flex<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>, which contain two copies of the *cp4 epsps* gene, glyphosate can be applied throughout the growing season (approximately 24 to 28 weeks). As part of its role in regulating pesticides, the APVMA may impose conditions on the use pattern of herbicides in order, for example, to limit residue levels or minimise the development of herbicide resistance.

110. A resistance management plan (RMP) for Bollgard II<sup>®</sup> cotton varieties grown south of latitude 22°S has been developed by the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers' Research Association in consultation with the APVMA (Farrell & Johnson 2005; Monsanto Australia Limited 2004). The APVMA requires implementation of this plan as a condition of registration. The RMP is designed to minimise the development of resistant insects and requires growers to employ a number of measures designed to achieve this objective. As part of the resistance management strategy, refuge crops must be grown, to allow Bollgard II<sup>®</sup>-sensitive insects to survive. A similar resistance management plan for areas north of latitude 22°S has been developed and cultivation of Bollgard II<sup>®</sup> cotton varieties would need to comply with this resistance management plan and any other relevant conditions that may be imposed by the APVMA (discussed further in Chapter 2, Event 32).

111. All other crop management practices, including application of water and fertiliser, are expected to be similar to those for non-GM cotton. However, cotton production practices in northern Australia are likely to differ from those currently used in south-eastern Australia, mainly due to the tropical climate which presents different environmental and growing conditions.

112. One key difference anticipated with cotton cultivation in northern Australia is winter (dry season) cropping, which may be necessary in certain areas to avoid periods of highest insect abundance (Australian Cotton Cooperative Research Centre 2004b). Additionally, the

wet season would impact adversely on cotton plant growth, cotton fibre quality, and the ability to access and operate machinery in the cotton fields.

113. It should be noted that any changes to agricultural systems arising from the introduction of cotton into new areas such as northern Australia will not be specific to the GM lines. Previous attempts of commercial cotton cultivation in northern Australia over the past 100 years have ended in failure. This has been attributed to a combination of factors including cultivation during the wet season (inconsistent rainfall, season too short), low plant populations, poor choice of soils, unsustainable insect pest management practices, lack of effective irrigation techniques, use of unsuitable cotton varieties, and pathogens (Australian Cotton Cooperative Research Centre 2004b; Lyn Craven pers. Comm., 2006). General overall problems were geographic distance, ignorance of the physical environment, and an aversion to learning from experience (Bauer 1985a, cited in Australian Cotton Cooperative Research Centre 2004b). Attempts at growing other large scale commercial agricultural crops in northern Australia in the past have also failed due to similar reasons (Australian Cotton Cooperative Research Centre 2004b).

114. The extensive land area of Australia north of latitude 22°S encompasses a range of climatic and environmental conditions, therefore region-specific production systems will need to be developed. One location that would require a notably different cotton production system is the west Kimberley region (eg Broome), where cotton would be grown on sandy loams and irrigated by sub-surface drip irrigation (Australian Cotton Cooperative Research Centre 2004b).

115. High levels of farm hygiene are commonly maintained on cotton farms (eg all equipment is cleaned on entry and exit to a field/farm to prevent the transfer of disease or the spread of weeds). Weeds and cotton volunteers on roads and irrigation structures are controlled by mechanical removal or non-glyphosate herbicides (Charles et al. 2002). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice may be implemented at some sites in northern Australia if GM cotton is commercially grown. At some potential cotton growing sites in northern Australia where tail water is not collected, experiments with growing Bollgard II® cotton have resulted in the development of efficient irrigation practices resulting in minimal run-off into river systems (Moulden et al. 2006).

#### **5.4 Presence of related plants in the receiving environment**

116. At present, there is no cotton (GM or non-GM) grown commercially in areas north of latitude 22°S in Australia. However, the Regulator has recently approved (August, 2006) the commercial release of the herbicide tolerant Liberty Link® GM cotton without containment measures for current cotton growing areas and potential cotton growing areas including northern Australia. Field trials with GM cotton lines containing the herbicide tolerance gene and/or the same or similar Cry proteins north of latitude 22°S have been conducted under limited and controlled conditions since 1997 and 1998, respectively. Currently, limited and controlled field trials with Bollgard II®, Roundup Ready®/Bollgard II®, and Roundup Ready Flex®/Bollgard II® cotton are being conducted in the Wyndham-East Kimberley and Broome regions of Western Australia, in areas around Katherine, Northern Territory, and in the Burdekin Shire, Queensland. Approvals for these and other previous field trials with relevant GM cotton lines in areas north of latitude 22°S are summarised in Section 6.

117. Cotton can persist as a perennial plant in tropical areas and small populations of naturalised cotton (*G. hirsutum* and *G. barbadense*) exist in northern Australia, particularly in



areas associated with a prolonged supply of fresh water (Hnatiuk 1990). The majority of naturalised *G. hirsutum* populations occur in the Northern Territory, while naturalised *G. barbadense* occurs mainly along the eastern regions of Queensland (data from Australian Virtual Herbarium, <http://www.anbg.gov.au/avh/>). Both *Gossypium* species are commonly found in littoral and watercourse habitats (Eastick 2002).

118. It has been noted by scientists over many years that the morphology of many of these naturalised cotton populations is distinct from that of the cultivated cotton varieties. When grown in a glasshouse, they tend to have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002).

119. Tufted seeded cotton plants were originally used when hand delinting was required, before the advent of mechanical saw gins in the late 18th century. Tufted seeded cotton plants were subsequently replaced by fuzzy seeded varieties with better lint characteristics and disease resistance. It seems likely, therefore, that many naturalised cotton populations result from attempts in the early 19th century to establish cotton industries in northern Queensland and the Northern Territory (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002).

120. Some naturalised cotton populations have been observed which appear to be from a more recent origin, but none seem to have originated from the current commercial types of *G. hirsutum* that have been cultivated since the 1970's (Eastick 2002).

121. There are 17 native species of *Gossypium* in Australia, most of which can be found in the Northern Territory and the north of Western Australia (OGTR 2002). *G. australe* is the most widely distributed species throughout northern Australia, occurring from the east to west coast and predominantly north of the Tropic of Capricorn (Australian Virtual Herbarium, <http://www.anbg.gov.au/avh/>). The native *Gossypium* species prefer well-drained sandy loams and are rarely found on heavy clay soils favoured by cultivated cotton (OGTR 2002). Generally, they are found in native vegetation and not in disturbed/modified habitats such as agricultural areas (Groves et al. 2002).

122. Well established genetic incompatibility prevents crossing of native cotton species with cultivated cotton (OGTR 2002). Refer to Chapter 2, Event 20 for more detail.

## 5.5 Presence of the same or similar proteins in the receiving environment

123. The same or similar CP4 EPSPS, Cry1Ac, GUS and NPTII proteins are widespread in the environment, through the presence of the bacteria from which they are derived. This forms part of the baseline data for assessing any risks from exposure to these proteins that may result from the proposed release of the GM cotton lines.

124. The CP4 EPSPS protein is produced naturally by the CP4 strain of the common soil bacterium *Agrobacterium* sp. (Padgett et al. 1996). This bacterium can also be found on plants and fresh plant produce. Similar EPSPS proteins are present in all plants, bacteria and fungi.

125. The Cry1Ac protein is naturally produced by the *kurstaki* variety of the common soil bacterium *B. thuringiensis* (*Bt*). The protein is also present in Btk (*B. thuringiensis* var *kurstaki*) microbial sprays which are used to protect crops from insect herbivory.

126. The Cry2Ab protein is not naturally expressed in soil bacteria or present in Btk sprays due to an ineffective promoter of the *cry2Ab* gene. However, the Cry2Ab protein expressed in the Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton is 88% identical at the amino acid sequence level to the Cry2Aa protein (Dankocsik et

al. 1990; Widner & Whiteley 1989). Like the Cry1Ac protein, the Cry2Aa protein is naturally expressed in *B. thuringiensis* var *kurstaki* and present in Btk sprays (information supplied by the applicant for DIR 012/2002).

127. Related Cry proteins are also produced by other varieties of *B. thuringiensis*. Spores from a range of *Bt* varieties and their crystal (Cry) toxins are found widely in both the agricultural and natural environment, including in soil, on plant leaves, in grain stores and dead insects (Meadows 1993).

128. The presence of Bt toxins in agricultural situations has increased over the past 35 years due to the commercial use of Btk microbial sprays to protect food crops from insect attack (ANZFA 1999). Residues of Btk proteins, including Cry1Ac and Cry2Aa (Dankocsik et al. 1990; Widner & Whiteley 1989), are present on a wide variety of foods, including lettuce and tomato, with no reported toxic or allergic responses in humans (ANZFA 1999).

129. The GUS and NPTII proteins are widespread in the environment since they are naturally produced by the common gut bacterium *E. coli*, which is widespread in human and animal digestive systems (Jefferson et al. 1986) as well as in the environment.

130. The GUS protein produced in GM plants is 99.8 % identical to the *E. coli* GUS protein. GUS activity is also found in a wide range of other bacteria, including other microorganisms of the digestive tract and many soil bacteria (Gilissen et al. 1998).

131. GUS enzyme activity has been detected in numerous plant and animal species, including species used as raw food (Gilissen et al. 1998). It is very common in almost all tissues of vertebrates and is also present in invertebrates such as molluscs, nematodes and insects. GUS activity has been detected in over 50 different plant species (Hu et al. 1990).

132. Humans (and, by implication, other animals) continually ingest kanamycin-resistant microorganisms, some containing the NPTII enzyme. The diet, especially raw salad, is the major source: estimated conservatively, each human ingests  $1.2 \times 10^6$  kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998) with Flavell et al. (1992) estimating approximately  $10^{12}$  per person. Kanamycin-resistant bacteria have been isolated from soil, river water and sewage (Smalla et al. 1993).

## Section 6 Previous releases

### 6.1 Australian approvals of the GM cotton lines

#### 6.1.1 Previous releases approved by GMAC or the Regulator

133. Under the current regulatory system all five GM cotton lines proposed for release in northern Australia have previously been approved for commercial release south of latitude 22°S (under DIRs 012/2002, 023/2002 and 059/2005) and for field trials (limited and controlled release) north of latitude 22°S (see Table 1.5).

• **Table 1.5 Previous approved limited and controlled trials north of latitude 22°S.**

DIR	GMO	Approved in	Size of area	Location
006/2001	Bollgard II® Roundup Ready® / Bollgard II®	2002	up to 90 ha over one season	WA, NT
009/2001	Bollgard II®	2002	up to 19.5 ha over one season	WA
012/2002	Bollgard II® Roundup Ready® / Bollgard II®	2002	up to 800 ha per season since 2002	WA, NT, QLD

035/2003	Roundup Ready Flex® Roundup Ready Flex® / Bollgard II®	2003	up to 30 ha per season over two seasons	WA, NT, QLD
055/2004	Roundup Ready Flex® Roundup Ready Flex® / Bollgard II®	2005	up to 45 ha per season over one season	WA, NT, QLD

134. Commercial releases have been restricted to areas south of latitude 22°S because of uncertainty about the potential weediness of the GM cotton lines in the northern tropical areas (refer to Chapter 4, Identified Risk 3 and the RARMP for DIR 012/2002 for details).

135. Numerous field trials in northern Australia of GM cotton plants, including plants containing the same or similar Cry proteins to those being proposed under this release, were conducted under the voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) beginning in 1998. These include trials carried out under PR-71, PR-71X, PR-71X(2), PR-89, PR-89X, PR-89X(2), PR-112, PR-112X, PR-112X(2), PR-131, PR-131X, PR-131X(2), PR-141, PR-143 and PR-144.

### 6.1.2 Approvals by other Australian government agencies

136. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements also have to be met in relation to the release of GMOs, including requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standard Australia New Zealand (FSANZ).

137. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously approved the use of oil and linters from Roundup Ready® (A355), Bollgard II® (A436) and Roundup Ready Flex® cotton (A553) in food (ANZFA 2001b; ANZFA 2002b; FSANZ 2005). No additional approvals are required by FSANZ for the stacked GM cotton lines.

138. APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. Roundup Ready® Herbicide has been registered for use on Roundup Ready® cotton since 2000 and on Roundup Ready Flex® cotton since September, 2006.

139. The APVMA registered the use of the insecticidal proteins produced by the insecticidal genes (*cry1Ac* and *cry2Ab*) in GM Bollgard II® cotton as insecticidal products in New South Wales (NSW) and Queensland (QLD) south of latitude 22°S in 2003. Any plantings north of latitude 22°S would also require APVMA approval. The APVMA is currently assessing an application from Monsanto to vary the label for Bollgard II® to remove the condition for restriction on planting Bollgard II® north of latitude 22°S.

## 6.2 International approvals

140. All the GM cotton lines proposed for release in the current application have been approved for commercial release, human consumption and/or animal feed in a number of other countries. Some of these approvals are listed below.

### Bollgard II® cotton

- In the USA, the Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA-APHIS) approved the commercial release in 2002, and the US Food and Drug Administration (US FDA) approved use in human food and animal feed in 2002.

- The Japanese Ministries of Health, Labour and Welfare, and Agriculture, Forestry and Fisheries approved use in human food in 2002, and in animal feed in 2003.
- Health Canada's Office of Food Biotechnology and the Canadian Food Inspection Agency (CFIA) gave approval for use in human food and animal feed in 2003.
- In the Philippines, the Bureau of Plant Industries, Department of Agriculture gave approval for use in human food and animal feed in 2003.
- In India, the Genetic Engineering Approval Committee gave commercial approval in 2006.
- Bollgard II® is listed on the Community Register of GM Food and Feed in the European Union (from 2003) and is pending approval by the European Commission under the current regulatory system.

#### Roundup Ready® cotton

- In the USA, the USDA-APHIS approved the commercial release in 1995, and the US FDA approved use in human food and animal feed in 1995.
- The Japanese Ministries of Health, Labour and Welfare, and Agriculture, Forestry and Fisheries approved the commercial release and use in human food in 1997, and in animal feed in 1998.
- Health Canada's Office of Food Biotechnology and the CFIA approved use in human food in 1996, and in animal feed in 1997.
- The European Union has approved use in human food in 2002 under the Novel Food Regulation (EC 258/97) and is considering approval for use as animal feed by the European Commission under the current regulatory system.
- In Argentina, the Secretary of Agriculture, Livestock, Fisheries and Food (involving CONABIA, the National Commission for Agricultural Biotechnology and SENASA, the National Service of Food Safety and Foreign Markets Agency) approved the commercial release in 1999, and the use in human food and animal feed in 2000/2001.
- Commercial release was approved by South Africa's Executive Council for Genetically Modified Organisms in 2000.
- China's Minister for Agriculture gave approval for use in human food and animal feed in 2003.
- In the Philippines, the Bureau of Plant Industries, Department of Agriculture gave approval for use in human food and animal feed in 2003.

#### Roundup Ready Flex® cotton

- In the USA, the USDA-APHIS approved the commercial release in 2004, and the US FDA approved use in human food and animal feed in 2005.
- Health Canada's Office of Food Biotechnology and the CFIA gave approval for use in human food and animal feed in 2005.

#### Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton

- Both stacked varieties are approved for commercial release in the USA. Under the current US regulatory system a stacked GMO is automatically approved if it was produced by conventional crossing of two GMOs, containing unrelated traits that have previously been approved in the USA.

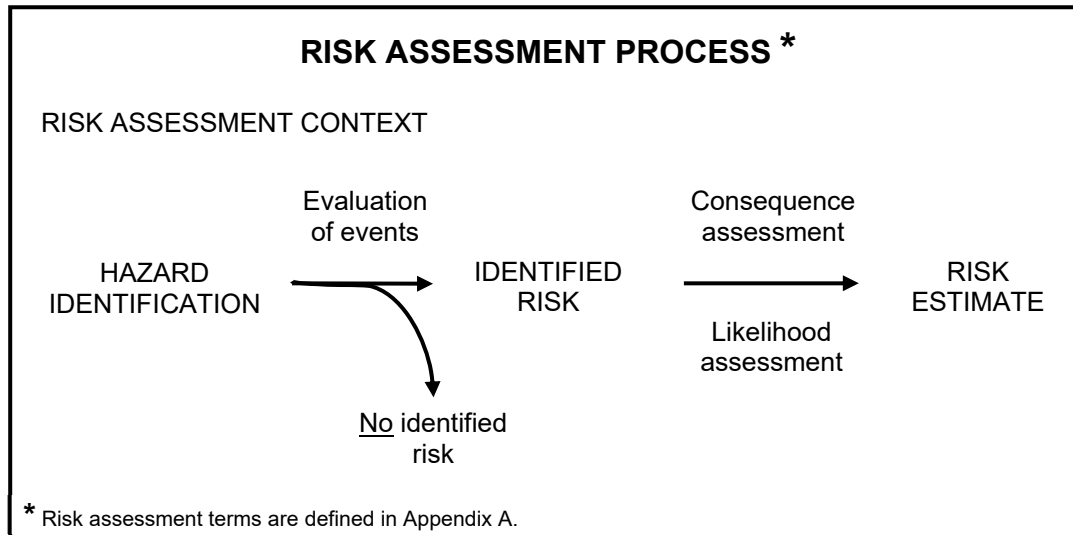
- The Japanese Ministry of Health, Labour and Welfare approved use in human food in 2003 for Roundup Ready<sup>®</sup>/Bollgard II and in 2005 for Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>.
- Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> is listed on the Community Register of GM Food and Feed in the European Union (from 2003) and is pending approval by the European Commission under the current regulatory system.
- In the Philippines, the Bureau of Plant Industries, Dept of Agriculture gave approval for the use of Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> in human food and animal feed in 2004.

## Chapter 2 Risk assessment

### Section 1 Introduction

141. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 2.1) considers risks from the proposed dealings with GMOs that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

**Figure 2.1 The risk assessment process.**



142. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during this release of the GMOs into the environment.

143. It is important to note that the word ‘hazard’ is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2005).

144. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMO and the receiving environment as a result of the proposed dealings.

145. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2005). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

146. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

## Section 2 Hazard characterisation

147. The list of events compiled during hazard identification are characterised to determine which events represent a risk to the health and safety of people or the environment posed by or as a result of gene technology.

148. A risk is identified only when there is some chance that harm will occur. Those events that do not lead to an adverse outcome or could not reasonably occur do not represent an identified risk and will not advance in the risk assessment process. Risks associated with the remaining events are assessed further to determine the seriousness of harm (consequence) and chance of harm (likelihood). The identified risks must be posed by or result from gene technology.

149. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2005). Harm is assessed in comparison to the parent organism and other GMOs previously approved for commercial release, in the context of the proposed dealing and the receiving environment. The risk assessment process focuses on measurable properties for determining harm.

150. The following factors are taken into account during the analysis of events that may give rise to harm:

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- comparisons with the non-GM parent(s)
- routes of exposure to the GMOs, the introduced gene(s) and the expressed product(s)
- potential effects of the introduced gene(s) and the expressed product(s) in the GMOs
- potential exposure to the introduced gene(s) and the expressed product(s) from other sources in the environment
- the presence of sexually compatible related species in the environment
- properties of the biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs
- the size, duration and regions of the release.

151. The GMOs proposed for release in the current application have previously been comprehensively assessed prior to the Regulator issuing a licence for their commercial release south of latitude 22°S under DIR 023/2002 (Roundup Ready<sup>®</sup> cotton), DIR 012/2002 (Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton) and DIR 059/2005 (Roundup Ready Flex<sup>®</sup> cotton and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton) in 2000, 2002 and 2006, respectively. Numerous limited and controlled releases of the same GMOs have also occurred in field trials approved under various licences (for details see Chapter 1, Section 6). There have been no reports of adverse effects on the health and safety of people or the environment resulting from any of these releases.

152. Events that are discussed in detail later in this Section are summarised below in Table 2.1. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. Thirty-five events were characterised, of which six are considered to lead to an identified risk that required further assessment.

153. The prevalence of the *nptII*, *aad* and *uidA* genes in the environment and the lack of evidence for toxicity or allergenicity of the NPTII and the GUS proteins were discussed in Chapter 1, Sections 4.2.3 and 4.2.4, respectively. In addition, the potential effects of the *nptII*, *aad* and *uidA* genes and their products were considered in detail in previous DIR applications including some for commercial releases of cotton (such as DIR 12/2002, DIR 022/2002, DIR 023/2002, and DIR 059/2005) and canola (DIR 021/2002). RARMPs for those DIR applications are available from the OGTR or from the website <<http://www.ogtr.gov.au>>. No risks have been identified from the expression of NPTII or GUS in GM cotton. Therefore, the potential effects of the *nptII*, *aad* and *uidA* genes will not be further assessed for this application.



**Table 2.1 Summary of events that may give rise to adverse outcomes**

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
<b>SECTION 2.1 Production of a substance toxic to people</b>	1. Ingestion of GM plant materials and food products containing the proteins encoded by the introduced genes.	Toxicity for people	No	No risk of toxicity for people from the proteins expressed by the introduced genes was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impacts have been reported. Thus, no risk for these events is expected as a result of growing the GM cotton lines north of latitude 22°S.  Refer also to Section 2.1, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.
	2. Contact with, or inhalation of, GM plant materials containing the proteins encoded by the introduced genes.	Toxicity for people	No	
	3. Consumption of honey produced by bees that pollinated GM plants.	Toxicity for people	No	
	4. Consumption of fungi cultivated on cotton trash/compost.	Toxicity for people	No	
<b>SECTION 2.2 Production of a substance allergenic to people</b>	5. Use of GM plant materials in food.	Allergic reactions in people	No	No risk of allergic reaction for people from the proteins expressed by the introduced genes was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impacts have been reported. Thus, no risk for these events is expected as a result of growing the GM cotton lines north of latitude 22°S.  Refer also to Section 2.2, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.
	6. Contact with items containing GM cotton fibre.	Allergic reactions in people	No	
	7. Contact with GM plant materials containing the introduced proteins.	Allergic reactions in people	No	
<b>SECTION 2.3 Production of a substance toxic to organisms other than people</b>	8. Ingestion of GM plant materials by vertebrates, including stock.	Toxicity for vertebrates	No	Following the commercial release of the GM cotton lines for areas south of latitude 22°S, GM cotton seed has been used as stockfeed in both southern and northern Australia since 2000 and 2002 for Roundup Ready® and Bollgard II®, respectively, with no report of adverse effects.  No risk of toxicity for vertebrates from the proteins expressed by the introduced genes was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impacts have been reported. Thus, no risks for these events are expected as a result of growing the GM cotton lines north of latitude 22°S.  Refer also to Section 2.3, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.
	9. Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins encoded by the introduced insect resistance genes by non-target invertebrates.	Toxicity for non-target invertebrates	Yes	

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	10. Direct or indirect ingestion of the CP4 EPSPS protein encoded by the herbicide tolerance gene by invertebrates.	Toxicity for invertebrates	No	No risk of toxicity from the proteins expressed by the introduced genes was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no adverse impacts have been reported (see also Section 2.3, Chapter 2 of the RARMP prepared for DIR 059/2005). Thus, no risk for these events is expected as a result of growing the GM cotton lines north of latitude 22°S. Invertebrates and microorganisms are also exposed to the proteins encoded by the introduced genes via the widespread occurrence of the bacteria from which they are derived and several large scale field trials in northern Australia since 1998. There have been no reports of adverse impacts on non-target invertebrates from these trials.
	11. Contact with the proteins encoded by the introduced genes by microorganisms.	Toxicity for microorganisms	No	
<b>SECTION 2.4 Spread and persistence of the GM cotton in the environment</b>	12. Tolerance to glyphosate due to expression of the <i>cp4 epsps</i> gene in the GM cotton plants.	Weediness	Yes	<b>See Chapter 4, Identified Risk 2.</b>
	13. Reduced lepidopteran herbivory due to expression of the <i>cry1Ac</i> and <i>cry2Ab</i> genes in the GM cotton plants.	Weediness	Yes	<b>See Chapter 4, Identified Risk 3.</b>
	14. Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the <i>cp4 epsps</i> , <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination in the GM cotton plants.	Weediness	Yes	<b>See Chapter 4, Identified Risk 4.</b>
	15. Presence of the regulatory sequences in the GM cotton plants.	Weediness	No	The presence of the regulatory sequences is not expected to have any influence on the spread and persistence of the GM cotton plants.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	16. Exposure of people and other organisms to the proteins encoded by the introduced genes as a result of spread and persistence of the GM cotton plants in the environment.	Toxicity/allergic reactions in people and other organisms	No	<p>The amount of exposure expected as a result of spread and persistence of the GM cotton lines would be small in comparison to the exposure from cultivation of the GM cotton lines in northern and southern Australia.</p> <p>People and other organisms (including non-target invertebrates) are already exposed to the proteins encoded by the introduced genes via the widespread occurrence of the bacteria from which the introduced genes are derived, and through several large scale field trials in northern Australia since 1998 and commercial release in southern Australia.</p> <p>There have been no reports of adverse impacts on people and other organisms (including non-target invertebrates) from the cultivation of the GM cotton lines. The same or similar proteins are already present in the environment.</p> <p>For more information on the consideration of toxicity or allergic reactions in people or toxicity to other organisms, refer to Sections 2.1, 2.2, and 2.3, Chapter 2; and Section 4.1, Chapter 1.</p> <p>For more information on the consideration of toxicity to non-target invertebrates, refer to Chapter 3, Identified Risk 1.</p>
<b>SECTION 2.5 Gene flow by vertical gene transfer</b>	17. Gene transfer between the GM cotton lines proposed for release.	Weediness	No	No new combinations of different genes will result. If gene flow between Roundup Ready® and Roundup Ready Flex® plants did occur, the progeny would contain three copies of <i>cp4 epsps</i> . However, no enhanced competitive ability is expected (see also Chapter 4, event 7 of RARMP for DIR 059/2005).
	18. Expression of <i>cp4 epsps</i> and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in naturalised <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants providing tolerance to glyphosate and/or reduced lepidopteran herbivory.	Weediness	Yes	<b>See Chapter 4, Identified Risk 5.</b>

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	19. Expression of <i>cp4 epsps</i> and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination with the <i>bar</i> gene (from Liberty Link® cotton) providing dual herbicide tolerance and reduced lepidopteran herbivory.	Weediness	Yes	<b>See Chapter 4, Identified Risk 6.</b>
	20. Gene transfer to native <i>Gossypium</i> species.	Weediness	No	Well established genetic incompatibility prevents vertical gene transfer to native <i>Gossypium</i> species.
	21. Exposure of organisms (including people) to the EP4 EPSPS, Cry1Ac and Cry2Ab proteins encoded by the introduced genes as a result of gene transfer to <i>G. hirsutum</i> or <i>G. barbadense</i> plants.	Toxicity for organisms, allergic reactions in people	No	<p>The amount of exposure expected as a result of vertical gene transfer would be small in comparison to the exposure from cultivation of the GM cotton lines in northern and southern Australia.</p> <p>People and other organisms are already exposed to the proteins encoded by the introduced genes via the widespread occurrence of the bacteria from which the introduced genes are derived and through several large scale field trials in northern Australia since 1998 and commercial release in southern Australia.</p> <p>There have been no reports of adverse impacts on people and other organisms from cultivation of the GM cotton lines.</p> <p>For more information on toxicity for organisms and allergic reactions in people refer to Sections 2.1, 2.2 and 2.3, Chapter 2.</p>
	22. Presence of the introduced regulatory sequences in <i>G. hirsutum</i> or <i>G. barbadense</i> plants as a result of gene transfer.	Unpredictable effects	No	The introduced regulatory sequences do not behave any differently than endogenous regulatory sequences in plants.
	23. Reduced choice of methods for controlling cotton volunteers due to tolerance to both glyphosate and glufosinate ammonium as a result of gene flow between the GM cotton lines and Liberty Link® cotton.	Weediness	No	Complete control of cotton volunteers using either glyphosate or glufosinate ammonium is limited to early growth stages. Other herbicides and/or mechanical cultivation are used to control volunteer cotton from seedling to mature stage.
	24. Tolerance to other herbicides as a result of gene flow between the GM cotton lines and Liberty Link® cotton.	Weediness	No	Unlikely that resistance to a new herbicide will develop. This hazard would be assessed by the APVMA.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
<b>SECTION 2.6 Gene flow by horizontal gene transfer</b>	25. Presence of the <i>cp4 epsps</i> , <i>cry1Ac</i> or <i>cry2Ab</i> genes, or the introduced regulatory sequences, in other organisms.	Toxicity, weediness, increased pathogenicity	No	<p>The introduced genes and regulatory sequences are already present in the environment due to the widespread occurrence of the bacteria from which the introduced genes are derived. Hence they are readily available for transfer via demonstrated natural mechanisms.</p> <p>Gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other natural sources of these genes.</p> <p>GM cotton containing the same introduced genes as the GM cotton lines proposed for release have been assessed previously in Section 2.6, Chapter 2 of the RARMP prepared for DIR 059/2005, and no risk resulting from horizontal gene flow was identified.</p>
<b>SECTION 2.7 Unintended changes in toxicity</b>	26. Altered levels of innate toxic or anti-nutritional compounds as a result of random insertion of the gene constructs into the cotton genome during development of the GM cotton lines.	Toxicity for people and/or other organisms	No	<p>Compositional analysis indicates that there are no significant changes in any of the toxic or anti-nutritional compounds that occur naturally in cotton seed or meal from Bollgard II<sup>®</sup> cotton, Roundup Ready<sup>®</sup> cotton or Roundup Ready Flex<sup>®</sup> cotton compared to non-GM cotton.</p> <p>For more details refer to RARMPs prepared for DIRs 012/2002, 023/2003 and 059/2005.</p>
	27. Altered metabolism of glyphosate in the GM plants expressing the CP4 EPSPS protein resulting in the production of toxic compounds.	Toxicity for people and/or other organisms	No	<p>The CP4 EPSPS protein functions in the same way (in the biosynthesis of aromatic amino acids) as the native enzyme in plants and is not involved in glyphosate metabolism.</p>
	28. Synergistic effects of the introduced proteins when ingested in combination resulting in altered toxicity.	Toxicity for people and/or other organisms	No	<p>No risk of toxicity for people and other organisms as a result of synergistic effects was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impact has been reported. Thus, no risks for this event are expected as a result of growing the GM cotton lines north of latitude 22°S.</p> <p>Refer also to Section 2.7, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.</p>

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
<b>SECTION 2.8 Unintended changes in biochemistry or physiology</b>	29. Altered biochemistry or physiology of the GM cotton plants resulting from insertion or expression of the introduced genes.	Toxicity for people and/or other organisms or weediness	No	<p>No adverse unintended secondary effects have been reported from numerous field trials and commercial releases, both in Australia and overseas, of all five GM cotton lines proposed for release.</p> <p>No risks of toxicity for people and other organisms as a result of altered biochemistry or physiology were identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impact has been reported. Thus, no risks for this event are expected as a result of growing the GM cotton lines north of latitude 22°S.</p> <p>Refer also to Section 2.8, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.</p>
<b>SECTION 2.9 Unintended effects on existing pests or weeds</b>	30. Increased prevalence of other insects due to decreased use of insecticide sprays on the GM Bollgard II® cotton varieties.	Increased non-lepidopteran insect herbivory	No	<p>Application of integrated pest management strategies developed by the cotton industry would be able to control any non-lepidopteran insect pests. These pests would be killed incidentally by broad-spectrum insecticides commonly used on non-GM cotton and GM cotton lines without an insect resistance trait.</p> <p>Experience from growing these GM cotton lines commercially south of latitude 22°S indicates that any increased herbivory resulting from increased prevalence of other insects due to decreased use of insecticide sprays can be successfully managed by the implementation of integrated pest management strategies.</p> <p>Similarly, no risks of increased disease burden due to expression of the introduced genes were identified and previous releases of the GM cotton lines proposed for release have not shown increased disease burden.</p> <p>Thus, no risks for these two events are expected as a result of growing the GM cotton lines north of latitude 22°S.</p> <p>Refer also to Section 2.9, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail.</p>
	31. Increased disease burden due to expression of the introduced genes.	Increased disease burden	No	

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
<b>SECTION 2.10 Secondary impacts</b>	32. Development of insects resistant to Cry1Ac and Cry2Ab proteins.	Loss of insecticidal efficacy	No	This issue is being considered by the APVMA. The applicant, in consultation with the Technical Group for Northern Australia Resistance Management (TGNARM) committee, developed a resistance management plan for cultivation of Bollgard II® cotton lines north of latitude 22°S, comparable to that for southern Australia. Cultivation of these varieties may require the implementation of this resistance management plan and all other relevant conditions that may be imposed by the APVMA.
	33. Secondary effects on populations of organisms that interact with lepidopteran insects.	Loss of food for other organisms	No	In non-GM cotton and GM cotton lines without an insect resistance trait, organisms that depend on lepidopteran insects would be killed by the broad-spectrum insecticides used to control lepidopteran insect larvae.  This event also relates to toxicity to non-target invertebrates and is addressed in Chapter 3, Identified Risk 1.
	34. Development of herbicide resistant weeds (in the agricultural environment) due to use of glyphosate on the GM cotton.	Emergence of weeds that are more difficult to control / development of herbicide resistant weeds	No	Development of herbicide resistant weeds is considered by the APVMA in assessing applications to register herbicides.  The Roundup Ready® and Roundup Ready Flex® cotton crop management plans specify integrated weed management strategies.  In addition, integrated weed management guidelines have been adopted by the Australian cotton industry.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	35. Consumption of animals that were fed GM plant material.	Toxicity for people	No	<p>As discussed in event 8, cotton seed from the GM cotton lines proposed for release has been permitted to be used as stockfeed particularly in areas south of latitude 22°S since 2000.</p> <p>Protein and DNA are rapidly broken down into smaller components in the digestive tract of animals that are fed cotton seed irrespective of whether it is GM or not. As a result, products from these animals would be no different to those from animals that were fed seed from non-GM cotton.</p> <p>There have been no reported adverse effects on people or animals resulting from the use of the GM cotton lines as stockfeed.</p>



## 2.1 Production of a substance toxic to people (Events 1 – 4)

154. Toxicity is the cascade of reactions resulting from exposure to a dose of a substance or chemical that is sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992). Toxicity may occur through ingestion, skin contact or inhalation. The level of acute toxicity is often expressed as the LD<sub>50</sub>. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

155. Toxicity assays generally use the purified toxin of interest rather than the product that contains the protein (eg GM plant material). This is necessary because the aim of an assay is to determine the concentration of toxin at which an adverse effect occurs. The level of protein/toxin in the product indicates the level of exposure to the toxin, and comparison to the results of the toxicity assay indicate whether or not this is a safe level of exposure (Konig et al. 2004; OECD 1998). The use of purified toxin also increases the reproducibility of the assays.

156. All five GM cotton lines proposed for release north of latitude 22°S have previously been approved for commercial release south of latitude 22°S (under DIRs 012/2002, 023/2002 and 059/2005). Products derived from the GM cotton plants have also been approved for use in food by FSANZ (see Section 6, Chapter 1). As part of these approval processes, the toxicity of the GM cotton lines has previously been evaluated.

157. The RARMP prepared recently for the commercial release of Roundup Ready Flex<sup>®</sup> cotton and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton, south of latitude 22°S, (DIR 059/2005) included the characterisation of a number of events that may lead to toxicity for people. In addition to these two GM cotton lines, the current application (DIR 066/2006) contains Roundup Ready<sup>®</sup> cotton, Bollgard II<sup>®</sup> cotton and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton. These three GM cotton lines contain the same introduced proteins which are expressed at the same or lower levels to those present in Roundup Ready Flex<sup>®</sup> or Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (see Table 1.1 and Section 5.5, Chapter 1). No toxicity risks were identified for DIR 059/2005 (see Section 2.1, Chapter 2 of RARMP for DIR 059/2005, which can be found at [http://www.ogtr.gov.au/ir/DIR\\_059.htm](http://www.ogtr.gov.au/ir/DIR_059.htm)) and no increased risks relating to toxicity for people are expected as a result of growing of the GM cotton plants north of latitude 22°S.

158. Events 1 through 4 relating to production of a substance toxic for people are identical to those characterised in the RARMP prepared for DIR 059/2005. On this basis, **no risk is identified** and the potential for toxicity for people, resulting from the expression of the introduced genes in the GM cotton lines will not be assessed further.

## 2.2 Production of a substance allergenic to people (Events 5 – 7)

159. Consideration has been given to the possibility that exposure of people to proteins expressed by the introduced genes, or their enzymatic products, in the GM cotton plants may result in an allergic reaction. Exposure to the CP4 EPSPS, Cry1Ac, or Cry2Ab proteins could occur via the oral, dermal or inhalation routes due to the consumption of food containing cotton products, accidental ingestion of material from the cotton plants, contact with clothing or household items containing cotton, or contact with material from cotton plants. Such exposures could occur to the general public or to workers handling GM cotton in an occupational setting.

160. As mentioned above, all five GM cotton lines proposed for release north of latitude 22°S have previously been approved for commercial release south of latitude 22°S, and

products derived from the GM cotton plants have been approved for use in food by FSANZ (see Section 6, Chapter 1). As part of the approval process, the allergenicity of the GM cotton lines has been evaluated.

161. Similarly, the RARMP prepared recently for the commercial release of Roundup Ready Flex<sup>®</sup> cotton and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton, south of latitude 22°S, (DIR 059/2005) included the characterisation of a number of events that may lead to allergic reactions in people. As noted above, these two GM cotton lines contain the same introduced proteins, which are expressed at the same or lower levels in the other three GM cotton lines proposed for release (see Table 1.1, Chapter 1, this RARMP). No risks related to allergenicity were identified for DIR 059/2005 and no increased risks relating to production of a substance allergenic to people are expected as a result of growing of the GM cotton plants north of latitude 22°S (refer to Section 2.1, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail).

162. Events 5 through 7 relating to production of a substance allergenic to people are identical to those characterised in the RARMP prepared for DIR 059/2005. On this basis, **no risk is identified** and the potential for allergenicity for people, resulting from the expression of the introduced genes in the GM cotton lines will not be assessed further.

### **2.3 Production of a substance toxic to organisms other than people**

163. A range of organisms (vertebrates, invertebrates and microorganisms) may be exposed directly to a toxic substance through feeding on the GM cotton plants, or indirectly through eating organisms that feed on GM cotton plants.

164. Tissue from both GM and non-GM cotton plants, particularly the seeds, can be toxic to mammals if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropanoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids).

165. The presence of gossypol and cyclopropanoid fatty acids in cotton seed limits the use of whole cotton seed as a protein supplement in animal feed, except for cattle which are less affected by these components. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine. The meal and hulls of cotton seed can also be used for cattle feed. The use of cotton seed as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

166. Neither cotton trash nor stubble is used as animal feed, due to the possible presence of pesticide residues.

#### **Event 8 Ingestion of GM plant material by vertebrates, including stock**

167. Mammals generally avoid feeding on cotton plants. In the field, seed cotton is present as large lint-covered bolls that are unattractive to avian species (OGTR 2002), so birds are not likely to be exposed to the proteins encoded by the introduced genes in the seeds of the GM cotton lines.

168. Cotton seed and pollen from the release are not expected to enter aquatic habitats in any significant quantities (OGTR 2002); therefore the level of exposure of aquatic vertebrates to the GM cotton lines will be low. Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice may be implemented at some sites in northern Australia if GM cotton is commercially grown. At some potential cotton growing sites in northern

Australia where tail water is not collected, experiments with growing Bollgard II<sup>®</sup> cotton have resulted in the development of efficient irrigation practices resulting in minimal run-off into river systems (Moulden et al. 2006).

169. The Cry1Ac and Cry2Ab proteins are known to bind to specific receptors located in the gut of the larvae of some insects. Current evidence indicates that only lepidopteran insects are affected (see Chapter 3), although it is possible that insect species from some other invertebrate orders may be sensitive. Due to this high degree of specificity, these proteins will not interact with receptors in vertebrates.

170. As discussed in Chapter 1, a number of studies have been performed to investigate the toxicity of the GM cotton lines on vertebrates, including: analysis of acute oral toxicity in mice of purified forms of the proteins encoded by the introduced genes (CP4 EPSPS, Cry1Ac, and Cry2Ab); feeding studies with plant material from Bollgard II<sup>®</sup> or Roundup Ready<sup>®</sup> cotton lines on rats, quail, catfish, chickens and dairy cows; and compositional analysis on the nutritional and anti-nutritional components of seed from Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines. These studies did not find any evidence of increased toxicity for the analysed GM cotton lines, compared to non-GM cotton.

171. The Roundup Ready<sup>®</sup>, Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines have been approved for use in stockfeed since 2000, 2002 and 2006, respectively. The use of cotton seed products derived from these GM cotton lines (particularly Roundup Ready<sup>®</sup> and Bollgard II<sup>®</sup>) has not shown any increased toxicity for livestock compared to non-GM cotton.

172. The proteins encoded by the introduced genes are widespread in the environment (see Chapter 1), and it is therefore likely that many vertebrates have been exposed to these or similar proteins through natural sources. Vertebrates have also been exposed to these proteins through their expression in the commercially released Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup> cotton lines (approved for commercial release south of latitude 22°S under DIR 012/2002 and DIR 023/2002, respectively) and via several field trials in northern Australia since 1998. No adverse effects have been reported from these releases.

173. The GM cotton lines proposed for release contain the same proteins as GM cotton lines approved for commercial release south of latitude 22°S (DIR 059/2005). No risks of toxicity for vertebrates were identified for DIR 059/2005 and thus, no risks for this event are expected as a result of growing of the GM cotton plants north of latitude 22°S (refer to Section 2.3, Chapter 2 of the RARMP prepared for DIR 059/2005 for further detail).

174. The proteins encoded by the introduced genes are not expected to be a novel source of harm for vertebrates. Therefore, **no risk is identified** and the potential for toxicity for vertebrates, including stock, resulting from the expression of the introduced genes in the GM cotton lines will not be assessed further.

**Event 9      *Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins encoded by the introduced insect resistance genes in combination by non-target invertebrates***

175. Non-target invertebrates in cotton growing regions south of latitude 22°S are already widely exposed to the Cry1Ac and Cry2Ab proteins through the commercially released Bollgard II<sup>®</sup> GM cotton. In the 2005–06 season, 90% of all commercial cotton was GM cotton, of which 81% was Bollgard II<sup>®</sup> cotton (B. Patterson, 2006, Monsanto Australia Limited, pers. comm.). The applicant proposes to conduct plant breeding, agronomic trials and seed production, and potentially commercial scale plantings of the GM cotton lines. Accordingly, invertebrates in the north of Australia would be exposed to the Cry proteins.

176. Although the primary targets of the Cry1Ac and Cry2Ab proteins are the two major pests of cultivated cotton plants, *H. armigera* and *H. punctigera*, some other lepidopteran species are also sensitive to these toxins including *Spodoptera litura* and *Pectinophora gossypiella*, also pests of cultivated cotton in northern Australia. Different invertebrate species or different numbers of invertebrates, from those that occur in the south, may be exposed to the Cry proteins if the GM cotton lines are grown in the north. Evidence also suggests that expression of different Cry proteins in combination can have synergistic or antagonistic effects on the toxicity for invertebrates (del Rincon-Castro et al. 1999; Schnepf et al. 1998). Therefore, **a risk is identified** for toxicity to non-target invertebrates resulting from the direct or indirect ingestion of the GM cotton. The level of risk of toxicity to non-target invertebrates from this event is estimated in **Chapter 3** as **Identified Risk 1**.

**Event 10      Direct or indirect ingestion of the CP4 EPSPS protein encoded by the introduced herbicide tolerance gene by invertebrates**

177. There is no evidence to suggest that the CP4 EPSPS protein or other similar proteins are toxic to invertebrates. The gene that encodes this protein is derived from common soil or gut bacteria (for details see Chapter 1). In addition, similar EPSPS enzymes are naturally present in all plants, bacteria, algae and fungi. It is therefore expected that invertebrates in northern Australia are already exposed to these proteins within the environment or via several field trials of GM cotton since 1998 without evidence of toxic effects.

178. Roundup Ready® and Roundup Ready Flex® cotton lines contain one and two copies of the *cp4 epsps* gene, respectively. No risks of toxicity to invertebrates through direct or indirect ingestion of the CP4 EPSPS protein were identified in the RARMP prepared for the commercial release of Roundup Ready Flex® cotton south of latitude 22°S (DIR 059/2005, Section 2.3, Chapter 2). Thus, no risks for this event are expected as a result of growing of the GM cotton plants north of latitude 22°S.

179. Therefore, **no risk is identified** and the potential of toxicity for invertebrates as a result of the presence of the CP4 EPSPS protein in the GM cotton plants will not be assessed further.

**Event 11      Contact with the proteins encoded by the introduced genes by microorganisms**

180. Soil microorganisms may be exposed to the proteins encoded by the introduced genes through cotton plant material tilled into the soil after harvest or as a result of root exudation. Research investigating the effect of these proteins on microorganisms has been discussed in significant detail in previous RARMPs prepared for the commercial release of the GM cotton lines south of latitude 22°S (DIRs 012/2002, 023/2002 and 059/2005). The genes that encode these proteins are derived from common soil or gut bacteria and similar EPSPS enzymes are naturally present in all plants, bacteria, algae and fungi (for details see Chapter 1). It is therefore expected that microorganisms in northern Australia are already exposed to these proteins within the environment and via several large scale field trials of GM cotton since 1998 without evidence of toxic effects. Nor have there been reports of adverse effects on microorganisms due to the commercial cultivation of the same GM cotton lines south of latitude 22°S.

181. In summary, no evidence has been found to suggest that the CP4 EPSPS, Cry1Ac, or Cry2Ab proteins or similar proteins are toxic to microorganisms including various species of protozoa, bacteria, fungi, algae and diatoms. An extensive search of the literature did not identify any reports of adverse effects on microorganisms despite the widespread, natural occurrence of these or similar proteins within the environment (Section 2.3, Chapter 2 of the

RARMP prepared for DIR 059/2005). Thus, no risks for this event are expected as a result of growing of the GM cotton plants north of latitude 22°S.

182. Therefore, **no risk is identified** and the potential for toxicity for microorganisms as a result of the expression of the introduced genes will not be assessed further.

## 2.4 Spread and persistence of the GM cotton in the environment

### **Event 12 Tolerance to glyphosate due to expression of the cp4 epsps gene in the GM cotton plants**

183. Roundup Ready® cotton plants produce sufficient CP4 EPSPS protein to provide tolerance to glyphosate up to the 4-leaf stage (ie prior to flower formation, approximately 3 to 5 weeks after planting). The Roundup Ready Flex® cotton plants produce higher amounts of CP4 EPSPS protein over a longer period and are tolerant to glyphosate throughout the growing season (approximately 24 to 28 weeks).

184. In environments where glyphosate is used to control weeds, the GM cotton plants would have some selective advantage that could lead to spread and persistence of the GM cotton lines. Therefore, **a risk is identified** for weediness of the GM cotton plants as a result of tolerance to glyphosate due to expression of the *cp4 epsps* gene. The level of risk of weediness from this event is estimated in **Chapter 4** as **Identified Risk 2**.

### **Event 13 Reduced lepidopteran herbivory due to expression of the cry1Ac and cry2Ab genes in the GM cotton plants**

185. Bollgard II®, Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton produce the Cry1Ac and Cry2Ab insecticidal proteins. These Cry proteins are toxic to lepidopteran insects, including major lepidopteran pests of cotton crops. In environments where lepidopteran herbivory is a significant limitation on the spread and persistence of cotton plants, the GM cotton lines could survive and may become persistent in the environment. Therefore, **a risk is identified** for weediness of the GM cotton lines as a result of reduced lepidopteran herbivory due to expression of the *cry1Ac* and *cry2Ab* genes. The level of risk of weediness from this event is estimated in **Chapter 4** as **Identified Risk 3**.

### **Event 14 Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the cp4 epsps, cry1Ac and cry2Ab genes in combination in the GM cotton plants**

186. Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton produce the Cry1Ac and Cry2Ab insecticidal proteins and the CP4 EPSPS protein which provides tolerance to glyphosate. These Cry proteins are toxic to lepidopteran insects, including major lepidopteran pests of cultivated cotton crops. In environments where glyphosate is used to control weeds and lepidopteran herbivory is a significant limitation to the spread and persistence of cotton plants, the GM cotton lines could survive and may become persistent in the environment. Therefore, **a risk is identified** for weediness of the GM cotton lines as a result of tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination. The level of risk of weediness from this event is estimated in **Chapter 4** as **Identified Risk 4**.

### **Event 15 Presence of the regulatory sequences in the GM cotton plants**

187. The presence of the introduced regulatory sequences in the GM cotton lines are not expected to have any impact on the spread or persistence of the GM cotton plants. The introduced regulatory sequences behave no differently to endogenous regulatory sequences in cotton. Therefore, **no risk is identified** and the potential for weediness of the GM cotton lines as a result of the presence of the introduced regulatory sequences will not be assessed further.

**Event 16 Exposure of people and other organisms to the proteins encoded by the introduced genes as a result of spread and persistence of the GM cotton plants in the environment**

188. The spread and persistence of the GM cotton plants in the environment could lead to increased exposure of people and other organisms (such as vertebrates, non-target invertebrates and microorganisms) to the proteins encoded by the introduced genes, which could result in toxicity or allergic reactions.

189. However, all of these proteins are of very low oral toxicity and the GM cotton lines are approved for use in food (see Section 4.2 of Chapter 1). Evidence also indicates that none of these proteins are allergenic. The same or similar proteins are widespread in the environment (see Section 5.5 of Chapter 1) suggesting that people and other organisms are already exposed to these proteins.

190. People and other organisms are also widely exposed to these proteins south of latitude 22°S through the commercial release of all five GM cotton lines and in northern Australia via field trials of these GM cotton lines since 1998. In the 2005–06 season, 90% of all commercial cotton grown in Australia was GM cotton. Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup> cotton varieties comprised 81% and 74% of the GM cotton, respectively (with stacked trait varieties contributing to these percentages). There have been no reports of adverse impacts from these commercial releases or field trials as a result of exposure to people or other organisms. The applicant proposes to conduct plant breeding, agronomic trials and seed production, and potentially commercial scale plantings of the GM cotton lines. Subsequently, people and other organisms in northern Australia would have further exposure to these proteins.

191. No risks of toxicity or allergic reactions to people or toxicity to other organisms (excluding non-target invertebrates) due to exposure to the GM cotton lines were identified in Sections 2.1, 2.2 and 2.3 of this Chapter. Toxicity to non-target invertebrates as a result of direct or indirect exposure to the GM cotton lines expressing the Cry proteins was assessed as an identified risk in Section 2.3 of this Chapter. This event was assessed further in Chapter 3 and estimated to be negligible and no risk treatment measures were proposed. However, the amount of exposure expected as a result of spread and persistence of the GM cotton lines would be small in comparison to the exposure from the proposed commercial release of the GM cotton plants and from the same GM cotton lines that are already commercially released south of latitude 22°S.

192. Therefore, **no risk is identified** and the potential for toxicity or allergic reactions in people or toxicity to other organisms (such as vertebrates, microorganisms and non-target invertebrates) as a result of spread and persistence of the GM cotton plants in the environment will not be assessed further.

**2.5 Gene flow by vertical gene transfer**

193. Transfer of genetic material to offspring by reproduction (vertical gene transfer) could result in the transfer of the introduced genes or their associated regulatory elements to other plants. The only sexually compatible species present in Australia that could receive genes from the GM cotton lines are *G. hirsutum* (including both cultivated (GM and non-GM) and naturalised cotton populations) and *G. barbadense*.

194. There is no cotton grown commercially in areas north of latitude 22°S, as of October 2006. However, the Regulator recently approved (August, 2006) an application for the commercial release of the herbicide tolerant Liberty Link<sup>®</sup> GM cotton into current cotton growing areas as well as potential future areas, including northern Australia (DIR 062/2005).

Liberty Link<sup>®</sup> Cotton contains the *bar* gene which provides tolerance to glufosinate ammonium (the active constituent in herbicides such as Basta<sup>®</sup> and Liberty<sup>®</sup>). Field trials with Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> (DIR 012/2002), and Roundup Ready Flex<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (DIR 055/2004) are being conducted under limited and controlled conditions in the Wyndham-East Kimberley and Broome regions of WA and in the shire of Burdekin in northern QLD.

**Event 17 Gene transfer between the GM cotton lines proposed for release**

195. Gene flow between the GM cotton lines proposed for release would not result in new combinations of introduced genes.

196. However, unintentional gene flow between Roundup Ready<sup>®</sup> or Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> (containing one copy of the *cp4 epsps* gene) plants and Roundup Ready Flex<sup>®</sup> or Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> (containing two copies of the *cp4 epsps* gene) could result in plants containing three copies of the *cp4 epsps* gene. This may result either in increased or decreased (due to gene silencing) expression of the CP4 EPSPS protein. Decreased expression would reduce the risk of weediness as compared to the GM cotton lines proposed for release. Plants with increased expression may be tolerant to higher concentrations of glyphosate. The limited effectiveness of glyphosate in controlling cotton beyond the seedling stage is discussed in Identified Risk 2 (Chapter 4) and would be no different for plants with tolerance to increased herbicide levels.

197. Monsanto has stated that it has no intention to cross GM lines containing the Roundup Ready<sup>®</sup> trait with lines containing the Roundup Ready Flex<sup>®</sup> trait. Roundup Ready Flex<sup>®</sup> varieties are expected to replace Roundup Ready<sup>®</sup> cotton varieties for commercial production in the near future.

198. Therefore, **no risk is identified** and the potential for weediness as a result of gene transfer between the GM cotton lines proposed for release will not be assessed further.

**Event 18 Tolerance to glyphosate and/or reduced lepidopteran herbivory in naturalised *G. hirsutum* or *G. barbadense* cotton plants due to expression of the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes**

199. Sexually compatible naturalised *G. hirsutum* or *G. barbadense* cotton plants expressing the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes as a result of vertical gene transfer could become tolerant to glyphosate and/or resistant to lepidopteran insects. This could confer a fitness advantage on the plants in environments where glyphosate is used to control weeds and/or cotton plants are limited by lepidopteran insects. Therefore, **a risk is identified** for weediness as a result of vertical gene transfer of the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes into naturalised *G. hirsutum* or *G. barbadense* plants. The level of risk of weediness from this event is estimated in Chapter 4 as **Identified Risk 5**.

**Event 19 Tolerance to glyphosate and/or reduced lepidopteran herbivory in combination with glufosinate ammonium due to expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene**

200. GM cotton plants expressing the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene present in Liberty Link<sup>®</sup> GM cotton, could become tolerant to glyphosate and/or resistant to lepidopteran insects as well as tolerant to glufosinate ammonium, as a result of vertical gene transfer. This could confer a fitness advantage on the plants in environments where cotton plants are limited by lepidopteran insects and where glyphosate and/or glufosinate ammonium is used to control weeds. Therefore, **a risk is identified** for weediness as a result of vertical gene transfer of the *cp4 epsps* and/or *cry1Ac*

and *cry2Ab* genes into Liberty Link<sup>®</sup> GM cotton. The level of risk of weediness from this event is estimated in **Chapter 4** as **Identified Risk 6**.

#### **Event 20 Gene transfer to native *Gossypium* species**

201. As discussed in the *Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002), Australian flora contains 17 native *Gossypium* species. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory.

202. Most Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from agricultural areas. However, some native *Gossypium* populations occur near roads where GM cotton seed may be transported and GM cotton volunteers may establish.

203. There is well established genetic incompatibility between native *Gossypium* species and the cultivated cotton (OGTR 2002). All native *Gossypium* species are diploid (C, G or K genomes), while the cultivated cotton species are tetraploid (AD genomes). The GM cotton lines proposed for release do not have increased ability to cross with native cotton species (compared to non-GM cotton).

204. The native cotton species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids between these two species have been produced under field conditions, without application of plant hormones, when plants were grown in close proximity to each other. However, these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations under natural conditions (OGTR 2002). There are no reports of hybrids between *G. hirsutum* and any other native *Gossypium* species occurring under natural conditions.

205. Hybrids between *G. hirsutum* and native *Gossypium* species have been produced under artificial conditions in the glasshouse (ie emasculation, hand-pollination and application of plant hormones) but the resulting hybrids were sterile, with the exception of six K-genome species which had some level of female fertility (Brubaker et al. 1999; Brubaker & Brown 2001). Backcrosses between the *G. hirsutum* x K-genome species (ADK) hybrids and *G. hirsutum* (AD) resulted in the production of pentaploid progeny (AADDK). These successful backcrosses were possible due to the production of unreduced gametes in the hybrid (Brubaker & Brown 2001). The pollen from these pentaploid plants was functionally sterile which would limit the possibility of introgression of genes into the native K-genome species.

206. The introgression of the introduced genes is further limited because the pentaploid hybrids would contain a single set of K-genome chromosomes, which cannot pair up during meiosis. Thus, in subsequent backcrosses to either cultivated GM cotton or the native *Gossypium* K-genome species, the K-genome chromosomes would be lost and this process would continue until all the K-genome chromosomes are lost. In addition, the introduced genes are carried on the A and/or D genomes of the GM cotton (*G. hirsutum* (AD)) and could only be maintained in the K-genome cotton if they are transferred to a balanced set of K-chromosomes. Transfer of introduced genes by recombination between chromosomes of different genomic origin is thought to be extremely rare, as demonstrated by studies in hexaploid wheat (Hedge & Waines 2004). This is likely due to the spatial separation of chromosomes from different genomes during the cell cycle as observed in hexaploid wheat which contains 3 genomes (Avivi et al. 1982) and the F1 hybrid generated by crossing barley and wild rye (Leitch et al. 1991). Thus, the potential for introgression of introduced genes into any of the K-genome *Gossypium* species is effectively zero.



207. Therefore, **no risk is identified** and the potential for weediness in these sexually incompatible species as a result of gene transfer will not be assessed further.

**Event 21 Exposure of organisms (including people) to the CP4 EPSPS, Cry1Ac and Cry2Ab proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants**

208. Expression of the introduced genes in sexually compatible plants (ie naturalised, volunteer or commercially grown *G. hirsutum* or *G. barbadense* cotton plants, including other GM cotton plants) could lead to increased exposure of people and other organisms to the expressed proteins. This could result in a greater probability of toxicity or allergenicity for people and toxicity for other organisms. Risks of toxicity and allergenicity of these proteins to people and other organisms are discussed in Sections 2.1, 2.2 and 2.3 of this Chapter.

209. However, all of the proteins encoded by the introduced genes are of very low oral toxicity and both GM cotton and other GM crops containing the same or similar introduced proteins are approved for use in food (see Section 2.1 of this Chapter). Evidence also indicates that none of these proteins are allergenic.

210. People and other organisms are already widely exposed to these proteins via bacteria expressing the proteins naturally or via commercially released GM cotton lines in southern Australia. Commercially released Roundup Ready<sup>®</sup> and Bollgard II<sup>®</sup> GM cotton containing the same introduced proteins are already widely grown, accounting for 90% of all commercial cotton in Australia (see Chapter 1 for details). People and other organisms are already exposed to these proteins via field trials of GM cotton containing these proteins in northern Australia since 1998. The applicant proposes to conduct plant breeding, agronomic trials and seed production, and potentially commercial scale plantings of the GM cotton lines. Subsequently, people and other organisms north of latitude 22°S would be exposed to these same proteins.

211. Compared to this level of exposure, the level of exposure expected as a result of vertical gene transfer to sexually compatible cotton plants would be minimal, since outcrossing of cotton is localised around the pollen source and decreases significantly with distance (OGTR 2002).

212. Therefore, **no risk is identified** and the potential for toxicity for people and other organisms or allergic reactions for people as a result of vertical gene transfer of the introduced genes into other *G. hirsutum* or *G. barbadense* plants will not be assessed further.

**Event 22 Presence of the introduced regulatory sequences in *G. hirsutum* or *G. barbadense* plants as a result of gene transfer**

213. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to cotton plants. The transfer of either endogenous or introduced regulatory sequences could potentially result in unpredictable effects. However, the impacts from the introduced regulatory elements would be equivalent to the endogenous regulatory elements. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of vertical gene transfer of introduced regulatory sequences will not be assessed further.

**Event 23 Reduced choice of herbicides for the control of cotton volunteers as a result of stacking of herbicide tolerance traits**

214. GM cotton plants expressing the *cp4 epsps* gene in combination with the *bar* gene (present in GM Liberty Link<sup>®</sup> Cotton recently approved for commercial release under DIR 062/2005) as a result of vertical gene transfer could become tolerant to both glyphosate

and glufosinate ammonium and therefore these herbicides could not be used to control the cotton volunteers.

215. The control of cotton volunteers is important both in cotton fields and outside the fields such as along roadsides and drains. There are three types of cotton volunteers that need to be controlled: seedling cotton, established cotton, and regrowth or ‘ratoon’ cotton.

216. Herbicides can be used to control seedling cotton volunteers. Glyphosate has been the most common herbicide used to control these volunteers but, with the uptake of Roundup Ready® GM cotton since 2000, alternative herbicides are being used, including glufosinate ammonium. However, the use of glufosinate ammonium is limited on cotton volunteers as its effectiveness on cotton seedlings at the 4 and 8 leaf stage offers incomplete control. Other herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective (Roberts et al. 2002). Cultivation is also a very effective method to control seedling cotton volunteers (Australian Cotton Cooperative Research Centre 2002a).

217. Established or ratoon cotton plants, whether GM or non-GM, are difficult to control by herbicides alone. For example, glyphosate is not generally used to control established cotton plants because it does not kill the plants and they can recover. Instead, established or ratoon cotton plants are most effectively controlled by mechanical methods involving mulching, root cutting and cultivation (Roberts et al. 2002).

218. Thus, glyphosate and glufosinate ammonium tolerance is not likely to impact on the control of cotton volunteers. Therefore, **no risk is identified** and the potential for reduced choice of herbicides to control cotton volunteers as a result of vertical gene transfer of the *cp4 epsps* gene to a GM cotton containing the *bar* gene will not be assessed further.

#### **Event 24 Tolerance to other herbicides as a result of stacking**

219. GM cotton plants expressing the *cp4 epsps* gene in combination with the *bar* gene present in a GM cotton as a result of vertical gene transfer could become tolerant to both glyphosate and glufosinate ammonium as well as other herbicides.

220. Herbicides containing glyphosate are classified into Group M and herbicides containing glufosinate ammonium are in Group N and these herbicides affect different biochemical pathways in plants. Therefore, it is unlikely that the presence of both the *cp4 epsps* and *bar* genes in cotton plants will result in unintended biochemical interactions and that the plants will develop resistance to a different type of herbicide.

221. A study on stacking of glyphosate and glufosinate ammonium herbicide tolerance traits in GM canola showed no tolerance to other, unrelated herbicides and no gene silencing (Senior et al. 2002).

222. Therefore, **no risk is identified** and the potential for tolerance to other herbicides as a result of vertical gene transfer of the *cp4 epsps* gene to GM cotton containing the *bar* gene will not be assessed further.

## **2.6 Gene flow by horizontal gene transfer**

#### **Event 25 Presence of the *cp4 epsps*, *cry1Ac*, or *cry2Ab* genes, or the introduced regulatory sequences, in other organisms**

223. It is unlikely that transfer of the *cp4 epsps*, *cry1Ac*, or *cry2Ab* genes, or the introduced regulatory sequences, from the GM plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) could occur without human intervention.

224. As indicated above, all five GM cotton lines proposed for release north of latitude 22°S have previously been approved for commercial release south of latitude 22°S and products

derived from the GM cotton plants have also been approved for use in food by FSANZ (see Chapter 1).

225. A RARMP has been prepared recently for the commercial release of Roundup Ready Flex<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton south of latitude 22°S (DIR 059/2005). These two GM cotton lines contain the same introduced genes and the same or similar regulatory sequences as are present in the five GM cotton lines proposed for commercial release in this application (see Table 1.2, Chapter 1).

226. In the RARMP for DIR 059/2005, it was considered whether the transfer of the *cp4 epsps*, *cry1Ac* or *cry2Ab* genes, or the introduced regulatory sequences, from the GM plants to other organisms could lead to an adverse outcome and whether it could reasonably occur. No risk was identified and no increased risks relating to gene flow by horizontal gene transfer are expected as a result of growing of the GM cotton plants north of latitude 22°S.

227. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of horizontal gene transfer will not be assessed further.

## 2.7 Unintended changes in toxicity

### **Event 26** *Altered levels of innate toxic or anti-nutritional compounds as a result of random insertion of the gene construct into the cotton genome during development of the GM cotton lines*

228. As previously discussed, tissue from either GM or non-GM cotton plants, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional compounds including gossypol and cyclopropenoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids). There is potential for the GM cotton plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the genetic manipulation.

229. Compositional analysis of Roundup Ready<sup>®</sup> cotton and Bollgard II<sup>®</sup> cotton was conducted prior to their commercial releases in 2000 (refer to DIR 023/2002) and 2002 (refer to DIR 012/2002), respectively, and no difference was detected compared to other commercially grown cotton varieties. FSANZ approved the use of oil and linters from Roundup Ready<sup>®</sup> and Bollgard II<sup>®</sup> cotton in food (ANZFA 2000; ANZFA 2002b).

230. Compositional analysis of processed cotton seed oil, raw cotton seed meal and delinted cotton seed from Roundup Ready Flex<sup>®</sup> cotton are summarised in the RARMP prepared for the commercial release of this GM cotton south of latitude 22°S (DIR 059/2005). Cotton seed oil derived from Roundup Ready Flex<sup>®</sup> cotton is considered to be compositionally equivalent to oil derived from non-GM cotton. There were no significant differences in any of the toxic or anti-nutritional compounds, and cotton seed and raw cotton seed meal from Roundup Ready Flex<sup>®</sup> cotton were considered to be compositionally equivalent to that derived from non-GM cotton.

231. A detailed compositional analysis of Roundup Ready Flex<sup>®</sup> cotton in comparison to the parental line was assessed by FSANZ in deciding to approve Monsanto's application to use food (oil and linters) derived from this GM cotton in human food. FSANZ concluded that food derived from Roundup Ready Flex<sup>®</sup> cotton is as safe as food derived from other cotton varieties (FSANZ 2005).

232. This event has also been assessed previously in the RARMP prepared for DIR 059/2005, for GM cotton containing the same introduced genes as the three GM lines discussed above, for areas south of latitude 22°S, and no risk was identified. This is not expected to be different for areas north of latitude 22°S.

233. These assessments of Roundup Ready<sup>®</sup>, Bollgard II<sup>®</sup>, and Roundup Ready Flex<sup>®</sup> cotton indicate that they are compositionally equivalent to other non-GM cotton lines, suggesting that they do not contain altered levels of innate toxic or anti-nutritional compounds. Therefore, **no risk is identified** and the potential for toxicity as a result of unintended changes in toxicity will not be assessed further.

**Event 27      Altered metabolism of glyphosate in the GM plants expressing the CP4 EPSPS protein resulting in the production of toxic compounds**

234. In plants, the native *epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene encodes an important enzyme (EPSPS) involved in the biosynthesis of aromatic amino acids. During this biosynthetic process the EPSPS enzyme catalyses the addition of the enolpyruvyl moiety of phosphoenolpyruvate to shikimate-3-phosphate. EPSPS performs this function in plants, bacteria, algae and fungi but is absent from mammals, which are not able to synthesise these aromatic amino acids (Bentley 1990; Padgett et al. 1993).

235. The CP4 EPSPS protein expressed in the GM plants functions in the same way, in the biosynthesis of aromatic amino acids, as the native enzyme in plants. The only difference is that it is insensitive to the effects of glyphosate (Padgett et al. 1993) and therefore is still able to perform its function in the presence of glyphosate. The CP4 EPSPS protein is not involved in glyphosate metabolism and as a result no new metabolic products are expected to occur in the GM plants (OECD 1999). Therefore, **no risk is identified** and the potential for toxicity as a result of altered metabolism of glyphosate in the GM plants expressing the CP4 EPSPS protein will not be assessed further.

**Event 28      Synergistic effects of the introduced proteins when ingested in combination resulting in altered toxicity**

236. Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton expressing glyphosate tolerance and insecticidal proteins was approved for commercial release south of latitude 22° S in 2002 (DIR 012/2002) and has been used in food and stockfeed since then. There have been no reports of any unexpected or unintentional adverse effects from this commercial release. All five GM cotton lines proposed for release north of latitude 22° S contain the same glyphosate tolerance and/or insecticidal proteins and all five have been approved for commercial release south of latitude 22° S (refer to DIR 012/2002, DIR 023/2002, and DIR 059/2005), including use of stockfeed anywhere in Australia (subject to conditions in northern Australia).

237. The CP4 EPSPS protein that confers tolerance to glyphosate and the Cry1Ac and Cry2Ab insecticidal proteins operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the herbicide tolerance and insecticidal proteins or their metabolic pathways, and no reason to expect that this is likely to occur. There is no evidence or reasonable expectation that synergistic effects are likely to occur from the combination of the two traits, or that they would result in new or increased risks relating to human health and safety or the environment.

238. Only the two insecticidal proteins, Cry1Ac and Cry2Ab, have related biochemical mechanisms and may have synergistic effects when ingested in combination, and potentially impact on toxicity for invertebrates. This is considered in **Chapter 3, Identified Risk 1**.

239. Therefore, **no risk is identified** and the potential for toxicity as a result of synergistic effects of the introduced proteins will not be assessed further.

## 2.8 Unintended changes in biochemistry or physiology

### **Event 29** *Altered biochemistry or physiology of the GM cotton plants resulting from insertion or expression of the introduced genes*

240. Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion for example, due to changes in chromatin structure, methylation patterns, and transcriptional read-through
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of an introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway of the introduced gene product.

241. Such unintended effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional or agronomic value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

242. Unintended changes in gene expression could alter either the biochemistry or the physiology of the GM cotton plants. Biochemical or physiological changes to the GM cotton lines proposed for release could occur either as a result of the expression of the introduced genes or of the transformation process itself. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

243. The GM cotton lines proposed for release were selected from a large number of initial transformants and were selected on the basis that they did not show any altered agronomic properties beyond expression of the introduced proteins (information supplied by the applicant).

244. As well as approval for commercial release south of latitude 22° S (under DIRs 012/2002, 023/2002 and 059/2005), the five GM cotton lines proposed for release were previously approved for field trials north of latitude 22° S (under DIRs 006/2001, 009/2001, 012/2002, 035/2003 and 055/2004).

245. In numerous trials in Australia and overseas, all five GM cotton lines proposed for release have not shown any adverse unintended secondary effects.

246. Four of the GM cotton lines, Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>, have been subjected to comparisons to non-GM cotton for numerous phenotypic characters. Phenotypic comparisons such as germination, growth habit, plant morphology, fibre characters (eg length, strength, and diameter), lint yield, disease susceptibility, oil content, fertility, seed composition and nutrition characters (eg proteins, ash, oils, carbohydrate, amino acid, fatty acid and calories) and anti-nutritional or toxic factors (eg gossypol and cyclopropenoid fatty acids) were made. Greater detail of these

comparisons is presented in the RARMPs of the DIRs mentioned above. In summary, the phenotypic characters for these four GM cotton lines were all within the range for commercially grown cotton varieties, indicating that biochemical pathways and plant physiology are not altered in the GM plants.

247. Unintended changes to biochemical pathways and plant physiology are not expected for Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton. Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton is derived from conventional cross breeding of Roundup Ready Flex<sup>®</sup> and Bollgard II<sup>®</sup> cotton, and thus contains the same introduced genes and proteins. As indicated above, the phenotypic characters for both Roundup Ready Flex<sup>®</sup> and Bollgard II<sup>®</sup> cotton lines are within the range for commercially grown cotton varieties, indicating that biochemical pathways and plant physiology are not altered in the GM plants. It is not expected that conventional breeding between these two GM cotton lines will result in altered phenotypic characters or adverse changes to biochemical pathways and plant physiology. This expectation is based on the fact that the introduced proteins Cry1Ac, Cry2Ab and CP4 EPSPS operate through independent, unrelated biochemical mechanisms. Fields trials of Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton (under DIR 055/2004) have not shown any adverse unintended secondary effects.

248. Therefore, **no risk is identified** and the potential for toxicity or weediness as a result of unintended changes in biochemistry or physiology will not be assessed further.

## 2.9 Unintended effects on existing pests or weeds

### ***Event 30 Increased prevalence of other insects due to decreased use of insecticide sprays on the GM Bollgard II<sup>®</sup> cotton varieties***

249. Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup>, and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton are resistant to the major lepidopteran pests of cultivated cotton crops, thus they are expected to require fewer applications of insecticide sprays than non-GM cotton crops. This has the potential to allow populations of other insects, normally controlled by insecticide sprays, to increase to problematic levels. This could not only result in an increased pest burden in the environment but may also increase the incidence of gene flow if pollinator species became more abundant.

250. Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton are already approved for commercial release south of latitude 22°S (licence for DIR 012/2002 issued in 2002) and have been widely grown in this area. In the 2005–06 season, 90% of the cotton grown in Australia was GM-cotton, and Bollgard II<sup>®</sup> cotton varieties (including the stacked variety Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup>) comprised 81% of all GM cotton grown. Bollgard II<sup>®</sup> cotton varieties require around 86% less insecticide spray than conventional non-GM cotton (Cotton Australia 2005).

251. Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton expresses the same Cry proteins as Bollgard II<sup>®</sup> cotton and the insecticidal trait of the two cotton lines is identical (Burns et al. 2004). Therefore, the reduced requirement for insecticide sprays is expected to be the same for Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> and Bollgard II<sup>®</sup> cotton. If Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> were to replace the currently grown Bollgard II<sup>®</sup> cotton varieties, there would be no increased risk for a higher prevalence of insect pests.

252. Insect abundance and diversity in Bollgard II<sup>®</sup> cotton fields have been assessed in the RARMP prepared for DIR 012/2002 (available at <<http://www.ogtr.gov.au>>). Integrated pest management guidelines developed by the cotton industry recommend that Bollgard II<sup>®</sup> cotton must be monitored regularly for pests, similarly to conventional non-GM cotton, to determine whether and what spraying is required (Australian Cotton Cooperative Research Centre 2004a; Johnson & Farrell 2004). The reduction of insecticide use in Bollgard II<sup>®</sup> cotton has

led to increased incidence of sucking pests, mainly mirids. These pests would be killed incidentally by broad-spectrum insecticides commonly used on non-GM cotton and other GM cotton lines without an insect resistance trait. Non-lepidopteran pest populations can be controlled in Bollgard II® by insecticide sprays if they reach a level where yield loss could occur.

253. Therefore, **no risk is identified** and the potential for increased non-lepidopteran insect herbivory as a result of decreased use of insecticide sprays on any of the Bollgard II® cotton varieties proposed for release will not be assessed further.

**Event 31      Expression of the introduced genes resulting in increased disease burden**

254. Expression of the introduced proteins (Cry1Ac, Cry2Ab, and CP4 EPSPS) is not expected to affect the disease status of the GM plants.

255. Roundup Ready® cotton, and Bollgard II® and Roundup Ready®/Bollgard II® cotton have been commercially released south of latitude 22°S under DIR 023/2002 and DIR 012/2002 since 2000 and 2002, respectively. There have been no reports of any increased disease burden of these commercially released GM cotton lines.

256. No differences were observed in the pest or disease status of GM and non-GM cotton during phenotypic evaluation of Roundup Ready Flex® cotton during Australian field trials conducted in the 2003–04 season (Dunn 2005) nor in fields trials conducted since with Roundup Ready Flex® and Roundup Ready Flex®/ Bollgard II® cotton (under DIR 055/2004). Similarly, no increase in pest potential was detected during agronomic performance testing conducted in the USA (Burns 2004).

257. Therefore, **no risk is identified** and the potential for increased disease burden as a result of the expression of the introduced genes will not be assessed further.

## **2.10      Secondary impacts**

**Event 32      Development of insects resistant to Cry1Ac and Cry2Ab proteins**

258. The APVMA has a complementary regulatory role in respect to this application due to its responsibility for agricultural chemical use in Australia, including insecticides, under the *Agricultural and Veterinary Chemicals Code Act 1994* (Ag Vet Code Act 1994).

259. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an insecticide, for example, for a limited period of time or for a limited area. In considering applications for registration or permits, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of an insecticide, and insect resistance management and, if necessary, imposes conditions in relation to these. The APVMA can impose conditions on both registrations and permits.

260. Widespread and long-term use of Bollgard II® cotton varieties, including Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton, could result in the emergence of resistance to the Cry1Ac and Cry2Ab proteins in the target species (*H. armigera* and *H. punctigera*), and other susceptible lepidopteran species feeding on cotton. This would result in a reduction in the efficacy of these GM cotton lines for the control of insect pests, and could also have impacts on the efficacy of Bt microbial sprays to control insects in other agricultural systems. Potential adverse effects include attenuation of the potential benefits of growing Bollgard II® cotton lines to the environment and human health.

261. It should be noted that Bollgard II<sup>®</sup> cotton was developed with the specific intention of reducing the risk of insects developing resistance to the Cry1Ac or Cry2Ab proteins. The expression of two insecticidal proteins (which differ sufficiently in their mechanisms of action) in Bollgard II<sup>®</sup> cotton, and the fact that the overall insecticidal activity is increased relative to INGARD<sup>®</sup> cotton (which contains only the Cry1Ac protein), is expected to delay the emergence of resistant insects. In 2004, INGARD<sup>®</sup> cotton was withdrawn from the market in Australia in favour of Bollgard II<sup>®</sup> cotton.

262. Bollgard II<sup>®</sup> cotton varieties (Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>) cotton fall under the Ag Vet Code Act 1994 definition of an agricultural chemical product, due to their production of two insecticidal substances, and are thus subject to regulation by the APVMA. The APVMA registered the use of the insecticidal proteins as produced by the insecticidal genes (*cry1Ac* and *cry2Ab*) in GM Bollgard II<sup>®</sup> cotton as insecticidal products for New South Wales (NSW) and Queensland (QLD) south of latitude 22°S in 2003. The APVMA is currently assessing an application from Monsanto to vary the label for Bollgard II<sup>®</sup> to remove the condition for restriction on planting Bollgard II<sup>®</sup> north of latitude 22°S. Further information about the APVMA can be obtained from <[www.apvma.gov.au](http://www.apvma.gov.au)>.

263. A resistance management plan (RMP) for Bollgard II<sup>®</sup> cotton varieties grown south of latitude 22°S was developed by Monsanto and endorsed by the TIMS committee of the Australian Cotton Growers' Research Association in consultation with the APVMA (Farrell & Johnson 2005; Monsanto Australia Limited 2004). The APVMA requires implementation of this plan as a condition of registration. The RMP is designed to minimise the development of resistant insects and requires growers to employ a number of measures designed to achieve this objective. As part of the resistance management strategy, refuge crops must be grown, to allow Bollgard II<sup>®</sup>-sensitive insects to survive.

264. A resistance management plan for potential growing areas of Bollgard II<sup>®</sup> cotton varieties north of latitude 22°S in Australia has been developed by Monsanto. The plan has been endorsed by the Technical Group for Northern Australia Resistance Management (TGNARM) committee. Cultivation of Bollgard II<sup>®</sup> cotton varieties may require its implementation along with all other relevant conditions that may be imposed by the APVMA.

265. Therefore, **no risk** to the health and safety of people or the environment **is identified** as the potential for decreased efficacy of the insect resistance trait is being actively managed by the APVMA.

### **Event 33      Secondary effects on populations of organisms that depend on lepidopteran insects**

266. Widespread and long-term use of Bollgard II<sup>®</sup> cotton varieties, including Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton, expressing the *cry1Ac* and *cry2Ab* genes could result in other organisms that depend on lepidopteran insects in the food web being adversely affected, which could result in loss of a food source.

267. Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton are already approved for commercial release south of latitude 22°S (licences for DIR 012/2002 and DIR 059/2005 issued in 2002 and 2006, respectively). Bollgard II<sup>®</sup> cotton varieties have been widely grown in this area. In the 2005–06 season, 90% of all commercial cotton grown in Australia was GM cotton and Bollgard II<sup>®</sup> cotton varieties comprised 81% of the GM cotton. The Bollgard II<sup>®</sup> cotton varieties all express the same Cry proteins. The effect of the expression of the *cry1Ac* and *cry2Ab* genes in Bollgard II<sup>®</sup> cotton on other organisms



that depend on lepidopteran insects has been assessed in the RARMP prepared for DIR 012/2002 (available at <<http://www.ogtr.gov.au>>).

268. The RARMP for DIR 012/2002 determined that there would be no increased risk for organisms that depend on lepidopteran insects, if Bollgard II<sup>®</sup> cotton lines were to replace non-GM cotton. This is because lepidopteran insect larvae are specifically killed upon ingestion of the insecticidal proteins in GM cotton expressing the Cry1Ac and Cry2Ab proteins (ie Bollgard II<sup>®</sup>, including stacked varieties), whereas in non-GM cotton, they are killed by commonly used broad-spectrum insecticides. Therefore, the secondary effects on other organisms that depend on lepidopteran insects in the food web will be no greater in GM cotton than in non-GM cotton.

269. In addition, invertebrates that depend on lepidopteran insects in the food web are likely to be adversely affected or killed by the use of broad-spectrum insecticides in non-GM cotton, as well as in other agricultural crops.

270. Therefore, **no risk is identified** and the potential for an adverse outcome for organisms that depend on lepidopteran insects, as a result of the expression of the *cry1Ac* and *cry2Ab* genes in Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton, will not be assessed further.

**Event 34 Use of glyphosate on the GM cotton lines resulting in development of herbicide resistant weeds (in the agricultural environment)**

271. Changes in agricultural practices such as adoption of minimal tillage or changes in herbicide use may cause changes to weed populations. For example, weed species that are inherently more resistant to a herbicide than other weed species may become more abundant (Nandula et al. 2005; Owen & Zelaya 2005). The development of herbicide resistant weeds may occur where Roundup Ready<sup>®</sup> Herbicide is used to replace other weed management practices and this could result in the emergence of weeds that are more difficult to control.

272. Herbicide use on weed communities can exert selective pressure that leads to the development of herbicide resistant weeds. The repetitive use of a single herbicide, or herbicide group, increases the chance that development of herbicide resistant weeds will occur. Integrated weed management practices help to avoid selection of resistant weed biotypes (Avcare 2003). Integrated weed management has prevented the development of herbicide resistant weeds in Australian cotton fields up to this point (Roberts & Charles 2002; Walker et al. 2006; Werth et al. 2006).

273. The first confirmed cases of glyphosate resistance in Australia were in populations of *Lolium rigidum* (rigid ryegrass) (Powles et al. 1998; Pratley et al. 1999). Subsequently, other resistant populations of *L. rigidum* have been verified, with 34 cases confirmed in Australia in 2003 (Preston 2003). The majority of these populations have developed in fallows or horticultural situations with intensive use of glyphosate.

274. Glyphosate resistance has also been reported in a number of other weed species around the world (Heap 2003; Nandula et al. 2005). To date, a total of eight weed species have developed resistance to glyphosate (Nandula et al. 2005).

275. As part of the Deed of Agreement between the Australian Government and Monsanto for the commercial release of Roundup Ready<sup>®</sup> and Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> cotton in 2000, Monsanto was required to develop a crop management plan designed to minimise the potential for development of glyphosate-resistant weeds (Monsanto Australia Limited 2001) (refer DIR 023/2002). This plan was endorsed by the herbicide resistance subcommittee of the TIMS committee. The plan includes a requirement to prevent seed set of weeds that have

survived exposure to Roundup Ready® Herbicide. Compliance with the current crop management plan and undertaking of a weed management audit endorsed by the TIMS subcommittee is encouraged by the APVMA in connection with the use of Roundup Ready® Herbicide and the continued commercial release of Roundup Ready® cotton, as noted in the licence for DIR 023/2002 issued by the Regulator in June 2003.

276. Data collected by Monsanto from growers of Roundup Ready® cotton as part of the weed management audit (provided in support of licence application for DIR 023/2002) indicates general compliance with this plan. Over three seasons of cultivation of these GM cotton lines, there has been no indication of development of glyphosate resistant weeds and no change in the level of grower satisfaction with this technology.

277. The APVMA recently approved (September 2006) Roundup Ready® Herbicide for use on Roundup Ready Flex® cotton. A Roundup Ready Flex® cotton crop management plan, which specifies an Integrated Weed Management Strategy and a weed management audit endorsed by the TIMS committee, is in place to minimise the potential for development of glyphosate-resistant weeds. Compliance with the crop management plan would be implemented through a Technology User Agreement between the grower and Monsanto.

278. Therefore, **no risk is identified** as the potential for the use of glyphosate on the GM cotton lines resulting in development of herbicide tolerant weeds will be assessed by the APVMA.

### **Event 35      Consumption of animals that were fed GM plant material**

279. Mammals generally avoid feeding on cotton plants because of the presence of gossypol and cyclopropanoid fatty acids in cotton seed which limits the use of whole cotton seed as a protein supplement in animal feed. Cattle are less affected by these components. Inactivation or removal of these components during processing enables the use of some cotton seed meal for farmed fish, poultry and swine. The meal and hulls of cotton seed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

280. The introduced proteins are rapidly degraded in mammalian digestive systems (see Section 4.2 of Chapter 1 for details). As a result, meat produced from animals fed GM cotton seed would be no different to meat from animals that were fed seed from non-GM cotton.

281. All five GM cotton lines proposed for release north of latitude 22°S have previously been approved for commercial release south of latitude 22°S (under DIRs 012/2002, 023/2002 and 059/2005). Products derived from the GM cotton plants have also been approved for use in food by FSANZ (see Section 6, Chapter 1). The introduced proteins present in the Roundup Ready Flex®, Roundup Ready Flex®/Bollgard II®, and Roundup Ready®/Bollgard II®, cotton lines are the same as those present in Roundup Ready® and Bollgard II® cotton lines. Meat from cattle that were fed seed from commercially released Roundup Ready® and Bollgard II® cotton lines has been consumed for several years (since 2000 and 2002, respectively) with no adverse effects reported.

282. Therefore, **no risk is identified** and the potential for toxicity for people as a result of consumption of animals that were fed GM plant material will not be assessed further.

## **Section 3 Risk estimate process for identified risks**

283. Six events from the hazard identification process (Identified Risks 1–6 in Table 2.1) are considered to lead to identified risks for the adverse outcomes of toxicity for non-target invertebrates and weediness.

284. Chapters 3 and 4 give detailed consideration to the consequences and likelihood of these six Identified Risks in order to obtain estimates of the level of risk. The risks are assessed against the baselines established by reference to characteristics of the parent organism and aspects of the receiving environment (including agronomic practices and GM Liberty Link<sup>®</sup> Cotton previously approved for commercial release in northern Australia).

285. Information contained in the application (including information required by the Act and the Regulations on the GMOs, the parent organism, the proposed dealings and potential impacts on the health and safety of people and the environment), current scientific knowledge, and submissions received during consultation with experts, agencies and authorities, and the public (Appendices B to E) were also considered.

286. The consequence assessment considers the seriousness of the harm that could potentially result from each event, while the likelihood assessment considers the chance of the event resulting in harm. Consequence and likelihood assessments are then combined to give an overall risk estimate using the Risk Estimate Matrix (Figure 2.2). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty that arise from a lack of data.

**Figure 2.2** The OGTR Risk Estimate Matrix (OGTR 2005)

		RISK ESTIMATE			
		Low	Moderate	High	High
LIKELIHOOD	Highly likely	Negligible	Low	High	High
	Likely	Negligible	Low	Moderate	High
	Unlikely	Negligible	Negligible	Low	Moderate
	Highly unlikely	Marginal	Minor	Intermediate	Major
		CONSEQUENCES			

**Risk Estimate Matrix:** A *negligible* risk is considered to be insubstantial with no present need to invoke actions for mitigation. A *low* risk is considered to be minimal but may invoke actions for mitigation beyond normal practices. A *moderate* risk is considered to be of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. A *high* risk is considered to be unacceptable unless actions for mitigation are highly feasible and effective.

287. Definitions of risk analysis terms used by the Regulator can be found in Appendix A.

288. After an estimate is obtained for each identified risk, risks higher than negligible are evaluated to determine if risk treatment measures are required to mitigate potential harm (see Chapter 5—Risk Management)

## Chapter 3 Risk estimate for toxicity for non-target invertebrates

289. This Chapter estimates the risk associated with one event (**Identified Risk 1** from Chapter 2) that could lead to the adverse outcome of toxicity for non-target invertebrates arising from this proposed release. The risk estimate is based on consequence and likelihood assessments for this event.

### Section 1 Background

290. The five GM cotton lines proposed for release express one to five proteins as a result of genetic modification. Events that may give rise to toxicity for non-target organisms as a result of the proposed release were considered in Chapter 2. Expression of the CP4 EPSPS, GUS and/or NPTII proteins is not expected to provide a novel source of harm to non-target organisms, as these and similar proteins are naturally present in the environment and are expressed by common bacterial species without any indication of toxicity for any organism. Evidence also indicates that the Cry1Ac and Cry2Ab proteins are not toxic to vertebrates or microorganisms and are expected to pose a risk only to some non-target invertebrates. Therefore, this Chapter will be limited to assessing the risk of toxicity for non-target invertebrates as a result of ingestion of the Cry1Ac and Cry2Ab proteins in the Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton lines.

291. Toxicity of the Cry proteins is highly specific due to their mode of action. Susceptible organisms must have the correct combination of gut conditions and suitable binding sites on the midgut cells (see Chapter 1 for details). The toxicity of the Cry1Ac and Cry2Ab proteins for insect pests of cultivated cotton, in particular the targets *H. armigera* and *H. punctigera*, is not considered to be an adverse outcome but rather the intent of the genetic modification.

### Section 2 Consequence and likelihood assessments

292. Consideration is given to Identified Risk 1 from Chapter 2 (Hazard identification) that may give rise to toxicity in non-target invertebrates. For this identified risk, the level of risk is estimated from assessment of the seriousness of harm (**consequence**—ranging from marginal to major) and the chance of harm (**likelihood**—ranging from highly unlikely to highly likely).

293. The Regulator can only consider risks to the health and safety of people or the environment posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMOs is considered relative to the baselines of toxicity of the non-GM parent to invertebrates and the environment in which the GM cotton plants are proposed for release. Therefore, other sources of the introduced genes or similar genes in the environment (such as from naturally occurring bacteria) and the widespread commercial plantings of Bollgard II<sup>®</sup> GM cotton in Australia are relevant to the risk estimate.

#### 2.1 Toxicity of non-GM cotton

294. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton lines being considered in this risk assessment. Cotton is a well-established field crop with a long history of use. A comprehensive review of the biology of non-GM cotton is provided in the document *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002).

295. Gossypol, a phenolic compound produced by cotton, is known to be toxic to insects (Percival et al. 1999). However, a study by Wilson et al. (1981) (cited in Percival et al. 1999) showed that high levels of gossypol, even in combination with morphological characteristics

that discourage insect infestations, such as okra or lacinate leaf forms, were not sufficient to provide protection against the pink bollworm (*Pectinophora gossypiella*), a major pest of cotton in the USA.

## **2.2 Identified Risk 1: Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates as a result of this release**

296. The Cry1Ac and Cry2Ab proteins are known to be specifically toxic to the two major lepidopteran pests of cultivated cotton in Australia, namely *H. armigera* and *H. punctigera* and some other minor pests such as the pink bollworm (*P. gossypiella*) and armyworms (*Spodoptera exigua* and *S. frugiperda*) (Perlak et al. 2001; Stewart et al. 2001; van Frankenhuyzen & Nystrom 2002). In northern Australia, the four target lepidopteran pests of Bollgard II<sup>®</sup> cotton are *H. armigera*, *H. punctigera*, *Spodoptera litura* and *P. gossypiella*, although *S. litura* is only moderately susceptible to Bt toxins and therefore can persist in low abundance in Bollgard II<sup>®</sup> cotton crops (Cotton Catchment communities CRC 2006; Strickland et al. 2000; Strickland et al. 2003). A number of Australian field studies have been performed both north and south of latitude 22°S to determine the ecological effects of GM cotton plants expressing the Cry1Ac and/or Cry2Ab proteins (ie INGARD<sup>®</sup> and Bollgard II<sup>®</sup>).

297. The invertebrate fauna of cultivated cotton consists of a wide range of species including a number of beneficial species that parasitise or prey on various cotton pests, including Lepidoptera. Unlike several parasitoid species, none of the known predators which attack Lepidoptera in cotton are specialists and can consequently feed on a range of other species (Fitt & Wilson 2002).

298. The risk of toxicity for non-target invertebrates from direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins would depend upon the level of toxicity of these proteins both individually and in combination (consequence assessment) and the probability of exposure to the proteins resulting in harm during this release (likelihood assessment). The risk is assessed against the baseline toxicity of the non-GM parent organism for insects, agronomic management practices for non-GM cotton, particularly the use of broad spectrum insecticides, and the toxicity due to the presence of the Cry proteins in the environment, either through common bacteria or through commercial plantings of Bollgard II<sup>®</sup> GM cotton. The risk is also assessed in the context of the large scale of the proposed release and the receiving environment for this proposed release (all areas north of latitude 22°S).

### **2.2.1 Consequence assessment**

299. Potential non-target toxicity of the Cry1Ac and Cry2Ab proteins has been assessed in detail in the risk assessment for the commercial release of Bollgard II<sup>®</sup> cotton (DIR 012/2002) and in the risk assessment for DIR 022/2002 for the continued commercial release of INGARD<sup>®</sup> cotton (expressing only the Cry1Ac protein). More recently, this issue was assessed again in the risk assessment prepared for DIR 059/2005 for the commercial release of Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton in areas south of latitude 22°S. These risk assessments are available at <<http://www.ogtr.gov.au>>. Summarised information from these RARMPs is presented below along with new information from the literature and information specific to this application.

#### Studies conducted under controlled conditions

300. A considerable number of non-target insect species from a range of orders, which occur on cultivated cotton, have been tested and shown to be insensitive to the Cry1Ac and/or the Cry2Ab protein, even at concentrations well above the expression levels found in the GM cotton lines. These studies were performed using direct ingestion of the Cry proteins and/or

indirect ingestion by being fed susceptible host/prey reared on cotton containing the Cry protein(s). Many of these studies were performed on key beneficial species such as:

- the parasitic wasps, *Microplitis mediator* and *Nasonia vitripennis* (order Hymenoptera), which parasitise the caterpillars of *H. armigera* (Liu et al. 2005; Palmer & Krueger 2000b)
- the ladybird beetle, *Hippodamia convergens* (order Coleoptera), a beneficial predatory insect which feeds on aphids and other plant insects commonly found on cotton (Palmer & Beavers 1993b; Palmer & Krueger 2000c; Sims 1994a)
- green lacewings, *Chrysoperla carnea* (order Neuroptera), a beneficial predatory insect whose larvae feed on *Helicoverpa* eggs, aphids and other soft-bodied insects commonly found on cotton (Palmer & Beavers 1993a; Palmer & Krueger 2000a; Rodrigo-Simon et al. 2006; Sims 1994a).

301. Results of the studies indicated that the Cry1Ac protein was found to be toxic only to insects of the order Lepidoptera. Cry2Ab also exhibits toxicity towards lepidopteran, and there have been reports that this protein may also be toxic to some dipteran species.

Ahmad et al. (1989) reported that the Cry2Ab was toxic to the dipteran insects, *Anopheles gambiae* (African malarial mosquito) and *Aedes aegypti* (yellow fever mosquito), although the concentration at which mortality was seen was not reported. Conversely, two additional studies reported the Cry2Ab protein was not toxic to *A. aegypti* (Dankocsik et al. 1990; Widner & Whiteley 1990; Widner & Whiteley 1989).

302. A study by CSIRO Entomology (commissioned by the OGTR) investigated the toxicity of Cry2Ab on three additional dipteran species: *Culex quinquefasciatus* (mosquito); *Musca domestica* (house-fly); and *Chironomus tepperi* (bloodworm). The study found no evidence of toxicity at exposure levels that were equivalent to, or higher than, those that would occur in fresh GM cotton leaves (Akhurst 2005).

303. Most of the studies above, address the toxicity of the Cry1Ac or Cry2Ab protein when they are ingested alone. Synergistic, additive or antagonistic effects can occur when different Cry proteins, or other insecticidal proteins, are ingested by an insect at the same time (del Rincon-Castro et al. 1999; Schnepf et al. 1998). Studies have not directly investigated whether or not synergistic or antagonistic effects occur when the Cry1Ac and Cry2Ab proteins are ingested together. If such effects occurred in the GM cotton lines, they could potentially increase the potency of the GM cotton lines towards lepidopteran insects (which are the target pests).

304. Studies have shown both synergistic and antagonistic effects between the Cry1Aa, Cry1Ab and Cry1Ac proteins for different lepidopteran species (Lee et al. 1996; van Frankenhuyzen et al. 1991) and have also suggested that the individual toxins may interact synergistically with the spores in spray formulations (Liu et al. 1998; van Frankenhuyzen et al. 1991). The spores in the spray formulations could be expected to contain the Cry2Aa protein, which is naturally expressed in the HD1 strain of *B. thuringiensis* from which the spray is formulated. The spores may also contain the VIP3A protein (Donovan et al. 2001), another insecticidal protein produced by some *B. thuringiensis* strains, including the HD1 strain.

#### Field studies on invertebrate biodiversity

305. Monsanto conducted studies in the field to investigate the ecological effects of the INGARD® and Bollgard II® cotton plants (Addison 2001a; Addison 2001b; Addison 2001c), which have been grown commercially in Australia since 1996 and 2002, respectively. The

abundance of non-target arthropod groups was compared on unsprayed Bollgard II<sup>®</sup>, INGARD<sup>®</sup> and non-GM cotton fields over two seasons in two locations, near Dalby, QLD and near Kununurra, in northern WA. At least 10 arthropod groups (which included at least 9 insect orders plus Araneida) were sampled and only the target group, Lepidoptera, showed any significant difference between treatments. These field studies have been discussed in more detail in the RARMP prepared for DIR 012/2002.

306. The field studies involving INGARD<sup>®</sup> cotton plants, expressing the Cry1Ac protein, showed no significant adverse impact on the abundance or variety of non-target invertebrate populations in GM cotton fields, as compared to non-GM cotton. Field studies using Bollgard II<sup>®</sup> cotton plants also found no significant impacts on non-target invertebrate populations, suggesting that even if there is a synergistic effect occurring between the Cry1Ac and Cry2Ab proteins, this does not result in significant toxicity for non-target invertebrates.

307. Studies were conducted at two different locations: near Dalby, in southern QLD (Addison 2001a; Addison 2001b); and near Kununurra, in northern WA (Addison 2001c). A comparison of at least 10 invertebrate orders recorded from collections at both sites suggests that invertebrate populations found on cultivated cotton grown in northern Australia are similar to those found in current cotton growing areas in the south of Australia.

308. A study conducted by the WA Department of Agriculture (Strickland & Annells 2005) compared invertebrate fauna in non-GM, INGARD<sup>®</sup> and Bollgard II<sup>®</sup> cotton grown at Kununurra, WA. The plots were managed for pests according to CottonLOGIC protocols based on the INGARD<sup>®</sup> genotype, with all plots receiving the same insecticide applications. Sixty-one arthropods groups from 10 orders were identified and counted. Results indicated that non-target invertebrate fauna was unaffected by the Cry1Ac and Cry2Ab proteins present in the GM cotton lines. However, some beneficial (mainly predatory) arthropods were more abundant mid season on non-GM and INGARD<sup>®</sup> cotton than on Bollgard II<sup>®</sup>, probably due to a lower availability of lepidopteran prey. Invertebrates collected in this study were representative of those collected by Addison (2001a; 2001b; 2001c).

309. A recently published study conducted in NSW examined over 100 species from 14 orders to compare the species diversity between unsprayed non-GM cotton, two unsprayed Bt cotton lines (INGARD<sup>®</sup> and a stacked Bt cotton line expressing the Cry1Ac and Cry2Aa proteins) and sprayed non-GM cotton using a conventional insecticide spray regime (Whitehouse et al. 2005). The greatest reduction on population diversity was caused by the spraying of insecticides on the non-GM cotton, while only small differences in the non-target invertebrate communities were observed between unsprayed non-GM and Bt cotton. These differences were slightly decreased numbers of dipteran (frit and fruit flies) and hemipteran species (damselflies and jassids). Damselflies are beneficial generalist predators, jassids are sometimes considered a cotton pest and the role of the frit and fruit flies in cotton is not known. There is no explanation for the decrease in dipteran and hemipteran species, however a decline in damselflies has been noted in other field studies (Naranjo 2005) and in commercial crops of Bt cotton (Addison 2003).

310. A six-year field study, carried out in the USA, compared the impact on 22 taxa of non-target invertebrates in non-GM and INGARD<sup>®</sup> cotton and found no long term effects due to the Cry1Ac protein (Naranjo 2005). This study reported a minor decline in the abundance of different generalist predators, which appeared to be associated with a reduction in lepidopteran prey. In contrast the application of insecticides on both the non-GM and Bt cotton caused much greater decline of many more taxa. Studies on commercially managed cotton fields (in the USA) also found Bt cotton containing the Cry1Ac had no significant

adverse effect on non-target invertebrate populations (Head et al. 2005; Torres & Ruberson 2005).

311. A recent review article (Romeis et al. 2006) evaluated all published peer-reviewed studies on the effects of various Bt proteins (including Cry1Ac and Cry2Ab), in different crops species (including cotton) on beneficial invertebrates based on feeding and field studies. The authors found no indication of direct toxic effects of the Cry proteins and only minor, transient or inconsistent effects of Bt crops when compared with a non-Bt control. When compared with insecticide-treated non-Bt crops, Bt crops were found to support higher populations of beneficial species.

312. Negative effects on invertebrates due to their interaction or dependence on the target lepidopteran pests are a consequence of an intended effect (ie control of a pest) and are common for all pest control methods including broad spectrum insecticides, biological control and conventional host-plant resistance (Boethel & Eikenberry 1986; Croft 1990).

313. In summary, laboratory experiments have demonstrated that the Cry1Ac and Cry2Ab proteins are toxic to lepidopteran caterpillar pests of cotton with no evidence of toxicity to invertebrates from other orders, except perhaps a few dipteran species at very high concentrations. Field studies both in Australia and overseas have found no significant effect on non-target invertebrate populations, although small declines in the abundance of different generalist predators have been reported and associated with the reduced availability of lepidopteran prey. There is a well documented reduction in the use of insecticides on Bt cotton crops compared to non-GM cotton, with a concomitant increase in overall insect populations. Studies conducted in both the north and south of Australia have revealed no substantial differences in invertebrate fauna and Bollgard II<sup>®</sup> cotton has been widely grown in southern Australia since 2002 with no evidence of significant adverse effects on non-target invertebrates.

### Conclusion

314. Expression of Cry1Ac and Cry2Ab proteins in the Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> GM cotton lines is not expected to cause significant toxicity for non-target invertebrates north of latitude 22°S and the consequence of toxicity as a result of direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates is assessed as **minor**.

### **2.2.2 Likelihood assessment**

315. The commercial release of Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton lines in regions north of latitude 22°S that are suitable for growing cotton could result in a large number of non-target invertebrates being exposed to the Cry1Ac and Cry2Ab proteins. Some exposure to Cry1Ac would already exist though the natural presence of this protein in the environment (see Chapter 1, Section 5.5) and exposure to the Cry proteins through field trials of GM cotton lines since 1998. Non-target invertebrates in regions south of this latitude are already exposed to these proteins through the commercial release of these cotton lines.

316. Non-target invertebrates may be directly exposed to the Cry1Ac and Cry2Ab proteins, through feeding on the GM cotton plants. Exposure may also occur in the soil either when cotton tissues break down following incorporation into the soil or as a result of exudation of the introduced proteins through the roots. Persistence of a similar Cry protein (Cry1Ab) in soil has been demonstrated for several weeks without loss of insecticidal activity (Stotzky 2004). Indirect exposure may also occur through eating other organisms, including the lepidopteran target pests, which have previously fed on the GM cotton plants.



317. Relative levels of exposure through these routes are discussed in the RARMP prepared for DIR 055/2004. The conclusions are that herbivorous species would have the highest level of exposure, with sap suckers, pollinators, pollen feeders, soil invertebrates and insectivores having far reduced exposure.

318. A recent study has indicated that some non-target herbivores and arthropod predators collected from a Bt corn field contained detectable levels of the Cry1Ab protein, which was present in the GM corn plants (Harwood et al. 2005). However, the study did not investigate whether there were any adverse effects resulting from this exposure to the Cry1Ab protein.

319. While variation in the invertebrate fauna present in cultivated cotton between the north and south of Australia has not been intensively investigated, field studies conducted in northern WA and southern Queensland (described in Section 2.2.1) found no evidence for different abundances of invertebrate orders. Similarly, the insects collected and identified from naturalised cotton populations at three NT locations were common to cotton in the south of Australia (Eastick 2002).

320. As discussed in Section 2.2.1, populations of non-target invertebrates do not appear to be particularly sensitive to the levels of Cry1Ac and Cry2Ab expressed in commercially released Bollgard II<sup>®</sup> plants. The insect resistance of the Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton plants have been shown to be similar to that of Bollgard II<sup>®</sup> plants (see Chapter 1, Section 4.4.2), suggesting that expression levels of Cry1Ac and Cry2Ab proteins are equivalent. Bollgard II<sup>®</sup> cotton has been widely grown (south of latitude 22°S) in Australia since 2002 with no evidence of significant adverse effects on non-target invertebrates.

### Conclusion

321. Within a commercially released GM Bollgard II cotton crop, a number of non-target invertebrates could be exposed to the Cry1Ac and Cry2Ab proteins. However, non-target invertebrates are insensitive to the levels of Cry1Ac and Cry2Ab proteins expressed in the Bollgard II<sup>®</sup> plants. The proposed dealings are not expected to cause greater impacts on non-target invertebrates than non-GM cotton and Bollgard II<sup>®</sup> cotton currently grown in the south. Therefore, the likelihood of toxicity for non-target invertebrates as a result of direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins is estimated to be **highly unlikely**.

### **Section 3 Risk estimates**

322. Risk estimates (which can range from negligible to high) are based on a combination of the consequences and likelihood assessments, using the Risk Estimate Matrix (see Chapter 2).

323. The risk estimates for the adverse outcome of toxicity for non-target invertebrates as a result of the proposed release of these GM cotton plants have been made relative to the baseline of the toxicity of non-GM cotton for invertebrates, agronomic management practices for non-GM cotton, particularly the use of broad spectrum insecticides and in the context of the widespread use of commercially released Bollgard II<sup>®</sup> cotton plants in Australia without evidence of significant adverse effects on non-target invertebrates.

324. The consequences of direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins in the GM cotton plants by non-target invertebrates has been assessed as **minor**, and the likelihood of this resulting in toxicity to them as **highly unlikely**. Therefore, the risk estimate for Identified Risk 1 is **negligible**.

325. As the risk of Identified Risk 1 leading to toxicity for non-target invertebrates is estimated to be **negligible**, there is no need to invoke actions for mitigation (OGTR 2005).

Therefore, no risk treatment measures for toxicity for non-target invertebrate organisms are proposed.

• **Table 3.1 Summary of risk assessment**

Event that may give rise to toxicity for non-target invertebrates	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 1</b> Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates.</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• The Cry1Ac and Cry2Ab proteins are toxic only to lepidopteran insects.</li> <li>• Field studies indicated that growing Bollgard II® cotton plants has no significant effect on non-target invertebrate populations when compared to unsprayed non-GM cotton.</li> </ul>	<p><b>Highly Unlikely</b></p> <ul style="list-style-type: none"> <li>• Exposure to the GM cotton lines and the Cry proteins would occur mostly to those non-target invertebrates consuming the GM cotton within the cotton field.</li> <li>• Non-target invertebrates are insensitive to the levels of Cry1Ac and Cry2Ab proteins expressed in the Bollgard II® plants.</li> </ul>	<p><b>Negligible</b></p>	<p><b>No</b></p>

## Chapter 4 Risk estimates for weediness

326. This Chapter estimates the risks associated with five events (**Identified Risks 2-6** from Chapter 2) that could lead to the adverse outcome of weediness arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

### Section 1 Background

327. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. In addition, plants may also be considered weeds if they are simply growing where they are not wanted.

328. Weediness in Australia is often correlated with weediness of the species, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999; Pheloung 2001). The chance of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for the plants to establish and spread into new environments (eg escapes of commonly used garden plants) (Groves et al. 2005; Mulvaney 2001).

329. Negative characteristics of weeds may include spread and persistence, competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. In addition, the spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative environmental impacts such as:

- reduced biodiversity (including genetic, species and ecosystem diversity) that results from lower abundance of desirable species, reduced species richness, or undesirable changes in species composition
- interference with the intended use of the land they occupy
- degradation of landscape/ecosystems, such as altered water or nutrient availability.

330. Complex interactions between a plant and its environment (including availability of water, nutrients and light) determine the degree to which that plant can spread and persist in the environment. A number of measurable properties of plants that may influence spread and persistence or competitiveness are listed below:

- germination, survival and reproduction under a wide range of environmental conditions
- rates of seedling growth
- rates of growth to reproductive stage
- degree of self-pollination
- use of non-specialist pollinators or wind when out-crossing
- period of seed production
- seed output
- degree of seed dispersal
- longevity of seed and degree of dormancy
- allelopathy (effect on the germination and/or growth of neighbouring plants through chemical exudates)

- resistance to pests or pathogens.

331. In the risk assessment, consideration is given to characteristics that may be expected to be altered as a result of the genetic modification and that may increase the spread and persistence of the GMOs, or of sexually compatible relatives that may receive the introduced gene(s). Alterations in these characteristics may indicate potential for weediness.

332. The GM cotton lines proposed for release express one to five proteins as a result of the genetic modification. Events that may give rise to weediness were considered in Chapter 2. Expression of the GUS and/or NPTII proteins in four of the GM cotton lines (Bollgard II<sup>®</sup>, Roundup Ready<sup>®</sup>, Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>) is not expected to have any impact on the weediness of the GM cotton. The toxicity of the Cry1Ac and Cry2Ab proteins for lepidopteran pests of cotton and/or the tolerance to glyphosate herbicide conferred by the CP4 EPSPS protein may lead to weediness of the GM cotton plants. Therefore, this Chapter will be limited to assessing the risk of weediness as a result of expression of the *cry1Ac*, *cry2Ab* and/or *cp4 epsps* genes in the GM cotton lines.

333. The risk of weediness as a result of expression of the *cry1Ac*, *cry2Ab* and/or *cp4 epsps* genes in the GM cotton lines with regard to their commercial release in areas south of latitude 22°S has previously been assessed in the RARMPs prepared for DIRs 012/2002, 023/2002 and 059/2005. These documents are available at <<http://www.ogtr.gov.au>>.

## Section 2 Consequence and likelihood assessments

334. Consideration is given to the five identified risks in Chapter 2 (Hazard identification) that may give rise to weediness (Identified Risks 2 to 6). For each identified risk the level of risk is estimated from assessments of the seriousness of harm (**consequence**—ranging from marginal to major) and the chance of harm (**likelihood**—ranging from highly unlikely to highly likely).

335. The Regulator can only consider risks posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMOs is considered relative to the baseline of weediness of the non-GM parent and the environment in which the GM cotton plants are proposed for release.

### 2.1 Weediness of non-GM cotton

336. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton lines being considered in this risk assessment. Attributes of non-GM cotton associated with potential weediness are discussed in the document *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002). This document concludes that non-GM cotton is not a serious weed in Australia. Firstly, cotton does not possess certain innate characteristics typically associated with problematic weeds such as prolonged seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long distance dispersal of seeds. Secondly, abiotic and biotic factors including temperature, soil moisture, nutrient levels and roadside management practices limit the establishment and/or persistence of cotton outside of agricultural and other disturbed environments.

337. Additional limiting abiotic and biotic factors that determine whether cotton will persist in the environment include frost, short summer seasons, soil type, fire, competition from other plants, herbivory (insects and other animals), and physical destruction such as trampling (Eastick 2002; Farrell & Roberts 2002). The relative impact of each of these factors is dependent on whether the cotton plants are in coastal or inland areas, as well as whether they

are in northern or southern areas of Australia. For example, frost is a major limiting factor in southern areas of Australia, whereas the reliable availability of water is a limiting factor in most areas of Australia.

338. In conservation areas, eg National Parks, where weeds may be defined as any naturalised alien/non-native plant, cotton (*G. hirsutum*) in the form of isolated populations may be considered as a weed (reviewed in (Eastick 2002). *G. hirsutum* is for example listed under the category ‘moderate to minor weed usually in small infestations’ in Kakadu National Park (Cowie & Werner 1987; Storrs 1996). However, these populations appear to be old style cultivars originating from the late 1800’s/early 1900’s (Curt Brubaker/DEH, 2006) and there is no evidence that these isolated cotton populations are invasive or have become problematic weeds.

339. As discussed in Chapter 1, small, persistent cotton populations have been observed, mainly in northern Australia. It has been noted by scientists over many years that the morphology of many of these naturalised cotton populations is distinct from that of the cultivated cotton varieties. When grown in a glasshouse, they tend to have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002).

340. Tufted seeded cotton plants were originally used when hand delinting was required, before the advent of mechanical saw gins in the late 1700’s. Tufted seeded cotton plants were subsequently replaced by fuzzy seeded varieties with better lint characteristics and disease resistance. It seems likely, therefore, that many naturalised cotton populations result from attempts in the early 1800’s to establish cotton industries in northern Queensland and the Northern Territory (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002).

341. A small number of other cotton plants appear to be of more recent origin, but none seem to have originated from the current commercial types of *G. hirsutum* that have been cultivated since the 1970’s (eg Eastick 2002). These naturalised cotton plants are confined to areas of disturbed land with at least a seasonal water supply: typical locations are above the high tide mark on beaches and near river banks in northern Australia.

342. Even though cotton has been grown previously in a number of places in northern Australia, only isolated cotton populations have been able to naturalise. For example, cotton has not persisted in the environment in the Ord River Irrigation Area following the abandonment of cotton farms, with actively growing cotton plants in the fields, in the 1960s and 70s (Eastick 2002). As discussed in Chapter 1, Section 5.3, previous attempts of commercial cotton cultivation in northern Australia ended in failure due to many reasons including wet season cultivation, poor choice of soils, unsustainable insect pest management practices and unsuitable cotton varieties (Australian Cotton Cooperative Research Centre 2004b; Lyn Craven pers. Comm., 2006).

343. Using the inferential modelling software package CLIMEX, a model has been developed to predict the areas that are climatically suitable for long-term survival of cotton (*Gossypium hirsutum*) in Australia (Rogers et al. 2006). Parameter values (relating to temperature, moisture, and cold, heat, dry and wet stress) used in this model were estimated from the literature on cotton physiology and growth, and adopted from known values of perennial *G. hirsutum* races native to Central America, the Caribbean and the US gulf coast. The final *G. hirsutum* model was substantiated by comparing the potential cotton distribution predicted by CLIMEX for West Africa with the known distribution of naturalised cotton populations in West Africa.

344. When this model is run for Australia, it indicates that dry stress is the major limiting factor for potential distribution of *G. hirsutum* in northern Australia. The model predicted that, in the absence of supplementary water, the coastal and sub-coastal areas of the east coast from Cape York to just south of the QLD/NSW border, but excluding the dry tropics, were the only climatically suitable areas for long term survival of cotton populations.

345. When overall soil fertility was considered in addition to climatic data, the area suitable for cotton was further restricted (ie even more closely limited to coastal areas). However, the majority of these most favourable areas for cotton either carry forests (with >50% canopy closure) or are already used for some form of managed agricultural system and it is therefore not expected that cotton plants would be able to establish in these areas. Weed competition and fire were also identified to further reduce the probability of permanent cotton populations establishing in the identified areas.

346. This modelling study also supports previous studies/reports which observed that survival of permanent *Gossypium hirsutum* populations in northern Australia seems to require access to supplementary water over the dry season.

347. An important indicator of potential weediness of a particular plant is its history of weediness in any part of the world and its taxonomic relationship to declared weeds (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). Cotton has been grown for centuries throughout the world without any reports that it is a serious weed. Likewise, cotton is not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2002; Groves et al. 2003). Worldwide, there are about 50 species of *Gossypium* (Craven et al. 1994; Fryxell 1992), none of which is listed as a serious weed anywhere in the world (Groves et al. 2003; Holm et al. 1979; Holm et al. 1997; Randall 2002).

348. The weed status of cotton has also been considered previously in many of the RARMPs produced during the assessment of a variety of GM cotton lines (eg DIRs 012/2002, 022/2002, 023/2002, 055/2004, 059/2005 and 062/2005). In addition to the information in the Biology and Ecology document (OGTR 2002), these RARMPs have considered new data that has been collected during previous releases of GM cotton lines in Australia.

349. Small quantities of *G. barbadense* (Pima cotton) are also commercially grown in Australia. Herbarium records for *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, mainly in Queensland (OGTR 2002). The presence of remnants of some of these populations has not been confirmed.

350. Currently, no non-GM or GM cotton is grown commercially in northern Australia. However, herbicide tolerant GM Liberty Link<sup>®</sup> Cotton was granted approval in August 2006 for commercial release in Australia and may therefore be planted in northern Australia in the near future.

## **2.2 Identified Risk 2: Tolerance to glyphosate due to expression of the *cp4 epsps* gene(s) in the GM cotton plants.**

351. The applicant is seeking approval to conduct plant breeding, agronomic trials and seed production and, if opportunities arise, commercial scale planting of the five GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures. This would include conventional breeding, sale of seed for planting, use in human food and stockfeed, sale of lint, export of seed and unrestricted transport. Therefore, GM cotton plants could potentially persist in the agricultural environment where originally grown, or GM cotton plants may establish and persist in the wider environment as a result of GM cotton seed dispersal via transport, stockfeeding, animals or flooding.

352. The expression of one copy of the *cp4 epsps* gene results in Roundup Ready<sup>®</sup> cotton being tolerant to glyphosate only up to the four-leaf stage of growth, whereas Roundup Ready Flex<sup>®</sup> cotton with two copies of the *cp4 epsps* gene is tolerant to glyphosate throughout the growing season.

353. The risk of weediness of the Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> GM cotton plants as a result of the expression of the *cp4 epsps* gene would depend on the weediness of non-GM cotton plants, the importance of the use of glyphosate in limiting the spread and persistence of cotton (consequence assessment), the scale of the release and the chance of progeny establishing as weeds (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release (all areas north of latitude 22°S).

354. Assessment of the risk of weediness of cotton plants in Australia resulting from expression of a single *cp4 epsps* herbicide tolerance gene is provided in the risk assessment documents for DIRs 023/2003 (Roundup Ready<sup>®</sup> and Roundup Ready<sup>®</sup>/INGARD<sup>®</sup>) and 012/2002 (Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup>), available at <<http://www.ogtr.gov.au>>. These risk assessments concluded that expression of a single *cp4 epsps* gene does not enhance the weediness potential of these GM cotton plants (in comparison to non-GM cotton plants) in the cotton growing regions of Australia south of latitude 22° S.

355. Assessment of the risk of weediness of cotton plants in Australia resulting from expression of two copies of the *cp4 epsps* herbicide tolerance gene is provided in the risk assessment document for DIR 059/2005 (Roundup Ready Flex<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>), available at <<http://www.ogtr.gov.au>>. This risk assessment concluded that expression of two copies of the *cp4 epsps* gene does not enhance the weediness potential of these GM cotton plants (in comparison to non-GM cotton plants) in areas south of latitude 22° S in Australia. The risk of weediness of the GM cotton plants as a result of the expression of two copies of the *cp4 epsps* gene was estimated as negligible.

### **2.2.1 Consequence assessment**

356. One or two copies of the *cp4 epsps* gene could confer a selective advantage in areas where glyphosate is used to control weeds. This could result in spread and persistence of the GM cotton lines in the environment.

357. As mentioned earlier (Section 2.1 of this Chapter), cotton is not a serious weed anywhere in Australia because it does not possess many of the characteristics associated with weediness and due to a number of abiotic and biotic factors which limit its spread and persistence. The use of glyphosate and whether it is significant in limiting spread and persistence of glyphosate tolerant GM cotton plants was previously considered in the RARMP prepared for DIR 059/2005 (commercial release of Roundup Ready Flex<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton south of latitude 22°S) and this is not expected to be different for areas north of latitude 22°S.

358. Glyphosate is the most widely used herbicide in Australia today, in both the agricultural and non-agricultural environment (Radcliffe 2002). It is approved for the control of a wide range of annual, perennial, tree, brush and woody weeds. There are currently 266 products registered that contain glyphosate as active constituent (APVMA Pubcris database available at <[http://www.apvma.gov.au/pubcris/subpage\\_pubcris.shtml](http://www.apvma.gov.au/pubcris/subpage_pubcris.shtml)>).

359. However, glyphosate is not generally used to control established cotton plants, whether GM or non-GM, because while the application of glyphosate beyond the seedling stage results in yield loss, including reduced boll formation, it does not kill the plants. (Roberts et al.

2002). Consequently, compared to non-GM cotton, there might be an increased number of Roundup Ready® or Roundup Ready Flex® plants surviving past the seedling stage only in areas where glyphosate is used.

360. In the presence of glyphosate, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects), that limit the spread and persistence of all cotton in northern Australia (discussed further in Section 2.2.2 of this Chapter).

361. Therefore, the consequence of tolerance to glyphosate due to expression of the *cp4 epsps* gene in the GM cotton plants proposed for release is assessed as **minor**.

### **2.2.2 Likelihood assessment**

362. As discussed in Section 2.2 of this Chapter, the applicant is seeking approval for a number of dealings, including potential commercial scale planting of the GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures, transportation of GM plant material and use of seed as stockfeed. Thus, GM cotton plants could potentially persist in the agricultural environment where grown and/or in the wider environment as a result of seed dispersal.

363. GM herbicide tolerant cotton plants are already being grown in the current cotton growing areas of NSW and southern QLD. The glyphosate tolerant Roundup Ready® GM cotton is widespread in the agricultural environment as a result of its commercial release (since 2000) in southern Australia and has not become a problematic weed. In the 2005-06 season, 327,000 ha of cotton was planted (38% in QLD and 62% in NSW) and 90% of this was GM cotton (Cotton Australia 2006). The GM cotton comprised 74% Roundup Ready® cotton, with stacked traits contributing to some of these percentages (B. Patterson, Monsanto Australia Limited, 2006). Roundup Ready Flex® cotton was approved for commercial release in southern Australia in February 2006 and is expected to replace Roundup Ready® cotton in the near future.

#### Agricultural environment

364. GM cotton seed will persist in the fields where the GM cotton lines are grown and harvested. Some dispersal of GM cotton seed may occur in areas where cotton seed is stored. Seed is stored on farms in various ways (eg in sheds) that maintain its quality and protect it from animals and weathering. Dispersal of seed during storage is expected to be restricted to areas immediately surrounding these areas.

365. Cotton volunteers are actively managed on-farm by mechanical methods involving mulching, root cutting and cultivation (using cultivators, graders, excavators or chippers), application of herbicides (if in the seedling stage) or burning (Australian Cotton Cooperative Research Centre 2002a; Charles et al. 2002; Roberts et al. 2002). Volunteer Roundup Ready® and Roundup Ready Flex® cotton plants could not be controlled by the application of glyphosate but could easily be controlled by other herbicides (if in the seedling stage) or these other methods.

366. In the on-farm environment, a range of herbicides may be used to control cotton volunteers (at the seedling stage) that emerge after harvest. Herbicides containing carfentrazone-ethyl or paraquat and diquat as active constituents are currently registered by the APVMA for control of volunteer cotton, including Roundup Ready® cotton volunteers (APVMA Pubcris database available at <[http://www.apvma.gov.au/pubcris/subpage\\_pubcris.shtml](http://www.apvma.gov.au/pubcris/subpage_pubcris.shtml)>).



367. Integrated weed management strategies stress the need to avoid relying on one control method (Roberts & Charles 2002). To avoid development of glyphosate resistant weeds for example, it is recommended that the application of glyphosate alone should not be used as the sole management strategy. Alternating various strategies would result in the destruction of glyphosate tolerant GM cotton volunteers. Consistent with this approach, the applicant has developed an integrated weed management strategy in the current Roundup Ready<sup>®</sup> and the proposed Roundup Ready Flex<sup>®</sup> cotton crop management plan that is being/would be implemented through a Technology User Agreement.

368. Therefore, the likelihood of Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton plants persisting in the agricultural environment is not expected to be greater than that of non-GM cotton.

#### Dispersal during transportation

369. Some GM cotton seed may be dispersed during transport of GM cotton seed for storage, planting, ginning, processing and stockfeed. The amount of cotton seed being transported and the distances transported would depend on the amount of the GM cotton grown each year and its end use. This can be highly variable. For example, the use of cotton seed as stockfeed increases significantly during drought.

370. As cotton does not compete well with other plants and has high water and nutrient requirements (see Section 2.1 of this Chapter), volunteer establishment is mainly expected in disturbed, favourable habitats such as ditches and roadside drains.

371. The type of seed dispersed has a large impact on the likelihood of germination (Eastick & Hearnden, in press). Black seed, which has been ginned and acid delinted and is used for planting, has the highest germination rate at >80%. This seed is unlikely to be accidentally dispersed as it is transported in smaller quantities and is of higher monetary value. Fuzzy seed, which has been ginned, is often transported and used for cattle feed. This germinates much less readily than the black seed. The seed cotton, directly harvested from the plant, has the highest potential for unintentional dispersal during transport but germinates relatively poorly.

372. A survey of the transport routes between Emerald (in the cotton growing region in central QLD) and the Atherton Tablelands (north of latitude 22°S in QLD), conducted in 2002, indicated that cotton plants had established in the roadside environment only infrequently, despite 12 years of use of these routes for transporting ginned seed (including GM cotton varieties since their respective commercial releases) for stockfeed (Farrell & Roberts 2002). Only four plants were observed in 1200 km of road surveyed north of latitude 22°S. Details of the study can be found in the risk assessment prepared for DIR 059/2005 (available at <<http://www.ogtr.gov.au>>). The study concluded that cotton volunteers tend to establish in highly and regularly disturbed environments and appear to have negligible ability to invade non-disturbed habitats (eg native bush). The following factors that limit survival of cotton volunteers in the roadside environment were identified: competition from already established vegetation, low quantity of seed escapes, high disturbance in areas requiring frequent maintenance and high rate of seed desiccation.

373. The above results are supported by the Eastick and Hearnden study (in press), where cotton seed germination was highest in disturbed habitats especially when the seed was buried rather than remaining exposed on the soil surface. Persistence of cotton plants for more than 1-2 years was only seen in habitats with increased water availability or nutrition such as cattle yards.

374. As part of the commercial release of Roundup Ready<sup>®</sup> and Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> cotton in 2000, Monsanto was required to conduct an environmental monitoring program. This three year program included monitoring for the incidence of volunteer GM cotton in non-agricultural situations (eg roadsides and non-crop areas). Details of the results were reviewed in the risk assessments prepared for DIR 023/2002 and DIR 059/2005 (available at <<http://www.ogtr.gov.au>>) and are only summarised here.

375. Roadside surveys conducted over three years in two traditional cotton growing regions south of latitude 22°S (Lower Namoi Valley and Darling Downs in NSW and QLD, respectively) showed that cotton was not a significant roadside weed in any of the regions surveyed. The number of volunteer cotton seedlings seems to be highly variable between seasons, indicating that it is most probably dependent on ideal germination conditions. The majority of cotton volunteers resulted from new germinations rather than survival of plants from the previous season. Survival of cotton volunteers seemed to be limited by plant competition, roadside slashing and predation.

376. Slashing appeared to be the common method of roadside weed control, and herbicide use tended to be limited to around fixtures (eg signs and guide posts) and drainage points where slashing is difficult. This suggests that glyphosate tolerance is not likely to provide a significant selective advantage.

377. Analysis of the correlation between the percentage of Roundup Ready<sup>®</sup> volunteers detected and the proportion of Roundup Ready<sup>®</sup> cotton grown per valley in the previous season also did not indicate a selective advantage of Roundup Ready<sup>®</sup> cotton compared to other cotton varieties.

378. Although some GM cotton plants may establish along transport routes, the expression of one or two copies of the *cp4 epsps* gene is not expected to alter susceptibility to the major abiotic and biotic factors that limit the establishment and persistence of cotton in these areas (eg reliable availability of water and nutrients, roadside slashing, plant competition, insect herbivory, soil type and fire).

#### Dispersal via use as stockfeed

379. In addition to seed dispersal during feeding, a small percentage of cotton seed consumed by stock can pass through the digestive system intact and is able to germinate (Eastick & Hearnden, in press). As a result, cotton volunteers could establish in areas where livestock is fed cotton seed (eg feedlots, cattle yards, paddocks or dairy farms), or grazes after being fed. Areas where stock is fed are nutrient rich, disturbed habitats and cotton volunteers are expected to establish. However, animal trampling and grazing are expected to reduce the chance of survival of volunteers in these areas.

380. The amount of cotton seed being used in stockfeed each year can be highly variable. For example, its use increases significantly during drought. However, the quantity of cotton seed used is generally limited to a relatively small proportion of the diet, and must be introduced gradually, to avoid potential toxic effects due to the presence of anti-nutrients (ie gossypol and cyclopropanoid fatty acids) that are normally present in cotton.

381. Roundup Ready<sup>®</sup> and Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> cotton have been in commercial cultivation since 2000 (DIR 023/2002), and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton since 2002 (DIR 012/2002). Since their commercial release, cotton seed from these GM cotton lines has been used as stockfeed in Australia, including in northern Australia. Over this period there has been no evidence that these GM cotton lines have become problematic weeds.

382. Farrell and Roberts (2002) found cotton volunteers at seven of nine dairy farms surveyed (Atherton Tablelands, March 2002), with GM cotton (Roundup Ready<sup>®</sup>, Roundup Ready<sup>®</sup>/INGARD<sup>®</sup> or INGARD<sup>®</sup> cotton) identified on four of these. Volunteers were all close to dairy infrastructure, suggesting that their ability to invade is negligible. Such volunteers generally do not complete an entire reproductive cycle to produce new seedlings, due to physical damage (eg trampling and grazing), disease and competition, and therefore do not spread into other areas of the farms or natural environment or lead to the development of self-sustaining populations. On farms where both GM and non-GM volunteers were found, there was no indication that the GM plants had enhanced survivorship or reproductive potential in any situation.

383. Eastick (2002) found that although cotton growing in cattle yards may reach reproductive maturity, persistence and seed dispersal from these areas is limited by trampling and grazing. No cotton volunteers were found in the undisturbed bush habitats surrounding these areas (Eastick 2002; Eastick & Hearnden, in press).

384. Results from a survey conducted over the 2002–03 cotton growing season (as part of research required under licences for DIR 022/2002 and DIR 023/2002) on the incidence of cotton volunteers in areas in northern Queensland where stock are fed cotton seed, or graze after being fed cotton seed, indicate that very little cotton seed is used as stockfeed. Where it has been used, the incidence of cotton volunteers was never observed to be problematic, and volunteer plants never reached flowering or maturity. Cotton seed had not been used for stockfeed in Northern Territory and northern Western Australia during the 2002-03 season and these areas were therefore not included in this survey.

385. Although some GM cotton plants may establish where stock is fed cotton seed or where stock grazes after being fed cotton seed, the expression of the *cp4 epsps* gene is not expected to alter susceptibility to the major abiotic and biotic factors that limit the establishment and persistence of cotton in these areas (eg trampling and grazing by cattle, reliable availability of water and plant competition). The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism.

#### Dispersal via animals

386. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants due to both the presence of toxic, anti-nutritional substances and the morphology of the plant (OGTR 2002), and therefore are unlikely to carry bolls any greater distance from the cotton fields. The cotton bolls are also unattractive to avian species, so birds are unlikely to transport seeds of the GM cotton (OGTR 2002).

#### Dispersal via flooding or other extreme environmental conditions

387. Some seed from the GM cotton plants may be dispersed from areas where the cotton is grown or harvested, or from areas used for stockfeed and storage of GM cotton seed, during flooding or other extreme environmental conditions such as cyclones. Seed may also be washed into drains, creeks, rivers and sinkholes close by. As a result, cotton volunteers may establish along waterways (eg drains, creeks and rivers) or in flood prone areas.

388. Given that flooding does sometimes occur in northern Australia, GM cotton seed may be dispersed by flooding. Much of this dispersed seed is not expected to survive as seeds of modern cotton varieties have been bred to be soft-seeded (Hopper & McDaniel 1999; Mauncy 1986) and the viability of cotton seed is affected by moisture (Hallowin 1975; Stephens 1958). Areas that get flooded regularly during the wet season may not be favourable for commercial

production, as cotton plants are poorly adapted to waterlogging (Hodgson & Chan 1982). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice may be implemented at some sites in northern Australia if GM cotton is commercially grown, which would reduce the dispersal of seed. At some potential cotton growing sites in northern Australia where tail water is not collected, experiments with growing Bollgard II<sup>®</sup> cotton have resulted in the development of efficient irrigation practices resulting in minimal run-off into river systems (Moulden et al. 2006), again suggesting that amount of seed dispersal would be reduced.

389. A number of glyphosate products are registered for use in aquatic areas such as drains, channels or the margins of dams (APVMA 2004; NRA 1996). However, as mentioned above, the use of glyphosate is only considered to be a limiting factor on the growth of cotton seedlings, not of established cotton plants. If glyphosate tolerant established cotton plants occurred near waterways, they can be effectively destroyed by mechanical means.

390. Although habitats close to waterways may be favourable for cotton establishment, tolerance to glyphosate is not expected to provide a significant selective advantage, compared to non-GM cotton, in these environments. Other environmental factors such as plant competition and herbivory by insects and other animals are expected to limit the establishment and persistence of cotton plants in these areas.

### Conclusions

391. The opportunity for any adverse outcome from dispersal of seed from the proposed release is diminished by the limited availability of suitable environmental conditions for germination of dispersed seed, and for survival and persistence of any resulting cotton plants.

392. Some GM cotton seeds may spread from the release sites, germinate and persist in the environment following the release. However, glyphosate is not used to control established cotton volunteers and other methods are available. The expression of the *cp4 epsps* gene is not expected to alter susceptibility to the abiotic and biotic factors that limit the spread and persistence of cotton in northern Australia, where reliable water and nutrient availability in particular are known to be major limitations (see Chapter 1, Section 5). The chance of volunteer Roundup Ready<sup>®</sup> or Roundup Ready Flex<sup>®</sup> plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism. Therefore, the likelihood of weediness as a result of Identified Risk 2 is assessed as **highly unlikely**.

### **2.3 Identified Risk 3: Reduced lepidopteran herbivory due to expression of the *cry1Ac* and *cry2Ab* genes in the GM cotton plants**

393. As discussed in Section 2.2 of this Chapter, the applicant is seeking approval for a number of dealings including potential commercial scale planting of the GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures. Thus, GM cotton plants could potentially persist in the agricultural environment where grown and/or in the wider environment as a result of seed dispersal.

394. The risk of weediness of the Bollgard II<sup>®</sup> GM cotton plants as a result of the expression of the *cry1Ac* and *cry2Ab* genes in combination would depend on the weediness of non-GM cotton plants, the importance of lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the scale of the release and the chance of progeny establishing as weeds (likelihood assessment). The risk is assessed against the baseline of the

low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release (all areas north of latitude 22°S).

395. A detailed assessment of the potential of the expression of the *cry1Ac* and *cry2Ab* genes to increase weediness is provided in the RARMP prepared for application DIR 012/2002 (commercial release of Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton south of latitude 22°S). This concluded that ‘the risk of Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton establishing as a weed in the southern cotton-growing regions of NSW and QLD is low, and not likely to be greater than that of non-GM cotton’. For areas north of latitude 22°S, the DIR 012/2002 RARMP concluded that ‘the risk of Bollgard II<sup>®</sup> or Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton establishing as a weed in the cotton-growing regions of northern WA and the NT is also likely to be low, however further information regarding potential weediness of Bollgard II<sup>®</sup> in northern Australia is required before this can be determined conclusively’. In the risk assessment prepared recently for application DIR 059/2006, the risk of Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton establishing as a weed in areas south of latitude 22°S was estimated as negligible.

396. The current application is for areas north of latitude 22°S and further studies since the initial assessment for the commercial release of Bollgard II<sup>®</sup> cotton lines under DIR 012/2002 are now available regarding the potential weediness of cotton, including GM insect resistant cotton, in these areas (Eastick 2002; Eastick & Hearnden, in press; Rogers et al. 2006).

### **2.3.1 Consequence assessment**

397. The *cry1Ac* and *cry2Ab* genes in combination could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in spread and persistence of the GM cotton in the environment.

398. As mentioned earlier (Section 2.1 of this Chapter), cotton is not a serious weed in northern Australia because it does not possess many of the characteristics associated with weediness and due to a number of abiotic and biotic factors which limit its spread and persistence. In northern Australia, reliable availability of water is a major limiting factor and thus, naturalised cotton populations mainly occur in areas with a supplementary fresh water supply (eg coastal habitats or on the banks of permanent water courses) (Eastick 2002; Hnatiuk 1990; OGTR 2002).

399. As discussed in Chapter 1, Section 5.3, previous attempts of commercial cotton cultivation in northern Australia ended in failure due to many reasons including wet season cultivation, poor choice of soils, unsustainable insect pest management practices and unsuitable cotton varieties (Australian Cotton Cooperative Research Centre 2004b; Lyn Craven pers. Comm., 2006).

400. A recent modelling study (Rogers et al. 2006) also concluded that dry stress is a major limiting factor for the long-term survival of cotton populations (*G. hirsutum*) in northern Australia (for details see Section 2.1 of this Chapter).

401. Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they do not seem to be a major limiting factor in naturalised cotton populations. The *cry* genes have been introduced into Bollgard II<sup>®</sup> cotton to protect the plants against damage of reproductive tissues (ie flower buds and bolls) by lepidopteran pests. Monitoring of seven naturalised cotton populations in the Northern Territory revealed abundant seed production, suggesting that these cotton plants were not significantly affected by lepidopteran pests (Eastick 2002). The major insect herbivores observed, particularly over the wet season, were grasshoppers. Grasshoppers are considered to be the most important

insect herbivores in tropical savanna ecosystems (OGTR 2002) and are unaffected by the Cry proteins present in Bollgard II<sup>®</sup> cotton.

402. When insects were sampled from three naturalised *G. hirsutum* populations in the Northern Territory, only 16% were from the order Lepidoptera of which none were confirmed to be a Noctuid, the insect family to which the target insects of Cry1Ac and Cry2Ab belong (Eastick 2002). The dominant insect order found was Hemiptera (28% of total insects) suggesting that sucking insects possibly influenced naturalised cotton populations more than lepidopteran insects. A number of non-lepidopteran pests, including sucking insects (which are not affected by the Cry proteins), also attack cultivated cotton and require pest management via insecticides (Farrell & Johnson 2005). As the lepidopteran pest *S. litura* is only moderately susceptible to Bt toxins, insecticides may also be needed to control heavy infestations of this species in Bollgard II<sup>®</sup> cotton varieties.

403. A naturalised cotton population at Beatrice Hill (near Adelaide River, NT) was used to conduct an insect enclosure study. Entire seedlings or individual fruiting branches of larger plants were enclosed in netted cages to protect them from insects. There was no difference between seedling survivorship and fruit production between caged and non-caged plants during the dry season. However, during the wet season, uncaged plants were attacked by grasshoppers (order: Orthoptera). Similarly, monitoring of Bt cotton volunteers in Kununurra (WA) showed considerable damage by leaf-eating insects during the wet season (Eastick 2002).

404. Lepidopteran insect pressure in cultivated cotton is highly variable across different regions, seasons or throughout an individual season. However, as discussed in Chapter 1, Section 5.3, previous attempts of commercial cotton cultivation in northern Australia ended in failure due to many reasons including wet season cultivation, poor choice of soils, unsustainable insect pest management practices and unsuitable cotton varieties (Australian Cotton Cooperative Research Centre 2004b; Lyn Craven pers. Comm., 2006).

#### Study on the potential weediness of GM insect resistant cotton in northern Australia

405. Results from a study on the potential weediness of GM insect resistant cotton in northern Australia conducted over four years indicate that lepidopteran insect pressure is not the critical factor limiting establishment and growth of cotton populations and that expression of the *cry* genes does not confer increased fitness (Eastick 2002; Eastick & Hearnden, in press). Results from the first two years of this study are also described in detail in the RARMP prepared for DIR 012/2002, available at <<http://www.ogtr.gov.au>>.

406. Seed from non-GM cotton and from GM cotton containing one (*cry1Ac*) or two (*cry1Ac* and *cry2Aa*, which is very similar to *cry2Ab*) *cry* genes were planted at 13 sites in four different habitat types (bush, roadside, cattle feedlot, waterway) where cotton seed could potentially disperse. The sites were in the Katherine (NT), Kununurra (WA) or Broome (WA) areas. For most of the 13 sites, over 3000 seeds were planted at each individual site. To achieve a 'worst case scenario', areas were cleared (removal of interspecific plant competition) before planting seed (hand-placed and then covered with soil) and plots were hand-watered for three weeks after sowing to maximize germination. All sites, excluding cattle feedlots, were fenced to exclude grazing cattle.

407. Germination, survival, fecundity and invasiveness of non-GM cotton and the two GM cotton lines were compared. Genotype (non-GM, or GM containing one or two *cry* genes) only had an effect on germination at two out of the 13 sites. At one of the waterway sites, germination of the non-GM fuzzy seed was lower than that of the GM cotton lines, although

there was no difference for the other two seed types (black seed and seed cotton). At another waterway site germination of non-GM seed was lower than that of GM seed.

408. There was no effect of genotype on cotton survival for all sites after the first year. After the second year, survival at the majority of sites was so low that the effect of genotype on survival could not be assessed. However, the low survival rates indicate that the *cry* gene/s did not provide a significant selective advantage. No effect of genotype was detected for the two sites (irrigation channel and cattle feedlot) where sufficient plants survived to assess an effect.

409. For the three sites (two irrigation channel sites and one cattle feedlot site) where a successive generation of cotton plants had established, data collection was continued for a further two years. Higher survival rates at these sites were consistent with higher nutrient and/or water availability (particularly during the dry season). However, survival continued to decline over the two years and at the end of the two years, plant numbers were less than 1% of plants remaining from sown seed.

410. Over the first two years, cotton plants at eight of the thirteen sites did not produce any fruiting structures, while plants at two sites produced a small number of open bolls (<15) and plants at only three sites (with adequate water and nutrient availability) produced relatively large numbers of open bolls (>150). After a further two years, the number of open bolls produced at these three sites had significantly declined and there was no consistent indication that the GM cotton plants produced significantly more fruit than the non-GM cotton (Eastick & Hearnden, in press).

411. Invasiveness values (ie rate of increase of a population) were calculated as the proportion of plants surviving after a given time, plus the addition of any new/recruited seedlings, compared to the number of plants present initially. The calculation of invasiveness incorporates the demographic information gathered throughout the life cycle of the plants (eg germination, survivorship and fecundity). Invasiveness values greater than one imply that the population is growing under the given set of environmental conditions. Invasiveness values less than one imply that the population is in decline.

412. The invasiveness values of the GM plants were not significantly greater than those of the non-GM plants over the two or four years studied, indicating that expression of the insecticidal genes did not increase fitness to lead to invasive populations. When invasiveness for the two sites conducive to cotton survival was calculated between survival after the first year and the final measurement (approximately three years later), there was no effect of genotype. Mean values for all genotypes were less than 0.12. These results demonstrate that, although some habitats such as irrigation channels and cattle yards are more suitable for establishment of cotton plants, cotton populations, whether GM or non-GM, will not be invasive.

413. Factors that influenced cotton plant survival during this four year study were: nutrient and water availability, plant competition, herbivory by non-lepidopteran insects, grazing and trampling by cattle, and fire.

414. Although northern QLD was not included in this weediness study, GM cotton expressing the *cry1Ac* and *cry2Ab* genes is not expected to have an increased selective advantage in this region compared to NT and northern WA. The climatic conditions and soil types in most of northern QLD, including the areas identified as potential cotton growing areas (Australian Cotton Cooperative Research Centre 2004b), are similar to those at one or more of the places (Katherine, Kununurra and Broome) where the studies on potential weediness of insecticidal GM cotton described above were conducted (Rogers et al. 2006). Nutrient and water availability, plant competition, herbivory by non-lepidopteran insects,

grazing and trampling by cattle, and fire are also considered to be limiting factors on cotton plant survival in northern QLD.

### Conclusion

415. Bollgard II<sup>®</sup> cotton plants, if they were to establish in the natural environment, are unlikely to have a significant selective advantage over non-GM cotton plants. A range of other biotic and abiotic factors seem to be far more important in limiting the spread and persistence of cotton.

416. Therefore, the consequences of the expression of the *cry1Ac* and *cry2Ab* genes increasing the spread and persistence of the GM cotton plants proposed for release through reduced lepidopteran herbivory are assessed as **minor**.

### **2.3.2 Likelihood assessment**

417. As discussed in Section 2.2 of this Chapter, the applicant is seeking approval for a number of dealings, including potential commercial scale planting of the GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures, transportation of GM plant material and use of seed as stockfeed. Thus, GM cotton plants could potentially persist in the agricultural environment where grown and/or in the wider environment as a result of seed dispersal.

418. Bollgard II<sup>®</sup> cotton is already being widely grown in the current cotton growing areas of NSW and southern and central QLD (south of latitude 22°S) as a result of its commercial release in southern Australia (since 2002) and has not become a problematic weed.

### Agricultural environment

419. As discussed in Section 2.2.2 in this Chapter, GM cotton seed is likely to persist in the fields where the GM cotton lines are grown and harvested, and near seed storage areas. Volunteer Bollgard II<sup>®</sup> cotton can be controlled in the same way as non-GM cotton ie by mechanical means (or if in the seedling stage) by the use of herbicides. Therefore, the likelihood of Bollgard II<sup>®</sup> cotton plants persisting in the agricultural environment is not expected to be greater than that of non-GM cotton.

### Dispersal during transportation

420. Some Bollgard II<sup>®</sup> cotton seed is likely to be dispersed during transport and therefore GM cotton volunteers may establish on roadsides. As cotton does not compete well with other plants and has high water and nutrient requirements (see Section 2.1 of this Chapter), volunteer establishment is mainly expected in disturbed, favourable habitats such as ditches and roadside drains.

421. As discussed for Identified Risk 2, a roadside survey in central and northern QLD found infrequent cotton volunteers which were generally found in highly and regularly disturbed environments (Farrell & Roberts 2002). Factors that limit survival of cotton volunteers in the roadside environment were competition from established vegetation, low quantity of seed escapes, high disturbance in areas requiring frequent maintenance and high rate of seed desiccation (Eastick 2002).

422. Although some GM cotton plants may establish along transport routes, the expression of the *cry1Ac* and *cry2Ab* genes is not expected to alter susceptibility to the major abiotic and biotic factors that limit the establishment and persistence of cotton in these areas (eg reliable availability of water and nutrients, roadside slashing, plant competition, soil type and fire).



### Dispersal via use as stockfeed

423. As mentioned previously, cotton volunteers could establish in areas where livestock is fed cotton seed or graze after being fed. However, animal trampling and grazing are expected to reduce the chance of survival of volunteers in these areas (Section 2.2.2 in this Chapter).

424. The amount of cotton seed being used in stockfeed each year can be highly variable. INGARD<sup>®</sup> cotton (containing the *cry1Ac* gene) has been in commercial cultivation since 1996 (DIR 022/2002), and Bollgard II<sup>®</sup> and Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton since 2002 (DIR 012/2002). Since their commercial release, cotton seed from these GM cotton lines has been used as stockfeed in Australia, including in northern Australia. Over this period there has been no evidence that these GM cotton lines have become problematic weeds.

425. As discussed for Identified Risk 2, surveys of diary farms and other areas where stock were fed cotton seed (both GM and non-GM) revealed limited capacity for cotton to invade as the cotton volunteers generally do not reach maturity due to trampling, disease and competition (Eastick 2002; Farrell & Roberts 2002).

426. Although some GM cotton plants may establish where stock is fed cotton seed or where stock grazes after being fed cotton seed, the expression of the *cry1Ac* and *cry2Ab* genes is not expected to alter susceptibility to the major abiotic and biotic factors that limit the establishment and persistence of cotton in these areas (eg trampling and grazing by cattle, reliable availability of water and plant competition). The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism.

### Dispersal via animals

427. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants and therefore are unlikely to carry bolls any greater distance from the cotton fields. The cotton bolls are also unattractive to avian species, so birds are unlikely to transport seeds of the GM cotton (OGTR 2002).

### Dispersal via flooding or other extreme environmental conditions

428. Some seed from the GM cotton plants may be dispersed via flooding or other extreme environmental conditions and as a result, cotton volunteers may establish along waterways (eg drains, creeks and rivers) or in flood prone areas (see Section 2.3.1 of this Chapter). However, seed dispersed by flooding will have reduced viability (Halloin 1975) and flooded areas are not favourable as cotton plants are poorly adapted to waterlogging (Hodgson & Chan 1982).

429. Although habitats close to waterways may be favourable for cotton establishment, reduced lepidopteran herbivory is not expected to provide a significant selective advantage, compared to non-GM cotton, in these environments. Other environmental factors such as plant competition and herbivory by non-lepidopteran insects and animals are expected to limit the establishment and persistence of cotton plants in these areas (see Section 2.3.1 of this Chapter).

### Conclusions

430. Some GM cotton seed may spread from the agricultural environment, germinate and persist in the wider environment. As cotton does not compete well with other plants and has high water and nutrient requirements, volunteer establishment is mainly expected in disturbed, favourable habitats. Bollgard II<sup>®</sup> cotton volunteers can be effectively controlled by mechanical means or, if still in the seedling stage, by the use of herbicides. Although

lepidopteran insects are the main insect pests of cultivated cotton, herbivory by other non-lepidopteran insects (eg grasshoppers and sucking insects which are not affected by the Cry1Ac and Cry2Ab proteins) is more significant in naturalised cotton populations. Hence, the expression of the insecticidal genes is not expected to alter susceptibility to the main factors that are known to limit the spread and persistence of cotton in northern Australia (eg reliable water and nutrient availability, see Section 2.1 of this Chapter). The chance of volunteer Bollgard II® plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism. Therefore, the likelihood of weediness as a result of Identified Risk 3 is assessed as **highly unlikely**.

#### **2.4 Identified Risk 4: Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination in the GM cotton plants**

431. The risk of weediness of the Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton plants as a result of the expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination would depend on the weediness of non-GM cotton plants, the importance of the use of glyphosate and lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the scale of the release and the chance of progeny establishing as weeds (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release (all areas north of latitude 22°S).

432. Discussions of the risk from expression of the CP4 EPSPS herbicide tolerance protein in combination with the two insect resistance proteins (Cry1Ac and Cry2Ab) increasing the weediness of cotton plants in Australia is provided in the risk assessment document for DIR 012/2002 (Roundup Ready®/Bollgard II®) available at <<http://www.ogtr.gov.au>>. This risk assessment concluded that expression of the proteins in combination does not enhance the weediness potential of these GM cotton plants (in comparison to non-GM cotton plants) in the cotton growing regions of Australia south of latitude 22° S. In the risk assessment prepared recently for application DIR 059/2006, the risk of Roundup Ready Flex®/Bollgard II® cotton establishing as a weed in areas south of latitude 22°S was estimated as negligible.

433. The expression of one copy of the *cp4 epsps* gene results in Roundup Ready®/Bollgard II® cotton being tolerant to glyphosate only up to the four-leaf stage of growth, whereas Roundup Ready Flex®/Bollgard II® cotton with two copies of the *cp4 epsps* gene is tolerant to glyphosate throughout the growing season.

##### **2.4.1 Consequence assessment**

434. The *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination could confer a selective advantage in areas where glyphosate is used to control weeds and lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in spread and persistence of the GM cotton lines (Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II®) in the environment.

435. However, cotton is not a serious weed in Australia and the limited effectiveness of glyphosate in controlling cotton beyond the seedling stage is discussed in Section 2.1 of this Chapter. Additionally, lepidopteran herbivory is not an important limiting factor for the spread and persistence of cotton plants/populations (see Section 2.3.1 of this Chapter for more detail). Therefore in the presence of both glyphosate and lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic

factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects), that limit the spread and persistence of all cotton in northern Australia.

436. The herbicide tolerance and insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the *cry* genes and the *cp4 epsps* gene, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur. There is no significant difference in the herbicide tolerance and insect resistance traits between GM cotton plants containing only one trait (Roundup Ready<sup>®</sup>, Roundup Ready Flex<sup>®</sup> and Bollgard II<sup>®</sup>) and GM plants containing both traits (Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup>) (refer RARMPs prepared for DIRs 023/2002 and 059/2005).

437. Therefore, the consequences of tolerance to glyphosate and reduced lepidopteran herbivory due to the expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination in the GM cotton plants proposed for release are assessed as **minor**.

#### **2.4.2 Likelihood assessment**

438. As discussed in Section 2.2 of this Chapter, the applicant is seeking approval for a number of dealings, including potential commercial scale planting of the GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures, transportation of GM plant materials and use of seed as stockfeed. Thus, GM cotton plants could potentially persist in the agricultural environment where grown and/or in the wider environment as a result of seed dispersal

439. Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> cotton is already being widely grown in the current cotton growing areas of NSW and southern and central QLD (south of latitude 22°S) as a result of its commercial release in southern Australia (since 2002) and has not become a problematic weed.

#### Agricultural environment

440. On-farm, cotton volunteers are actively managed by the application of herbicides (if in the seedling stage) or by mechanical methods (Roberts et al. 2002). Volunteer Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton plants could not be controlled by the application of glyphosate but could easily be controlled by other herbicides (if in the seedling stage) or by mechanical methods. Therefore, the likelihood of Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton plants persisting in the agricultural environment is not expected to be greater than that of non-GM cotton.

#### Dispersal of GM cotton seed

441. GM cotton seed may be dispersed via methods such as seed transportation, during use as stockfeed, animals, flooding, or other extreme environmental conditions. The likelihood of weediness as a result of dispersal of Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup>, and Bollgard II<sup>®</sup> seed via the above methods is discussed for Identified Risks 2 and 3, respectively. The combination of the herbicide tolerance and insect resistance trait in Roundup Ready<sup>®</sup>/Bollgard II<sup>®</sup> and Roundup Ready Flex<sup>®</sup>/Bollgard II<sup>®</sup> cotton plants is not expected to increase the likelihood compared to that of the GM cotton lines containing the single traits.

442. Although some GM cotton plants may establish due to seed dispersal, the expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination is not expected to alter susceptibility of GM cotton to the major abiotic and biotic factors that limit the establishment and persistence of non-GM cotton (see Section 2.1 of this Chapter). The chance of volunteer GM

plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism.

### Conclusions

443. As cotton does not compete well with other plants and has high water and nutrient requirements, volunteer establishment is mainly expected in disturbed, favourable habitats. GM cotton volunteers can be effectively controlled by mechanical means, if still at the seedling stage, by the use of alternative herbicides. The herbicide tolerance and insecticidal genes operate through independent unrelated biochemical mechanisms and the expression of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination is not expected to alter susceptibility to the main factors that are known to limit the spread and persistence of cotton in northern Australia (eg reliable water and nutrient availability, see Section 2.1 of this Chapter). The chance of volunteer Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® plants establishing as weeds by finding suitable ecological niches would be no greater than for the non-GM parent organism. Therefore, the likelihood of weediness as a result of Identified Risk 4 is assessed as **highly unlikely**.

## **2.5 Identified Risk 5: Tolerance to glyphosate and/or reduced lepidopteran herbivory in naturalised *G. hirsutum* or *G. barbadense* cotton plants due to expression of *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes**

444. The risk of weediness as a result of transfer of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes to naturalised *G. hirsutum* or *G. barbadense* plants would depend on the importance of the use of glyphosate and/or lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release (all areas north of latitude 22°S).

445. It should be noted that Bollgard II® was generated from two genetic modification events and, as expected, the *cry1Ac* and *cry2Ab* genes have been inserted into different regions of the plant genome and therefore segregate independently of one another. This means, after any initial outcrossing of Bollgard II® to non-GM cotton, any subsequent generations of cotton volunteers may contain either both *cry* genes, one *cry* gene or no *cry* genes. However, this does not impact on the assessment for weediness as a result of gene transfer of the introduced *cry* genes to non-GM cottons because any GM cotton lines produced from outcrossing containing either one *cry* gene or no *cry* genes will have equivalent or less insecticidal efficacy than a GM cotton volunteer with both *cry* genes. For example, INGARD® cotton, containing only the *cry1Ac* gene, has reduced insecticidal efficacy at later stages of plant growth. Therefore, segregation of the *cry* genes will not be considered further.

### **2.5.1 Consequence assessment**

#### Transfer of *cp4 epsps* gene(s)

446. Transfer of one or two copies of the introduced *cp4 epsps* gene to naturalised *G. hirsutum* and *G. barbadense* plants could result in the expression of the CP4 EPSPS herbicide tolerance protein in these plants. As discussed in Section 2.2.1 in this Chapter, the *cp4 epsps* gene could confer some selective advantage in areas where glyphosate is used to control weeds. This could result in spread and persistence of these cotton plants in the environment.

447. As discussed in Section 2.1 of this Chapter, cotton is not a serious weed in Australia. As an alien/non-native species, isolated naturalised cotton populations may be considered a weed in conservation areas such as National Parks. However, there is no evidence that current isolated cotton populations in northern Australia are invasive or have become problematic weeds.

448. The use of glyphosate is only considered to be a limiting factor on the growth of cotton seedlings, but not of established cotton plants. (see Section 2.2.1 in this Chapter)

#### Transfer of the *cry1Ac* and *cry2Ab* genes

449. Transfer of the *cry1Ac* and *cry2Ab* genes to naturalised *G. hirsutum* and *G. barbadense* plants could result in the expression of the Cry1Ac and Cry2Ab proteins in these plants. As discussed for Identified Risk 3, the *cry1Ac* and *cry2Ab* genes could confer some selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in spread and persistence of these cotton plants in the environment.

450. The consequence of transfer of the *cry1Ac* and *cry2Ab* genes to naturalised *G. hirsutum* and *G. barbadense* plants would be no different to the consequence of GM cotton containing these same genes, that is, whether these genes would confer a selective advantage. However, lepidopteran herbivory is not an important limiting factor for the spread and persistence of cotton plants (see Section 2.3.1, this Chapter). In the presence of lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects), that limit the spread and persistence of all cotton in northern Australia.

451. Although data on the lepidopteran insect pressure on naturalised cotton plants in northern Queensland are not available, lepidopteran insect pressure on cotton in northern QLD is not expected to be significantly higher than in NT and northern WA (refer to Identified Risk 3 for further detail).

452. Therefore, similar to GM cotton, naturalised cotton plants expressing the *cry1Ac* and *cry2Ab* genes are unlikely to have a significant selective advantage over non-GM plants. A range of other biotic and abiotic factors seem to be far more important in limiting the spread and persistence of cotton.

#### Transfer of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes

453. Transfer of the introduced *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination to naturalised *G. hirsutum* and *G. barbadense* plants could result in the expression of the CP4 EPSPS, Cry1Ac and Cry2Ab proteins in these plants. As discussed for Identified Risk 4, the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination could confer some selective advantage which could result in spread and persistence of these cotton plants in environments where the use of glyphosate and lepidopteran herbivory are the major constraints on cotton survival.

454. The consequence of the transfer of these genes in combination to naturalised *G. hirsutum* and *G. barbadense* plants would be no different to the consequence of GM cotton containing these same genes, that is, whether these genes would confer a selective advantage. However, lepidopteran herbivory is not an important limiting factor for the spread and persistence of cotton plants and the effectiveness of glyphosate in controlling cotton plants is limited to the seedling stage (see Sections 2.3.1 and 2.2.1 of this Chapter, respectively).

Furthermore, there is no evidence of interaction between the herbicide tolerance and insecticidal genes.

455. Therefore, naturalised *G. hirsutum* and *G. barbadense* plants expressing the introduced genes are unlikely to have a selective advantage over non-GM cotton.

### Conclusion

456. There are a number of abiotic and biotic factors that limit the spread and persistence of cotton plants and cotton does not possess certain innate characteristics typically associated with problematic weeds (see Section 2.1 of this Chapter). The presence of the *cp4 epsps*, and/or *cry1Ac* and *cry2Ab* genes in naturalised cotton is not expected to alter the susceptibility of cotton to these environmental factors. Therefore, the consequences of tolerance to glyphosate and/or reduced lepidopteran herbivory in naturalised *G. hirsutum* or *G. barbadense* cotton plants due to expression of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes are assessed as **minor**.

### **2.5.2 Likelihood assessment**

457. The adverse outcome of weediness resulting from an increase in the spread and persistence of naturalised cotton plants is contingent on both of the following steps:

- transfer of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes to naturalised cotton plants via outcrossing
- weediness of the recipient plants as a result of expression of the introduced gene(s).

458. There is limited data on the existence and proximity of naturalised cotton populations to potential commercial cotton growing regions in northern Australia. This information would be useful for determining the opportunity for, and possible frequency of, gene transfer between GM cotton crops and these populations. If GM cotton volunteers establish in areas adjacent to existing naturalised populations, eg along certain transport routes, the chance of transfer of the introduced genes to these naturalised populations would increase. The different ways of potential GM seed dispersal and the chance of GM cotton volunteers establishing and surviving are discussed for Identified Risks 2, 3 and 4.

459. However, cotton is primarily self-pollinating with pollen that is large, sticky and heavy and not easily dispersed by wind (Jenkins 1992; OGTR 2002). Overseas studies have shown that insect pollinators can transfer pollen to other nearby cotton plants at rates up to 80% (eg Oosterhuis & Jernstedt 1999). However, cotton pollen dispersal studies conducted in Australia consistently show that outcrossing is localised around the pollen source and decreases significantly with distance (OGTR 2002 and references therein). For example, levels of outcrossing between cotton plants in adjacent rows is in the order of 1-2% (Llewellyn & Fitt 1996; Mungomery & Glassop 1969; Thomson 1966). Therefore, gene transfer from the GM cotton to other cotton plants is only expected to occur in close proximity and at low frequencies.

460. In Australia, honeybees and native bees are the most likely insects responsible for any cross-pollination in cotton (OGTR 2002). A study on the fate of pollen on *H. armigera* as a possible vector for long distance pollen transport showed the quality and quantity of cotton pollen decreased rapidly in contact with *H. armigera* proboscis and therefore this is unlikely to promote wide pollen dispersal (Richards et al. 2005). For vertical gene transfer to occur, the GM cotton lines (either planted or volunteers) would need to be within pollination distance of naturalised *G. hirsutum* or *G. barbadense* plants. The plants would also need to be flowering simultaneously with the GM cotton.

461. The requirement of multiple applications of insecticides would further limit the amount of insect pollination in cultivated Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton plants. However, Bollgard II<sup>®</sup> cotton varieties require less insecticidal sprays and therefore insect pollinators may be more abundant, which may increase the incidence of gene flow.

462. *G. barbadense* is the closest relative of *G. hirsutum* occurring in Australia (OGTR 2002). It is commercially grown on a small scale in Australia. Hybridisation can occur naturally between these two species (Brubaker et al. 1999). Hybrid progeny exhibit characteristics intermediate to the parents but typically with a lower capacity to produce cotton bolls. *G. barbadense* and hybrids are not weedier or more difficult to control than *G. hirsutum* (Warwick Stiller & Greg Constable, CSIRO, pers. comm., 2002).

463. Cotton can persist as a perennial plant in tropical areas and small, naturalised cotton (*G. hirsutum* and *G. barbadense*) populations exist in northern Australia, particularly in areas associated with a prolonged supply of fresh water (Hnatiuk 1990). The majority of naturalised *G. hirsutum* populations occur in the Northern Territory, while naturalised *G. barbadense* occurs mainly along the eastern regions of Queensland (data from Australian Virtual Herbarium). Both *Gossypium* species are commonly found in littoral and watercourse habitats (Eastick 2002). The herbarium records for *G. hirsutum* and *G. barbadense* may not indicate current naturalised populations of these plants.

464. Some naturalised cotton populations may be better adapted to environmental stresses than cultivated modern cultivars. However, the expression of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes is not expected to alter susceptibility to the abiotic and biotic factors that limit the spread and persistence of naturalised cotton populations in northern Australia, where reliable water availability in particular is known to be a major limitation. Other factors include nutrient availability, plant competition, herbivory by non-lepidopteran insects, animal grazing and fire (see Chapter 1). The chance of naturalised cotton plants that express *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes establishing as weeds by finding suitable ecological niches would be no greater than for naturalised non-GM cotton.

465. Following transfer of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes to naturalised cotton plants, the likelihood of it causing weediness in these plants is expected to be the same as for the GM cotton plants (see Identified Risks 2, 3 and 4). As naturalised *G. hirsutum* and *G. barbadense* species are commonly found in littoral and watercourse habitats (Eastick 2002), glyphosate is not expected to be widely used in these areas, offering no selective advantage. GM cotton volunteers can be effectively controlled by mechanical means, or if still at the seedling stage, by the use of alternative herbicides.

466. Therefore, the likelihood of weediness as a result of Identified Risk 5 is assessed as **highly unlikely**.

## **2.6 Identified Risk 6: Tolerance to glyphosate and/or reduced lepidopteran herbivory in combination with tolerance to glufosinate ammonium (from Liberty Link<sup>®</sup> Cotton)**

467. The risk of weediness as a result of transfer of the introduced genes to the commercially approved Liberty Link<sup>®</sup> Cotton (DIR 062/2005), which is tolerant to the herbicide glufosinate ammonium, would depend on the importance of the use of the relevant herbicides and lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The level of risk is assessed against the baseline of the low weediness potential of the non-GM parent organism and in the context of the large scale of the proposed release, the distribution of other commercially approved

GM cotton plants growing in the vicinity of the crops and the conditions necessary for cross-pollination.

468. Liberty Link<sup>®</sup> Cotton is the only GM cotton currently approved for commercial release Australia-wide, including north of latitude 22°S. Liberty Link<sup>®</sup> Cotton is tolerant to herbicides containing glufosinate ammonium due to the introduction of the *bar* gene. It should be noted that approval was only granted in August 2006 and no commercial plantings have occurred anywhere in Australia as yet. The cultivation of Liberty Link<sup>®</sup> Cotton in rotation with the Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton lines (including stacks) may offer an additional option in the implementation of integrated weed management and herbicide resistance management strategies.

469. Segregation of the *cry* genes was discussed in Section 2.5 of this Chapter, and was not considered a risk so it will not be considered for this Identified Risk.

### 2.6.1 Consequence assessment

470. Transfer of the introduced *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination to Liberty Link<sup>®</sup> Cotton plants, which is tolerant to the herbicide ammonium glufosinate, could result in the expression of the PAT, CP4 EPSPS, Cry1Ac and Cry2Ab proteins in the same cotton plant. The *bar*, *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination could confer some selective advantage which could result in spread and persistence of these cotton plants in environments where the use of glyphosate and/or glufosinate ammonium and lepidopteran herbivory are the major constraints on cotton survival.

471. The stacking of the *bar* gene in combination with the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes was considered in the risk assessment for DIR 062/2005. The risk assessment estimated that the level of risk to health and safety of people or the environment for this event was negligible in areas south of latitude 22°S. The basis for this negligible risk estimate were: (1) the limited effectiveness of both glyphosate and/or glufosinate ammonium in controlling established plants, and (2) evidence that lepidopteran herbivory is not an important limiting factor for the spread and persistence of cotton plants/populations (see also Sections 2.3.1 and 2.2.1 of this Chapter). There is no evidence to suggest that stacking of the *bar* gene in combination with the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes would be different in northern areas of Australia compared to southern areas.

472. Additionally, the herbicide tolerance and insecticidal genes operate through independent, unrelated biochemical mechanisms. There is no evidence to suggest that the *cry* genes or the *cp4 epsps* genes will interact with the *bar* gene, the PAT protein or any metabolic pathways, resulting in unintended effects and no reason to expect that this is likely to occur. Hence, cotton volunteers containing any or all of the introduced genes in combination with the *bar* gene (from commercially approved GM Liberty Link<sup>®</sup> Cotton) are expected to be able to be controlled by other herbicides or by cultivation, similar to the parental GM cotton lines.

473. Therefore, the consequences of the expression of the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene increasing the spread and persistence of the GM cotton plants proposed for release through tolerance to glyphosate and/or reduced lepidopteran herbivory as well as glufosinate ammonium are assessed as **minor**.

### 2.6.2 Likelihood assessment

474. The adverse outcome of weediness resulting from an increase in the spread and persistence of stacked cotton plants is contingent on both of the following steps:

- transfer of the *cp4 epsps* and/or the *cry1Ac* and *cry2Ab* genes to Liberty Link<sup>®</sup> Cotton plants via outcrossing



- weediness of the recipient plants as a result of expression of the introduced gene(s).

475. As discussed earlier, the applicant is seeking approval to conduct plant breeding, agronomic trials and seed production and, if opportunities arise, commercial scale planting of the GM cotton lines in any area north of latitude 22°S where environmental conditions are suitable without specific containment measures. However, plantings would be limited to areas that are suitable for cotton cultivation.

476. Extensive cultivation of the GM cotton lines would increase the occurrence of gene transfer events. Some GM cotton seeds may also spread from the release sites, germinate and persist in the environment following the release. However, cotton is primarily self-pollinating and the main mechanism for gene transfer, via insect mediated pollen flow, would only be expected to occur in close proximity and at low frequencies. Furthermore, wide spread commercial planting of GM cotton in northern areas of Australia may be limited by the current lack of infrastructure that will restrict potential planting of the GM cotton lines proposed for release. Additionally, plantings of Liberty Link<sup>®</sup>, Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton in northern Australia will be limited because they are not insect resistant, and the major insect pests are highly likely to impact on cotton productivity if insecticides are not constantly applied throughout the growing season. These factors would further decrease the likelihood of gene transfer, due to both the limited number of Liberty Link<sup>®</sup> plants present in the environment and the requirement to apply insecticides which reduces the amount of insect mediated pollen flow. Therefore, the likelihood of gene transfer between the GM cotton lines proposed for release and Liberty Link<sup>®</sup> Cotton may be limited in northern areas of Australia.

477. As mentioned earlier, cotton is not a serious weed in Australia because of a number of abiotic and biotic factors and cotton does not possess certain innate characteristics typically associated with problematic weeds (see Section 2.1 of this Chapter). As discussed for Identified Risks 2, 3 and 4, the likelihood of the GM cotton lines proposed for release becoming weedy is estimated to be negligible. The risk assessment prepared for DIR 062/2005 concluded that the risk of Liberty Link<sup>®</sup> Cotton plants becoming weedy in Australia (including northern areas) is negligible. The expression of the *cp4 epsps* and/or *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene is not expected to alter susceptibility to the environmental conditions that limit the spread and persistence of cotton in northern Australia.

478. Cotton volunteers with glufosinate ammonium tolerance in combination with glyphosate tolerance and/or insect resistance would be effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.

479. The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches would be no greater than for GM cotton lines proposed for release, Liberty Link<sup>®</sup> Cotton or non-GM cotton. Therefore, the likelihood of weediness as a result of Identified Risk 6 is assessed as **highly unlikely**.

### **Section 3 Risk estimates**

480. Risk estimates (which can range from negligible to high) are based on a combination of the consequences and likelihood assessments, using the Risk Estimate Matrix (see Chapter 2).

481. The risk estimates for the adverse outcome of weediness of the GM cotton lines as a result of the expression of one or two copies of *cp4 epsps* gene, the *cry1Ac* and *cry2Ab* genes in combination, or the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination, have been made relative to the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this

proposed release (all areas north of latitude 22°S). Consideration has also been given to the current widespread use of the Roundup Ready<sup>®</sup> (containing the one copy of the *cp4 epsps* gene) and Bollgard II<sup>®</sup> (containing the *cry1Ac* and *cry2Ab* genes) cotton lines in commercial cotton crops in southern Australia.

482. The consequences of increased spread and persistence of cotton resulting from the presence of the *cp4 epsps* gene(s) in the GM cotton lines (Identified Risk 2) have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

483. The consequences of increased spread and persistence of cotton resulting from the presence of the *cry1Ac* and *cry2Ab* genes (Identified Risk 3) have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

484. The consequences of increased spread and persistence of cotton resulting from the presence of the *cp4 epsps*, *cry1Ac* and *cry2Ab* genes in combination (Identified Risk 4) have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

485. The consequences of increased spread and persistence resulting from the presence of the *cp4 epsps*, and/or the *cry1Ac* and *cry2Ab* genes in other *G. hirsutum* and *G. barbadense* cotton plants, as a result of gene transfer (Identified Risk 5), have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

486. The consequences of increased spread and persistence resulting from the presence of the *cp4 epsps*, and/or the *cry1Ac* and *cry2Ab* genes in combination with the *bar* gene (from commercially approved GM Liberty Link<sup>®</sup> Cotton), as a result of gene transfer (Identified Risk 6), have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

487. The risks of the five events (above) that may lead to weediness are estimated to be negligible and therefore, no risk treatment measures for weediness are proposed.

• **Table 4.1 Summary of risk assessment**

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 2</b> Tolerance to glyphosate due to expression of the <i>cp4 epsps</i> gene(s) in the GM cotton plants</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>• In the presence of glyphosate, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Glyphosate tolerant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>• The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> <li>• Glyphosate tolerant cotton is not likely to be cultivated as extensively as lepidopteran resistant cotton in northern Australia (unless stacked with lepidopteran resistant cotton) due to the requirement for multiple insecticide applications.</li> </ul>	<b>Negligible</b>	<b>No</b>
<p><b>Identified Risk 3</b> Reduced lepidopteran herbivory due to expression of the <i>cry1Ac</i> and <i>cry2Ab</i> genes in the GM cotton plants</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of herbicides.</li> <li>• The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> </ul>	<b>Negligible</b>	<b>No</b>

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 4</b> Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the <i>cp4 epsps</i>, <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination in the GM cotton plants</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of both glyphosate and lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interaction.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Glyphosate tolerant and lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>• The chance of volunteer GM plants arising from unintended seed dispersal (eg transportation, use as stockfeed, via animals or flooding) finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton.</li> </ul>	Negligible	No

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 5</b> Expression of the <i>cp4 epsps</i>, and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in naturalised <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants providing glyphosate tolerance and/or reduced lepidopteran herbivory</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Although glyphosate is the most widely used herbicide in Australia today, it is not generally used to control established cotton plants as the herbicide is not effective on cotton beyond the seedling stage.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of glyphosate and/or lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interaction.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies.</li> <li>• Glyphosate tolerant and/or lepidopteran resistant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> <li>• The chance of volunteer GM plants finding suitable ecological niches and establishing as weeds would be no greater than for the non-GM parent.</li> </ul>	Negligible	No

Event that may give rise to weediness	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
<p><b>Identified Risk 6</b> Expression of the <i>cp4 epsps</i>, and/or <i>cry1Ac</i> and <i>cry2Ab</i> genes in combination with the <i>bar</i> gene (from Liberty Link<sup>®</sup> Cotton) providing dual herbicide tolerance and reduced lepidopteran herbivory</p>	<p><b>Minor</b></p> <ul style="list-style-type: none"> <li>• Cotton does not have weedy characteristics and is not considered a serious weed anywhere in Australia.</li> <li>• Neither glyphosate nor glufosinate ammonium are effective in controlling established cotton plants.</li> <li>• While lepidopteran herbivory impacts adversely on productivity in commercial cotton crops, it is not an important limiting factor on the spread and persistence of cotton in northern Australia.</li> <li>• In the presence of glufosinate ammonium, and glyphosate and/or lepidopteran herbivory, the small competitive advantage of the GM cotton is offset by susceptibility to the abiotic and biotic factors (such as water and nutrient availability, plant competition and herbivory by non-lepidopteran insects) that limit the spread and persistence of all cotton in northern Australia.</li> <li>• The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interactions.</li> </ul>	<p><b>Highly unlikely</b></p> <ul style="list-style-type: none"> <li>• Cotton is primarily self-pollinating and gene transfer to other cotton plants is only expected to occur in close proximity and at low frequencies.</li> <li>• If Liberty Link<sup>®</sup>, Roundup Ready<sup>®</sup> or Roundup Ready<sup>®</sup> Flex cotton lines were to be cultivated in northern Australia, they will require multiple insecticide applications resulting in limited gene flow because of the reduced numbers of insect pollinators.</li> <li>• Cotton volunteers with glufosinate ammonium tolerance in combination with glyphosate tolerance and/or lepidopteran resistance would be effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides.</li> </ul>	<p><b>Negligible</b></p>	<p><b>No</b></p>

## Chapter 5 Risk management

488. This Chapter evaluates the risks assessed in Chapters 3 and 4 to determine whether specific treatments are required to mitigate harm that may arise during the proposed release. The roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are also explained. In addition, other considerations relevant to the Regulator's obligations under the Act are addressed.

### Section 1 Background

489. 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. All licences are required to be subject to three conditions prescribed in the Act.

490. Section 63 requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example section 64 requires that licence holders provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

491. Mandatory conditions contemplating ongoing oversight are supported by general conditions which require the licence holder to collect and provide further information related to the progress of the dealing. Requests for further information of this nature may be prompted by reports from the licence holder, or may be requested on the Regulator's initiative where it is necessary for her to do so in order to meet her statutory obligations as they arise. The Regulator will ensure that requests to collect and provide information are made reasonably having regard to consistency with the Act and relevance to its purposes.

492. It is a further requirement that the licence be 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 the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

### Section 2 Other Australian regulators

493. Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, NICNAS, NHMRC and AQIS. Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies.

494. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. The *Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purposes of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

495. FSANZ has approved the use of oil and linters from Roundup Ready<sup>®</sup> and Bollgard II<sup>®</sup> cotton in food (ANZFA 2000; ANZFA 2002b). No additional approvals are required by FSANZ for the stacked GM cotton lines.

496. APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. Roundup Ready<sup>®</sup> Herbicide is currently registered for use on Roundup Ready<sup>®</sup> and Roundup Ready Flex<sup>®</sup> cotton varieties.

497. The APVMA registered the use of the insecticidal proteins as produced by the insecticidal genes (*cry1Ac* and *cry2Ab*) in GM Bollgard II<sup>®</sup> cotton as insecticidal products for New South Wales (NSW) and Queensland (QLD) south of latitude 22°S in 2003. Any plantings north of latitude 22°S would require APVMA approval. The APVMA is currently assessing an application from Monsanto to vary the label for Bollgard II<sup>®</sup> to remove the condition for restriction on planting Bollgard II<sup>®</sup> north of latitude 22°S.

498. The Regulator has liaised closely with FSANZ and the APVMA during the assessment of applications pertaining to this commercial release of GM cotton lines.

### **Section 3 Specific licence conditions**

499. The detailed risk assessment of Identified Risks 1 to 6 contained in Chapters 3 and 4 concluded that the risk estimates are negligible for all six events. These events were considered in the context of the large scale of the proposed release and the receiving environment for this proposed release, including the other commercially approved GM cotton Liberty Link<sup>®</sup> Cotton.

500. The *Risk Analysis Framework* (OGTR 2005), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. Therefore, no specific licence conditions have been imposed.

### **Section 4 General licence conditions**

#### **4.1 Other risk management considerations**

501. All DIR licences contain a number of general conditions. These include, for example:

- applicant suitability
- auditing and monitoring provisions
- compliance plan
- reporting structures.

##### **4.1.1 Applicant suitability**

502. 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 (both individuals and the body corporate)
- 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 applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

503. Before making the decision to issue a licence for this application (DIR 066/2006), the Regulator determined that Monsanto Australia Ltd is suitable to hold a licence.

504. Conditions in the licence include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.



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

#### **4.1.2 Auditing and Monitoring Provisions**

506. 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 observe a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

#### **4.1.3 Compliance plan**

507. The licence requires Monsanto to submit a plan detailing how it intends to ensure compliance with the licence conditions and document that compliance. This plan is required before the planting of the GM cotton lines occurs.

508. Monsanto is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic material in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

#### **4.1.4 Reporting structures**

509. The licence obliges the licence holder to immediately report any of the following to the Regulator:

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

510. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing the above information and on the extent of cultivation of the GMO(s) approved for release.

511. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release.

### **Section 5 Conclusions of the RARMP**

512. The risk assessment concludes that this commercial release of herbicide tolerant and/or insect resistant GM cotton lines in northern Australia poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

513. The risk management plan concludes that the negligible risks do not require risk treatment measures and therefore no specific risk management conditions have been imposed. The licence contains general conditions relating to ongoing licence holder suitability; auditing and monitoring provisions; and reporting requirements, including a compliance plan, annual report and other relevant information, that enable the Regulator to maintain oversight of the licensed dealings in accordance with her statutory obligations.

### **Section 6 DIR 066/2006 Licence**

514. The licence DIR 066/2006 is available on the OGTR website (<http://www.gov.au/gmorec/ir.htm#table>, following the path to DIR 066/2006).

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## Appendix A Definitions of risk analysis terms used by the Regulator

(\* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

### **Consequence**

outcome or impact of an adverse **event**

*Marginal*: there is minimal negative impact

*Minor*: there is some negative impact

*Major*: the negative impact is severe

### **Event\***

occurrence of a particular set of circumstances

### **Hazard\***

source of potential harm

### **Hazard identification**

the process of analysing hazards and the **events** that may give rise to harm

### **Intermediate**

the negative impact is substantial

### **Likelihood**

chance of something happening

*Highly unlikely*: may occur only in very rare circumstances

*Unlikely*: could occur in some circumstances

*Likely*: could occur in many circumstances

*Highly likely*: is expected to occur in most circumstances

### **Quality control**

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

### **Risk**

the chance of something happening that will have an undesired impact

*Negligible*: risk is insubstantial and there is no present need to invoke actions for mitigation

*Low*: risk is minimal but may invoke actions for mitigation beyond normal practices

*Moderate*: risk is of marked concern requiring mitigation actions demonstrated to be effective

*High*: risk is unacceptable unless actions for mitigation are highly feasible and effective

### **Risk analysis**

the overall process of **risk assessment, risk management and risk communication**

**Risk analysis framework**

systematic application of legislation, policies, procedures and practices to analyse **risks**

**Risk assessment**

the overall process of **hazard identification** and **risk estimation**

**Risk communication**

the culture, processes and structures to communicate and consult with **stakeholders** about **risks**

**Risk Context**

parameters within which risk must be managed, including the scope and boundaries for the **risk assessment** and **risk management** process

**Risk estimate**

a measure of **risk** in terms of a combination of **consequence** and **likelihood** assessments

**Risk evaluation**

the process of determining risks that require treatment

**Risk management**

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

**Risk management plan**

integrates **risk evaluation** and **risk treatment** with the decision making process

**Risk treatment\***

the process of selection and implementation of measures to reduce risk

**Stakeholders\***

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

**States**

includes all State governments, the Australian Capital Territory and the Northern Territory governments

**Uncertainty**

imperfect ability to assign a character state to a thing or process; a form or source of doubt

## **Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities<sup>6</sup> on application DIR 066/2006**

All issues relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence that was used in the preparation of the RARMP.

Issues raised relating to the Risk Assessment:

- Human health effects (see Chapters 1, 2 and 3)
- Risk of enhanced spread and persistence (weediness) (see Chapters 1, 2 and 4)
- Risks arising from gene flow to non-GM and GM cotton plants including native cotton species (see Chapters 2 and 4)
- Environmental effects (see Chapters 1, 2, 3 and 4)
- Use of GM products in human food and animal feed (see Chapter 2).

Issues relating to the Risk Management Plan:

- Need for integrated weed management and insect resistance management strategies which may differ from the strategies for southern Australia (the Australian Pesticides and Veterinary Medicines Authority considers these issues—refer Chapters 2 and 4)
- Licence conditions to manage any identified risks (Chapters 3, 4, and 5).

Issues that were outside the scope of assessments conducted under the *Gene Technology Act 2000*:

- Moratorium on GM cotton production in WA

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<sup>6</sup> GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and Local councils where the release may occur.

## **Appendix C Summary of issues raised in submissions received from the public on application DIR 066/2006**

Five submissions from the public were received. All issues relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence.

Issues raised relating to the Risk Assessment:

- Human health effects (see Chapters 1, 2 and 3)
- Environmental effects (see Chapters 1, 2, 3 and 4)
- Use of GM products in human food and animal feed (see Chapter 2)

Issues that were outside the scope of assessments conducted under the *Gene Technology Act 2000*:

- Expansion of agriculture into new areas
- Benefits of GM technology
- Marketing issues.

## **Appendix D Submissions received from prescribed experts, agencies and authorities on the consultation RARMP**

None of the experts, agencies and authorities prescribed for consultation under the Gene Technology Act 2000, raised any issues on the RARMP regarding risks to human health and safety and the environment that required further consideration.

Three of the 122 local councils located north of latitude 22°S provided submissions, two supported the release and one opposed the release with no reason given.

## Appendix E Summary of public submissions received on the consultation RARMP

The Regulator received 50 submissions from the public on the consultation RARMP. All were analysed in detail. Thirteen were from organisations with interest in cotton growing that supported the application. Thirty-two were from individuals that supported the application and one that was a neutral opinion. Four were from interest groups and individuals that raised a range of concerns about the use of the GM cotton lines. These included issues regarding the use of agricultural chemicals and the development of resistance that fall within the regulatory responsibilities of the APVMA.

All issues raised relating to risks to human health and safety and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. The submissions, and how they were considered, are summarised in order of receipt, in the table below.

### Abbreviations used:

**Issues raised:** **A:** allergenicity; **AG:** agricultural production; **AR:** antibiotic resistance; **B:** benefits of gene technology; **C:** consultation; **CCI:** confidential commercial information; **DP:** democratic process; **EC:** economic issues **EN:** environmental risks; **ET:** ethical issues; **GS:** genetic stability; **GT:** gene transfer; **H:** human health and safety; **HR:** herbicide resistance; **HU:** herbicide use; **InR:** insect resistance; **IU:** insecticide use; **LC:** licence conditions; **M:** marketing concerns; **Mor:** moratoria; **RA:** risk assessment; **Res:** further research; **RM:** risk management; **RMP:** Monsanto's resistance management plan; **S:** gene stacking; **T:** toxicity; **W:** weediness

**Other abbreviations:** **APVMA:** Australian Pesticides and Veterinary Medicines Authority; **Ch:** chapter; **FSANZ:** Food Standards Australia New Zealand; **GM:** genetically modified; **GMO:** genetically modified organism; **IR:** Identified Risk; **OSA:** outside scope of the assessment; **RARMP:** risk assessment and risk management plan

<sup>a</sup> Submission from: **A:** agricultural/industry organisation; **IG:** Interest Group; **I:** Individual.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
1	A	Supports Monsanto's application	None	Noted.
		The current commercial releases of the GM cotton line in southern Australia have both cost and environmental benefits to cotton growers and the community at large (eg reduced pesticide use).	B, E, EC,	Noted. The <i>Gene Technology Act 2000</i> (the Act) requires the identification and management of risks that may be posed by the use of gene technology. Positive environmental effects and economic issues are outside the scope of assessments conducted under the Act.
		These benefits are crucial to commercial cotton in northern Australia, and would provide additional benefits such as expanded crop rotation options for disease and weed control, seed for cattle feed, future bio-fuel developments, and provide a boost to cotton breeding, agronomic traits and seed production. General release would reduce compliance costs associated with meeting stringent licence conditions.	B, AG, EC	Agricultural production benefits and economic issues are also outside the scope of assessments conducted under the Act.



Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
2	I	Question about whether the chemical that acts on the cotton pests (ie cry proteins) is in the cotton seed and whether this can harm livestock fed on GM seed or humans either consuming GM cotton seed oil or eating the meat from animals fed GM cotton seed.	H, EN	The Cry proteins are present in most tissues of the cotton plants including seeds (discussed in Ch 1 and the RARMP for DIR 012/2002). The potential toxicity of the Cry proteins is considered in Sections 2.1 and 2.3, Ch2 of this RARMP. Consumption of animals that were fed GM plant material is considered in Event 35 in Ch2. GM cotton seed has been fed to livestock since the commercial release of Bollgard II <sup>®</sup> cotton lines in southern Australia in 2002. FSANZ has approved the use of oil and linters derived from Bollgard II <sup>®</sup> for use in food.
		Question about how can Roundup Ready cotton be controlled in native pastures if it will not die from any chemicals and is concerned about the GM cotton becoming a weed.	W	Roundup Ready <sup>®</sup> cotton is only tolerant to herbicides containing glyphosate and can be controlled by mechanical means or by other herbicides if still in the seedling stage. Weediness potential of GM cotton tolerant to glyphosate is considered in IR 2 in Ch4.
3	I	Concerned about GM seed blowing into neighbouring properties and Monsanto's ability to sue for illegally growing the GM seed.	M	OSA. Liability issues are outside the scope of assessments conducted under the Act. Note: Cotton seed is relatively heavy and unlikely to be dispersed by wind.
		Concerned about Monsanto's monopoly of seed reserves.	M	OSA. Marketing issues are outside the scope of assessments conducted under the Act.
4	I	Supports the release of GM cotton because it gives alternative opportunities for sugarcane growers if sugar cane is no longer financially viable.	EC	Noted. Economic issues are outside the scope of assessments conducted under the Act.
5	I	Supports the release of GM cotton because it is important to have alternative options for sugarcane growers to protect against pests and to control weeds.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.
		The current compliance rules that apply to growing GM cotton in the region is making it difficult for practical farming operations.	EC, AG	Noted. The licence conditions that are applied to field trials limit the spread and persistence of the GMOs while information is gathered about their behaviour in the environment.
6	A	Supports release as Bollgard <sup>®</sup> II and Roundup Ready <sup>®</sup> cotton varieties have the advantage of pest protection and weed control assisting in the control of Heliothis and Nut Grass.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.
7	I	Supports release as the advantages of pest protection and weed control offered by Bollgard <sup>®</sup> II and Roundup Ready <sup>®</sup> cotton varieties would be a great tool to incorporate in our farming system allowing the growing of a lower risk crop in relation to problems such as Heliothis and weeds.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.
8	I	Supports release as it is important to have other viable industries in the Burdekin region and have options to grow alternative crops in rotation with sugarcane to maintain profitable farming businesses	EC	Noted. Economic issues are outside the scope of assessments conducted under the Act.
9	I	General support for the benefits of pest protection and weed control advantages it would provide to local growers	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
10	I	Supports release as it offers an alternative rotation crop, the trials of GM cotton in the Burdekin region indicates good yields and quality, and has the advantage of pest protection and the ability to control in crop weed situations.	AG	Noted. Economic issues and production benefits are outside the scope of assessments conducted under the Act.
11	I	Supports release because, as a cotton picking contractor, believes the Burdekin region has the resources to sustain a cotton industry and each farming region should have the opportunity to grow other crops that benefit farming practices such as improved pest and weed control.	B, AG	Noted. Economic issues and production benefits are outside scope of assessments conducted under the Act.
12	I	Supports release as it creates alternative crops to sugarcane in the Burdekin region.	AG	Noted. Economic issues are outside the scope of assessments conducted under the Act.
13	I	Supports release as it provides variety for crop rotation as sugarcane is unsustainable as a monoculture	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
		The district requires access to the latest technology and the advantages offered by the GM cotton lines (such as pest protection and improved weed control) to be competitive internationally.	B, EC	Noted. Production benefits and economic issues are outside scope of assessments conducted under the Act.
14	A	Supportive of the uptake of GM crops if they can be shown to pose acceptable risk to the environment and positive benefit for society.	EN, B	The risk assessment concluded that the proposed release of the five GM cotton lines poses negligible risk to the health and safety of people and the environment. Socioeconomic benefits are outside scope of assessments conducted under the Act.
		Supports continued research into trialling and testing of individual agricultural products including GM in plant breeding which has the potential to offer significant benefits in production and addressing agronomic difficulties.	B, AG	Noted. Production benefits are outside scope of assessments conducted under the Act.
		Supports the comprehensive and rigorous science-based assessment of GM species and products.	None	Noted.
		Supports the OGTR in ensuring that research trials be strictly contained within the legislated guidelines to ensure its human health and environmental safety.	None	Noted.
		Supportive of the application and sees no reason why the GM cottons should cause any detrimental effects.	None	Noted.
		Supports grain growers having access to an affordable choice of the latest research technology that is best suited to their production needs.	None	Noted.
		In supporting this application, the organisation hopes to improve understanding and acceptance amongst the general population of future grain GM varieties and the benefits they may bring to society.	None	Noted.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		The benefits that may come from this release are significant for rural and regional Australia and is vital to the future productivity of the grains industry of QLD.	B, EC	Noted. Production benefits and economic issues are outside the scope of assessments conducted under the Act.
15	A	Supports the release and is committed to supporting growers in the Burdekin region to build and sustain a cotton industry.	None	Noted.
		Have participated in the cotton trials in the Burdekin to ascertain planting dates, varieties and to observe if cotton would perform in these conditions which have indicated that cotton can be grown in the region with little difficulty.	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
		The GM cotton will provide an alternate crop for local growers to maintain financial viability and provide the benefits of pest protection and weed control.	AG, EC	Noted. Economic issues and production benefits are outside the scope of assessments conducted under the Act.
16	I	Supports the release as has recently moved into the area and has grown GM cotton previously and would like to be able to do so again.	None	Noted.
17	I	Supports release as it provides variety for crop rotation and will improve pest and weed management.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.
18	A	Fully supports the continuation of research development of GM cotton crops above the 22 <sup>nd</sup> parallel	None	Noted.
		Urges the WA State Government to grant GM exemption from the conditions of the GM Free Areas Act. The review of the moratorium on GM cotton due in 2008, will be too late for Expressions Of Interest for the Ord Stage 2. If cotton is included in the expressions of interest there are opportunities for growers to have monetary returns on their crops within 12 months as opposed to sugar which is more like 18 months. NSW also have a moratorium on GM crops but have a specific exemption for cotton hence they have a Billion dollar cotton industry in QLD and NSW.	Mor, EC	Noted. The <i>Gene Technology Act 2000</i> and corresponding State and Territory legislation focuses upon the assessment and management of risks to human health and safety and the environment that may be posed by GMOs. The laws establishing moratoria on growing GM crops are the responsibility of individual jurisdictions.
		GM cotton would be beneficial to local economy and growers because: <ul style="list-style-type: none"> <li>- Financial return within 6 months of planting</li> <li>- Gives region 12 month agricultural period</li> <li>- Provides more consistent employment</li> <li>- No water shortage in Ord Valley region</li> <li>- Supplement sugar income with cotton</li> <li>- Cotton being high input, high employing industry will have regional multiplier benefits to the whole community</li> <li>- Cotton seed can be used as cattle feed.</li> </ul>	B, EC, AG	Noted. Agricultural production issues and economic benefits are outside the scope of assessments conducted under the Act.
19	I	Supports release as it provides alternative and profitable crops in rotation with sugarcane	AG	Noted. Economic benefits are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
20	I	Supports release as it will provide a high security rotation crop with sugarcane and was involved with GM cotton trials in the Burdekin and acknowledges the capabilities of the GM such as aid in the control of Heliothis and nut grass.	AG	Noted. Agricultural production issues and benefits are outside the scope of assessments conducted under the Act.
21	I	Supports release as it provides an alternative for crop rotation, and will aid in the control of Helicoverpa and grasses in a crop situation. Have been impressed by the field trials of GM cotton in the Burdekin.	AG	Noted. Agricultural production issues and benefits are outside the scope of assessments conducted under the Act.
22	I	Strongly supports the release as thinks “the pseudo claims and anti-intellectual claims” against GM crops “should not be listened to any more”.	None	Noted.
		After reading the RARMP, a PNAS article and newspaper clipping re GM cotton in Arizona and China (attached with submission), does not see any honest objection to the proposed release.	None	Noted.
23	I	Objects to the proposed release because of:		
		- Inherent instability of GMOs in the environment	GS	These GM cotton lines have been trialled extensively in both northern and southern Australia. Three of the GM cotton lines have been grown commercially south of latitude 22°S (Roundup Ready <sup>®</sup> since 2000, and Bollgard II <sup>®</sup> and Roundup Ready/Bollgard II <sup>®</sup> since 2002) and currently comprise 90% of the Australian cotton crop. The genetic modifications have been shown to be stable over many generations and in a variety of genetic backgrounds when conventionally crossed with various cotton cultivars.
		- Insect and plant resistance development	InR, HR	OSA. The development of insect and herbicide resistance is considered by the APVMA as it comes under the scope of assessments conducted under the <i>Agricultural and Veterinary Chemicals Code Act 1994</i> . Management practices adopted by the cotton industry have effectively managed the potential for development of insect and herbicide resistance in cotton crops in Australia.
		- Effect on soil biota not thoroughly investigated	Res	The introduced genes are derived from common soil and gut bacteria and therefore are naturally present in the environment. Extensive data suggests that the proteins are not toxic to microorganisms. Therefore the GM cotton lines are not expected to disrupt soil ecology (Refer Event 11 in Ch2).
		- Residues of cotton fed to meat and milk-producing stock which people with religious objections will have problems avoiding. Causing stress to conscientious objectors who may suffer health decline avoiding milk or eggs.	H	No risk was identified for the consumption of products from animals fed GM cotton (See Event 35 in Ch2). Protein and DNA are rapidly broken down into smaller components in the digestive tract of animals irrespective of whether they are GM or not. As a result, products from these animals would be no different to that from animals that were fed seed from non-GM cotton.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		- References to published scientific experimentation on GM cotton are not provided in the GTR advice.	Res	The assessment conducted by the Regulator was based on scientific evidence and supported by peer-reviewed publications. The list of references is located at the end of the RARMP (prior to the Appendices).
		- Claims of “negligible risk” and “stability” of GMO’s not backed up with scientific literature references	Res	See earlier responses regarding stability and references. The assessment of the application is based on the <i>Risk Analysis Framework</i> , which applies international best practice and relies on both quantitative and qualitative data.
		- Effects of insect resistant cotton on insect life at base of food chain and undermining ecology may result in ill effects for humans.	EN	The Cry proteins contained in the GM cotton lines are specifically toxic for <i>Lepidopteran</i> insects and do not impact on other insects (See Event 9). The routine use of insecticides on non-GM cotton crops has a greater impact on all insect populations.
		- Many people have religious taboos against GM life forms and want an intact genetic heritage, not engineering for profit. Stress and ill health follow the flouting of human deeply held values.	ET, H	The development of new varieties is inherent to agricultural practice. The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by the release of GMOs. For this application these were determined to be negligible. The Gene Technology Ethics Committee is in place to advise the Regulator and to identify and explore any ethical issues relating to gene technology.
24	I	Supports release as it will provide growers with opportunities to branch into another viable crop and boost economic growth in the area.	EC	Noted. Economic issues are outside the scope of assessments conducted under the Act.
25	A	Supports the regulators cautious approach to the use of GM cotton in Australia and has encouraged the Cotton CRC’s research and as a result of which believes the risk posed by the introduction of GM cotton north of 22°C south to be minimal.	None	Noted.
		The organisation has followed research on potential weediness by Rowena Eastick and John Rogers and believes the work answers the key scientific questions about potential environmental risk.	EN	Noted.
		Roundup Ready cotton, INGARD cotton and more recently Bollgard II cotton have been grown commercially for around a decade now from an industry perspective all the impacts appear to be positive in relation to health. <ul style="list-style-type: none"> <li>- Bollgard II cotton has reduced insecticide use by around 86%</li> <li>- Roundup Ready has lessened need for hand weeding and thus reduced labourers exposure</li> <li>- together these reduce intense pressure of pest management on growers and their families</li> </ul>	B, AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p>In relation to the environment, Roundup Ready cotton has reduced herbicide use by 31% and thus reduced residual cotton herbicides in the environment.</p> <p>Reduced overall tillage and improved tillage timing thus enabling improved soil structural and conservation practices.</p> <p>ACGRA believes in 10 years of commercial use of GM cotton there have only been positive environmental outcomes.</p>	B, EN	Noted. The Act requires the identification and management of risks to people and the environment. Environmental benefits of gene technology are outside the scope of assessments conducted under the Act.
26	I	Supports release as was involved with GM cotton trials in the Burdekin and believes that the environmental conditions and high yield show cotton is well suited to the area, provided the Bollgard II technology is available to help relieve the financial burden placed on the growers by high heliothis pressure.	B	Noted. Economic benefits are outside the scope of assessments conducted under the Act.
27	I	Supports the application as has seen the benefits of GM cotton in Central Queensland and believes that it has no more impact on the environment than any other irrigated pulse or cereal crop, thanks to GM technology.	B	Noted. Benefits of gene technology are outside the scope of assessments conducted under the Act.
		Market analysts predict worldwide cotton shortage in 3-5 years and as such cotton could become a very lucrative commodity and a prosperous industry.	EC	Noted. Economic issues are outside the scope of assessments conducted under the Act.
		Imperative that Monsanto be allowed to continue their research work in areas above the 22 <sup>nd</sup> parallel to further advance a cotton industry that will be able to use the abundant and renewable resources of Northern Australia without impacting on the pristine natural environment	Res	Noted.
28	I	Supports the application as has benefited from Bollgard II and Roundup Ready cotton and feels that other growers need the opportunity to use and experience these technologies to provide alternate cropping option and help them remain competitive in a difficult farming environment.	AG, EC	Noted. Agricultural production and economic issues are outside the scope of assessments conducted under the Act.
29	I	Supports the application as has been involved in the trial in the Burdekin area and has seen the opportunity that cotton offers growers as an alternative to sugar cane and make farming more sustainable and profitable.	AG, EC	Noted. Agricultural production and economic issues are outside the scope of assessments conducted under the Act.
		Bollgard II and Roundup Ready varieties have the advantages of pest protection against the high heliothis pressure and controlling weeds in the warmer climate.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
30	A	Supports the application as, in the past, cotton can be grown successfully in the area, but a huge chemical requirement is necessary to manage the enormous pest pressure. Trials in Kununurra and Broome have shown that GM technology will allow cotton to be grown successfully with much lower chemical application which benefits both the grower and the environment.	IU, EN, B	Noted. Environmental and production benefits are outside the scope of assessments conducted under the Act.
		These extended trials have also clearly shown that the GM cotton does not pose a threat to the environment.	EN	Noted.
		Access to GM cotton will provide industry with a much needed broad acre crop that can be grown in rotation with other crops like sugar cane and as such will help maintain the soil health of the region.	AG, EN	Noted. Agricultural production issues and environmental benefits are outside the scope of assessments conducted under the Act.
31	A	Supports the application as believes that the risks to the health and safety of people and the environment are negligible based on the performance of these technologies in southern localities and the comprehensive risk assessments now conducted in north Australia.	EN	Noted.
		Benefits of technology include: <ul style="list-style-type: none"> <li>- increased productivity and yields due to better and more reliable control of lepidopteran pest species and weeds</li> <li>- decreased environmental footprint due to large reductions in pesticide use</li> <li>- reduction in human health issues for the communities associated with cotton production</li> <li>- improved access for seed production and plant breeding programs.</li> </ul>	B, AG	Noted. Benefits to people and environment from the use and development of gene technology, and agricultural production issues are outside the scope of assessments conducted under the Act.
		Cotton seed is an important stock feed, especially in times of drought and cattle producers in the north will benefit from having restrictions removed.	None	Noted. Economic issues are outside the scope of assessment conducted under the Act.
		Cotton industry takes responsibility for stewardship and the on-going sustainability of these technologies very seriously and has resistance management plans (RMP) and crop management plans (CMP) to accompany their introduction to the north. Both industry and the scientific community have worked closely to ensure the RMP and CMPs for the north are robust and scientifically sound. The plans will be improved and updated based on latest research outcomes.	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act. Resistance management relates to product efficacy which is regulated by the APVMA.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
32	A	Supports the conclusions reached by the Regulator that the “proposed commercial release of the five herbicide tolerant and/or insect resistant GM cotton lines in northern Australia poses negligible risk to the health and safety of people and the environment as a result of gene technology”.	None	Noted.
		Have provided sites for Cotton Seed Distributors for several seasons to assist with their contra-season cotton breeding and seed production activities. The extra generations provided by this production leads to a significant reduction in the time needed to develop new and improved varieties.	None	Noted.
		Experience with growing the GM cotton in the Ord supports the conclusions reached by the Regulator that there is little likelihood that it will establish as a troublesome weed.	W	Noted
33	I	Supports the application as has seen the economic benefits of the technology and believes that rural producers in Northern Australia should have the choice and opportunity to use Bollgard II and Roundup Ready cotton.	EC	Noted. Economic issues are outside the scope of assessments conducted under the Act.
		This would present growers in the Burdekin region with some flexibility and give them further scope to diversify into alternate crops, to assist with long term sustainability.	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
34	I	Supports the application as involvement in the 2005 GM cotton trials in the Burdekin has provided an understanding of how a GM cotton crop will perform in the Burdekin and an appreciation of its pest protection and weed control attributes. Looks forward to being able to grow GM cotton commercially.	AG	Noted.
35	A	Supports the application as believes that the availability of biotech enhanced varieties of cotton to growers in northern Australia will provide growers with similar opportunities to those in other cotton growing regions who have benefited from advanced insect protection and weed control attributes provided by these technologies.	AG	Noted. Production benefits are outside the scope of assessments conducted under the Act.
		A recent review of the global impact of GM crops (Brookes and Barfoot 2005, AgBioForum 8 (2&3) 187-196) has shown that since 1996 <ul style="list-style-type: none"> <li>- net benefits to farm US\$27 billion</li> <li>- pesticide spraying reduced by 172 million kg</li> <li>- environmental footprint from pesticides down by 14%</li> <li>- reduced green house gas emissions from agriculture equivalent to removing five million cars from the road.</li> </ul>	B, E, EN	Noted. Economic and environmental benefits of gene technologies are outside the scope of assessments conducted under the Act.



Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		In Australia more than 95% of cotton growers chose to plant GM cotton in 2004/5 and the new varieties have reduced pesticide use by 85% and significantly reduced the environmental footprint of the production system.	EN	Noted. Production and environmental benefits are outside the scope of assessments conducted under the Act.
36	I	Notes RAMRP is full of qualified statements and assumptions about safety, suggests no risks were found due to incomplete scientific data and proposes more extensive testing be carried out.	Res	Extensive scientific literature and studies have been used by the Regulator to assess the safety of the 5 GM cotton lines. The risks to people and the environment from this release have been assessed as negligible.
		Focuses on the hypothetical statement in the RARMP “There is potential for the GM cotton plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the genetic manipulation.” Feels there should be proper, scientific controlled feeding and breeding trials. Questions validity of extrapolating safety data on individual lines GM lines to that of the stacked GM cotton lines.	Res, T, GS	Detailed analyses of the GM cotton lines have been conducted, including compositional and molecular analysis and in some cases feeding studies to determine if there will be any toxic or allergenic effects (See Ch 1 and 2 of the RARMP for details) As the various genes operate through independent biochemical pathways, it is considered unlikely that there will be any unintended effects from stacking of the genes.
		Notes that Monsanto’s tests on the safety of the GM cotton lines as animal feed (p.45 para 167 & 168 of consultation RARMP) only looked for evidence of toxicity and not allergenicity and questions whether the cotton can be classified as safe without the allergenicity tests. Mentions two GM safety trials (CSIRO GM Pea and Russian GM Soy fed to rats) showing negative safety outcomes for the GM products being tested. Calls for long term feeding and breeding trials of all of the GM cotton lines before claims of safety can be made.	Res, H, T, A	Internationally validated tests based on compositional analyses and molecular similarities provide more accurate indications as to whether there will be any toxic or allergenic effects than feeding studies. Toxicity data and comparison of the introduced proteins with known allergens support the conclusion that the GM cotton lines are not toxic or allergenic (See Sections 2.1, 2.2 and 2.3, Ch2). Food products from the GM cotton lines have been approved for use by FSANZ. There are no reports of toxic or allergenic effects from either field trials or their commercial release in southern Australia.
		Notes that the WA Government is independently conducting feeding trials to determine the safety of GM crops.	None	Noted.
		Questions claim “no reports of any unexpected or unintentional adverse effects” p54.233 - What precisely are the symptoms of an unexpected adverse effect? - To whom would such reports be made?	H	It is a statutory condition of all DIR licences that any unexpected or unintended effects of the GMO be reported to the Regulator. If any person has evidence that the presence of a GMO is having an adverse impact on the health and safety of humans, other organisms or the environment with regards to toxicity, allergenicity, weediness or some other factor, then they should contact either the licence holder or the OGTR directly.
Questions claims of “Substantial equivalence” for Bollgard II cotton given that it is a registered insecticide regulated by APVMA and contains two insecticidal substances.		The Bollgard II cotton line is considered to be compositionally similar to other cotton varieties with exception of the introduced genes and the protein encoded by the genes, indicating that the genetic modification does not affect any other nutritional attribute of the cotton.		

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
37	I	Supports the application as GM cotton offers endless opportunities for the Ord River Irrigation Area and all Northern Australia including: <ul style="list-style-type: none"> <li>- agricultural industry growth</li> <li>- diverse farming enterprises</li> <li>- business and employment opportunities</li> <li>- potential to improve the state's future economic growth</li> </ul> as evidenced from the excellent results from the GM cotton trials in the Kununurra.	B, AG, EC	Noted. Production and economic benefits are outside the scope of assessments conducted under the Act.
38	I	Supports the application as is currently growing cotton in the Ord Irrigation Area and in the last year of production used 40 applications of pesticides with a yield of 4.3 bales per hectare as opposed to the GM cotton trial which yielded 10 bales per hectare with zero pesticide applications. The benefits to a healthy and sustainable environment and economic production are enormous.	AG, EN, EC	Noted. Agricultural production issues and environmental and economic benefits are outside the scope of assessments conducted under the Act.
39	I	Many of the growers in the Gwydir Valley / Moree district use Bollgard II and Bollgard II / Roundup Ready and would like to see this application approved so that contra-season research and breeding of cotton varieties can be conducted.	Res	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
40	IG	Opposes the release and recommends rejection of Monsanto Australia Ltd's application DIR066/2006. Supports the existing ban on commercial cotton in northern Australia and is concerned it will be lifted with insufficient robust scientific evidence to support the policy change.	Res, Mor	Noted. The RARMP concludes that the 5 GM cotton lines are as safe to people and the environment as non-GM cotton. Restrictions on the cultivation of GM or non-GM crops on marketing grounds are the responsibility of the individual jurisdictions.
		Fundamentally opposed to the establishment or expansion of broad scale irrigated agriculture in northern Australia as it is unsustainable and causes significant and unacceptable impacts. Opposed to the introduction of GM cotton and other crops through trials or commercial release in northern Australia.	AG	Noted. The sustainability of agricultural practices is beyond the scope of assessments conducted under the Act.
		The risk management measures proposed by the RARMP will fail to adequately manage the risks posed to the environment of northern Australia.	RM, EN	The RARMP concluded that the risks posed by the release to people and the environment are negligible and therefore no specific risk treatment measures are required. The licence contains a number of general conditions to enable the Regulator to maintain oversight of the release.
		<b>Availability and quality of research from field trial:</b>		

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p>Questions the lack of scientifically robust, publicly available data from the GM cotton trials in Northern Australia, criticises selected studies and states there is insufficient evidentiary basis for the Regulator to assess and quantify the risks to the environment and human health posed by the commercial release of the GM cottons in northern Australia. Questions the justification for CCI status on results from field trials.</p>	Res, CCI	<p>A diverse range of both published and unpublished data (some of which has subsequently been published) was comprehensively reviewed in preparing the RARMP for this application and deciding to issue a licence. Unless a declaration of commercial confidential information (CCI) is made, all information submitted by an applicant is available to the public. CCI is available to all experts and agencies prescribed for consultation under the Act.</p>
		<p>Notes that previous licence for DIR 12 required research on weediness; the effect of GMOs on pest and beneficial insects as well as soil microorganisms; and potential pest resistance to insecticidal activity. Annual reports on this research were also to be provided.</p> <p>Request the OGTR to supply copies of the foregoing trial reports. Also request copies of:</p> <ul style="list-style-type: none"> <li>- Annual Reports that are not already publicly available.</li> <li>- Research that has not been published in peer reviewed journals such as weediness reports (Eastick, 2002).</li> <li>- Research on the effects of GMOs on soil microorganisms.</li> <li>- Research does not explore the effects of GMOs on other factors relevant to measuring risks to the environment and human health in northern Australia, e.g. effects on native species including reptiles, interactions with disease, soil chemistry and function.</li> </ul>	Res, W, T, InR	<p>Other research (cited in the RARMP) has superseded the requirement for some of these studies specified in the DIR 012/2002 licence.</p> <p>All information not declared CCI is available to the public and the requested documents will be supplied. Most of the literature cited has been published in scientific journals or is available from websites (eg Eastick 2000 at <a href="http://cotton.pi.csiro.au/Assets/PDFFiles/TB3051.pdf">http://cotton.pi.csiro.au/Assets/PDFFiles/TB3051.pdf</a> and Strickland and Annells 2005 at <a href="http://cotton.pi.csiro.au/Assets/PDFFiles/NthNews/IGBGIns.pdf">http://cotton.pi.csiro.au/Assets/PDFFiles/NthNews/IGBGIns.pdf</a>). The Eastick and Heardan study cited in the RARMP is currently in press and will be forwarded as soon as it is available.</p> <p>The OGTR carefully assesses all research data irrespective of whether it is published or unpublished. Internationally accepted testing methodology for toxicity focus on impacts on indicative species.</p>
		<p>The Monsanto Australia Ltd reports attached to the DIR66 licence application:</p> <ul style="list-style-type: none"> <li>- Lack references to other research, reports and data to substantiate claims.</li> <li>- Experiments are often not replicated and small scale and limited duration</li> <li>- Conclusions are drawn from very limited data with little or no statistical significance.</li> </ul> <p>Request that the OGTR make all raw data from these trials publicly available and accessible to people commenting on the application.</p>	Res	<p>The invitations to comment issued by the Regulator indicate how copies of the RARMP and other documents including the application can be obtained. Unless a declaration of CCI is made, all information submitted by an applicant is available to the public upon request.</p> <p>The Regulator does not rely solely on material provided by the applicant and has used a wide range of scientific references and information for the risk assessment.</p>

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Applicant's Suitability:</b> Believes that Monsanto Australia Ltd is not a suitable applicant to be granted this licence for commercial release of the GM cottons in northern Australia for a number of reasons including:</p> <ul style="list-style-type: none"> <li>- Monsanto were charged with violating the <i>Foreign Corrupt Practices Act</i> (US)</li> <li>- has several times breached GMAC advice, guidelines and licence conditions in Australia relating to GM cotton.</li> <li>- Greenpeace had to bring a court case in Germany to force Monsanto to publicly release its data from a 90-day trial that involved feeding genetically manipulated insect resistant maize to rats (MON863).</li> </ul>	Applicant suitability	The Regulator has complied with the Act in reaching a determination that Monsanto Australia Ltd is a suitable licence holder. Any issues relating to compliance with GMAC advice or licence conditions have been determined as posing negligible risks to the safety of people or the environment.
		<p><b>Impacts on native species</b> No research on insectivorous ectothermic or poikilothermic species such as reptiles and amphibians endemic to Northern Australia that could be exposed to the GM plants through their food source and any secondary impacts. In the absence of this data it is difficult to see how the Regulator can accurately assess the risks of this release to the northern Australian environment.</p>	Res, EN, T	The GM cottons are unlikely to exert direct adverse effect on reptiles and amphibians because they are not known to consume cotton. The same or similar proteins are widespread in the environment. All published data shows that they have extremely low toxicity for all organisms tested (other than the targeted pest species). There have been no reports of adverse impacts due to unexpected interactions or secondary impacts from the commercial release of the GM cotton lines in southern Australia, field trials in northern Australia or other GM crops expressing these proteins from trials conducted around the world.
		<p>States the applicant should have been required to gather and publish scientific experimental evidence that the gene and its products would not have impacts on species,</p> <ul style="list-style-type: none"> <li>- Reports of sheep and goats death following grazing on GM Bt cotton plants in India (Parsai 2006) and questions why equivalent studies were not undertaken for these cotton strains.</li> <li>- Studies showing increased mortality in offspring of rats fed GM crops before, during and after conception in Russia (Ermakova 2005).</li> </ul>	Res, EN, T	Extensive scientific evidence supports that the proteins encoded by the introduced genes present in the GM cotton lines are not toxic or allergenic to any organisms (with the exception of toxicity of the Cry proteins to the target insect species) (see Ch 2, Sections 2.1, 2.2 and 2.3).

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Impacts on humans</b></p> <p>Raises concerns over human safety citing:</p> <ul style="list-style-type: none"> <li>- studies regarding allergenicity of GM foods which conclude that <i>“in the absence of new and reliable methods for allergenicity testing, particularly the lack of good animal models, it is at present almost impossible to definitely establish whether a new GM crop is allergenic or not in advance of its release into the human/animal food/feed chain”</i> (Ho et al, 2006).</li> <li>- research evidence indicates that Bt toxins may cause allergic skin sensitisation (Vacquwz-Padron, R.I. et al, 1999)</li> <li>- research into the toxicity of a particular Bt strain killed mice within eight hours from clinical toxic-shock syndrome (Herandez, E., et a,1999).</li> </ul>	H, A, Res	Extensive scientific evidence supports that the proteins encoded by the introduced genes present in the GM cotton lines are not toxic or allergenic to any organisms (with the exception of toxicity of the Cry proteins to the target insect species) (see Ch 2, Sections 2.1, 2.2 and 2.3).
		<p>Concerns that antibiotic resistance could be transferred from the GM cotton to bacteria harmful to humans that could render future use of antibiotics redundant have been dismissed by Monsanto Australia Ltd in their licence application.</p> <p>Notes European Union and the FAO/WHO are phasing out all antibiotic resistance marker genes from all GM crops across Europe and recommends that the Regulator follow this path in Australia.</p>	AR	The risk arising from such transfer from GMOs to pathogenic organisms is negligible. Phasing out their use would have no effect on current levels of antibiotic resistance or curtail the development of further resistance. Factors important in the development of antibiotic resistance include prescribing antibiotics to be used prophylactically and use as growth promotants in animals. The Act requires the Regulator to assess risks to human health and safety and the environment posed by gene technology, including GMOs containing antibiotic resistance maker genes. Internationally, it has been determined that genes commonly used to confer antibiotic resistance in GM plants pose negligible risks to the health and safety of people and the environment. The risk posed by antibiotic resistance marker genes has been assessed in many previous RARMPs (refer to Ch 2, Section 2)
		<p>Lack of long term research on the impacts of GM feed on animals with plant DNA being detected in the muscle of chickens indicating that DNA transfer is possible (Einspanier, R. et al (2001). Related issues with GM animal feed and human food, particularly the feeding of GM cottonseed to cattle include the lack of independent studies, long term in this area (Pryne, I.F and Lembeke, R. (2003). Further the discovery of large fragments of functional Bt toxin in cattle excrement is also a major cause for concern with potential build up in soil of Bt protein with unknown effects (Espianier, R. et al, 2004).</p>	Res, EN,T	<p>Extensive scientific evidence indicates that DNA does not transfer from GM plants to animals (see Ch 2, Event 25). No risk of toxicity for vertebrates from the proteins expressed by the introduced genes was identified when these GM cotton lines were approved for commercial release south of latitude 22°S and no such adverse impacts have been reported (see Ch 2 Event 8).</p> <p>Soil microorganisms are already exposed to Bt toxins due to the widespread occurrence of the organism from which Bt genes are derived in the environment.</p>

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		There remains great uncertainty about the impact of the GM cotton on human health and requests to be informed of the evidential basis on which the Regulator will assess these risks in the face of this uncertainty	H, RA, Res	Humans only consume cotton in the form of highly processed oil or linters, neither of which contain any detectable protein. No risk of toxicity or allergenicity in humans has been identified. The GM cotton lines (Roundup Ready, Bollgard II and Roundup Ready/Bollgard II cotton lines have been grown commercially in southern Australia (since 2000 and 2002 respectively, currently comprising 90% of the Australian crop), and around the world, with no reports of adverse effects
		People in northern Australia do not want Bollgard II®/ Roundup Ready Flex® cotton genes in their food or in their soil and do not want GM crops or food licensed for commercial scale release. The Regulator's assessors, and applicants and scientists should not have the last say in whether GM cotton is grown and utilized in northern Australia, especially if it does not reflect the consensus of ordinary Australians. We are outraged that the OGTR consistently exercises its discretions in favour of applicants, rather than Australian citizens who still fund the OGTR, without full accountability.	H, C, DP	Submissions received from the public consultation process expressed a range of views, including some from residents of northern Australia, that are strongly supportive of the release of GM cotton. The <i>Gene Technology Act 2000</i> requires the Regulator to identify and manage risks to human health and safety and the environment posed by the release of GMOs. Each application is assessed case-by-case using science based analyses. Extensive consultation with prescribed experts, agencies, authorities and the public is required to ensure all relevant matters are comprehensively considered. For this release risks were determined to be negligible.  The decision as to whether the GM cotton lines will be cultivated commercially is a matter for State and Territory governments to determine in consultation with industry and the broader community.  Monsanto has indicated it does not anticipate commercial scale planting until a range of industry community and infrastructure issues are resolved.
		<b>Impacts on soil microorganisms and soil function</b> There is no data or analysis ascertaining the nature of the risk associated with the release of the GM cotton lines on soil structure, micro-organisms and function in potential northern Australian commercial cotton sites, preventing full assessment and consideration of the risks posed by the release. Any broad assumptions that universal exposure to the Bt protein in all soil types at the same levels means that there are no risks to soil micro-organisms must be substantiated.	EN	The introduced genes and encoded proteins (or similar proteins) are naturally present in soil and all published data shows that the proteins have extremely low toxicity for all organisms tested (except for the target insect species). Therefore the GM cotton lines are not expected to disrupt soil ecology (Refer to Sections 21 and 2.3, Ch 2 of RARMP).
		There is research that has highlighted the possibility of Bt toxins enhancing the persistence of glyphosate in soil (Accinelli et al, 2004 p.497). This study was only concerned with a single activated Bt toxin and impacts may be intensified with double Bt cotton (Bollgard II®).	EN, Res, HU	Bt toxins are naturally present in soils and are widely used as insecticide sprays and hence the GM cotton lines are not a novel source of the Bt toxins. Issues relating to herbicide use falls under the scope of assessments done by the APVMA.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Impacts on disease and pests</b> The DIR066/2006 Application states that Bollgard II®/Roundup Ready Flex® Cotton has no greater susceptibility to or tolerance of pathogens, however no references to scientific research to substantiate this assertion are included and therefore the Regulator should not accept this claim. <i>Fusarium</i> wilt, <i>Alternaria</i> leaf spot, aphids, cluster caterpillars, cotton whitefly as well as a range of other fungus and bacterial diseases favour warm, wet conditions and are potential threats to cotton production in Northern Australia. Understanding the potential interaction between the soil borne fungal disease and the GM cotton lines is critical to identifying whether any management measures can be taken.</p>	EN, Res	Unintended effects, such as an increased disease burden in the GM cotton lines were considered in Chapter 2, Event 31. The diseases mentioned exist in the current cotton growing areas of southern Australia and it is well known that susceptibility to these diseases is very much cultivar dependent. Previous commercial releases of the same GM cotton lines in southern areas did not show increased disease burden. No differences were observed in pest or disease status during field trials of these GM cotton lines in northern Australia.
		<p><b>Impacts on weeds</b> The use of glyphosate on the GM cotton lines in northern Australia at commercial scales will change the types, numbers and rates of emergence of weeds i.e. the weed spectrum, therefore new integrated weed management strategies will be required. How will the risks from changes to weed species when the GM cotton lines be ascertained and assessed? How will effective, enforceable, adaptive herbicide resistance management systems be developed and complied?</p>	HU,HR	OSA. The effect of changed herbicide use and the development of herbicide resistant weeds are part of the APVMA's assessment of an application by Monsanto to change the registration of glyphosate to include use on Roundup Ready or Roundup Ready Flex cotton in Australia.
		<p><b>Other environmental impacts</b> Fundamentally opposed to all broad-scale irrigated agriculture in northern Australia as it is not sustainable. Notes the limitations of the matters the Regulator is to consider when assessing whether to approve a GMO however believes that the requirement for the Regulator to be satisfied that the GMO does not pose a significant risk to the environment includes consideration of the broader environmental impacts of cropping eg pesticide levels in tail water, water use issues</p>	AG,EN	Noted. Sustainability of agricultural practices is outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Site Characteristics</b></p> <p>The Licence Application fails to explore the potential risks associated with northern Australian cotton sites established adjacent to intact bush land, as required by s.2.1.5 (j) of the <i>Gene Technology Regulations 2001</i> (Cth). Although sites in the Ord or the Burdekin are likely to be contained within the existing irrigated agricultural matrix, it is entirely likely that many other sites would be cleared to establish cultivation thus increasing opportunities for interaction with native species, increased chances of dispersal of seeds through irrigation and/or high rainfall events and so on. In relation to risks associated with the commercial release of the GM cotton lines the Regulator needs to consider scientific data which elucidates the key characteristics of potential release sites in northern Australia.</p>	AG, W, Res	<p>Cotton is highly domesticated and has difficulty establishing in non-agricultural environments where there is limited water/nutrients, competition with native plants and insect pressure. There may be increased dispersal of seed from plantings due to irrigation and/or high rainfall events, but dispersal of the GM cotton seed would be no greater than for non-GM cotton. The risk of weediness of the GM cotton lines from seed dispersal is considered in detail (see Ch 4 of RARMP, IR 2, 3 and 4).</p> <p>The assessment of this release has considered all potential areas suitable for cotton growing in northern Australia.</p>
		<p><b>Weediness, volunteers and seed dispersal</b></p> <p>Limited number of weediness studies including Rowena Eastick's 2002 report. The Eastick research does not put beyond reasonable doubt that an advantage is conferred by the Bt genetic manipulation. At best it provides an ambiguous result. There are significant shortcomings to the Eastick report.</p>	W	<p>Additional research (Eastick and Hearnden, in press) conducted pursuant to the initial Eastick 2002 study supported the conclusion of the original report. Cotton is not considered a problematic weed in Australia. The GM cotton lines are susceptible to the same biotic and abiotic factors that limit the persistence of other GM and non-GM cottons (Refer to Ch 4 of RARMP for detailed discussion).</p>
		<p>Reference to a study by Farrell and Roberts (2002) into cotton volunteers from cotton seed transported from Emerald to Atherton in the RARMP fails to quantify the level of risk based on: (1) the overall quantity of seeds transported and provided to the dairies, (2) the proportion of GM cotton seeds of the overall quantity provided to the dairies, and (3) the number of trips to transport the seed.</p> <p>Quantifying the risk of cotton volunteers establishing in these circumstances would facilitate accurate extrapolation for increases in volunteers establishing as a result of increased transport of cotton seed resulting from an approval for commercial release of the GM cottons. The report has not been published in a peer reviewed journal.</p>	W	<p>As discussed in Ch 4, IR 2, 3 and 4, the likelihood of spread and persistence of the GM cotton lines due to expression of the introduced genes was assessed as highly unlikely and would not be greater than the spread and persistence of non-GM cotton.</p> <p>Expression of the introduced proteins would only offer a selective advantage in areas where glyphosate is used to control cotton plants and/or there is high Lepidopteran insect pressure.</p> <p>The occurrence of volunteer plants along roadsides following GM seed transport is expected to be transient rather than indicative of the potential to establish self-sustaining or invasive populations.</p> <p>The OGTR determined that the methodology for this study was sound.</p>
		<p>Concerned over potential seed dispersal:</p> <ul style="list-style-type: none"> <li>- Ability of cotton seed to pass through the gut of non feedlot cattle and subsequently germinate.</li> <li>- Increased transport leading to increased risk of volunteers</li> <li>- Seed dispersal through water particularly flooding events.</li> </ul> <p>Urges the Regulator to seek further information on seed dispersal risks at northern Australian sites, particularly management of seed dispersal during high rainfall events, to inform her decision.</p>	W	<p>As stated above, the presence of volunteer plants arising from the cultivation of a commercial crop and seed transport and dispersal is not expected to lead to the establishment of self-sustaining or invasive populations. The risk of weediness of the GM cotton lines is estimated to be negligible (refer to Ch 4, IR 2, 3 and 4).</p>



Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Gene transfer in the Broome Region</b> From RARMP, <i>G. hirsutum</i> “would be grown on sandy loams” in the Broome region. This is the same soil type preferred by <i>G. rotundifolium</i>. Gene transfer could potentially occur between these species if they were growing in the same region.</p>	GT	As discussed in Ch 2, Event 20, there is negligible risk from outcrossing with native cotton due to genetic incompatibility.
		<p><b>Pollination and pollinators</b> Out crossing rates for cotton may vary seasonally or regionally. Work on pollinators and pollination rates on GM cotton in Australia have provided a recommendation for buffer zones of 20m for field trials. It is well acknowledged that small scale trials cannot provide sufficiently accurate information on potential gene flow rates of larger releases, therefore these recommended buffers are unlikely to be effective in containing biological flows from large scale commercial releases.</p>	GT	The proposal in DIR 066/2006 is for an unrestricted commercial release of the 5 GM cotton lines and therefore no pollen trap is required. Cotton is primarily self-pollinated and cross-pollination occurs at low frequencies within a few metres of the cotton plant. No other sexually compatible plant species occur or are cultivated in northern Australia. The risk of an adverse impact on human health and safety or the environment arising from gene flow to other cottons is estimated as negligible. (see Ch 4, IR 5 and 6).
		<p>The only research in northern Australia on pollination and pollinators was undertaken in the Ord, Kununurra in the 1960s (Thompson 1966). Due to the changes in cultivation in the Ord River Irrigation Area over the last 40 years this information has limited or no application to the current potential pollination of GM cotton crops, or native or weedy relative of cotton in the area. There will be regional differences in pollinator insect populations and different pesticide applications affecting pollinator populations and rates of pollination.</p>	GT	The RARMP concluded that there is negligible risk from outcrossing with either non-GM or other GM cottons, irrespective of whether there is high or low pollinator activity (See Ch 4, IR 5 and 6).
		<p><b>Insect resistance</b> Queries makeup of the “Technical Group for Northern Australia Resistance Management” Could the Regulator please clarify the process for developing Monsanto’s Resistance Management Plan (RMP) and its status as far as binding licence holders?</p>	InR,	Technical Group for Northern Australia Resistance Management is an independent committee that has been established predominantly for the consideration of insect resistance management and consists of scientists and industry representatives. Monsanto’s RMP has been developed with input from this group and compliance would be implemented through a Technology User Agreement between the grower and Monsanto. The Regulator does not require compliance with this RMP as resistance management is an efficacy issue that is regulated by the APVMA. The APVMA requires the implementation of the RMP as part of its registration for Bollgard II®.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p>Monsanto's RMP states that "<i>all refuge requirements have been determined through rigorous scientific research and are based on the equivalent 10% unsprayed cotton refuge</i>" (p.1) however no sources are provided to support this position. Other issues of concern with the RMP indicating its inadequacy in managing insect resistance include:</p> <ul style="list-style-type: none"> <li>- -the use of Bt preparations</li> <li>- -requirement for refuge crops</li> <li>- - requirements for non-GM cotton (when it is not grown in northern Australia)</li> </ul>	IU	OSA. Insecticide use and the management of the development of insect resistance are subject to regulation by the APVMA.
		<p>Queries the planting windows mentioned in Monsanto's RMP and whether planting in both the wet and dry seasons would provide a year long habitat for <i>Heliothis</i> and potentially increase in the development of resistance to Bt toxins.</p>	InR	OSA. Management of the development of insect resistance is subject to regulation by the APVMA.
		<p>Feedback obtained from growers indicates that they do not accept the benefit of watering a refuge crop which is seen by some as a cost burden without a commercial value (CRDC, 2006, p.9). There has also been anecdotal evidence gathered that indicates large percentages of refuges were 'unattractive' to <i>Heliothis amigera</i> throughout the 2004/05 growing season (Cotton Consultants Association, 2005a). The question then of the efficacy of refuge crops that may not be maintained as required by Monsanto's RMP needs to be addressed.</p>	InR	OSA. Management of the development of insect resistance is subject to regulation by the APVMA.
		<p>Field resistance to both Cry1Ac and Cry2Ab has been reported and may pose a considerable threat to the future efficacy of Bt resistant GM crops worldwide. How will the Regulator sufficiently address the risk to the environment and other cropping activities from the development of resistance to Bt toxins? And how will the development of insect resistance be monitored?</p>	InR	OSA. Management of the development of insect resistance is subject to regulation by the APVMA.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p><b>Herbicide resistant weeds</b></p> <p>There is a risk of weeds developing resistance to Roundup Ready® Herbicide due to repeated exposure particularly as:</p> <ul style="list-style-type: none"> <li>- Roundup Ready Flex® Cotton allows the use of Roundup Ready® herbicide throughout the growing season not just before the true 4 leaf stage;</li> <li>- Monsanto's RMP permits the growing of Roundup Ready® Cotton as a refuge crop thereby allowing Roundup Ready® herbicide to be extensively utilized on this crop;</li> <li>- Glyphosate is utilized on other crops and therefore weeds in agricultural areas such as the Burdekin and the Ord River Irrigation Area may already be developing a tolerance to the herbicide, impacting on other agricultural crops;</li> <li>- there are already three glyphosate resistant plants in Australia with one, Annual ryegrass (<i>Lolium rigidum</i>), developing resistance to six different groups of herbicides.</li> </ul> <p>Glyphosate resistant plants are a real problem in cropping situations The Regulator may assess this risk as low, however it must be effectively managed to minimise the likely development of resistance, for example through licence conditions (depending on efficacy of compliance with conditions by growers).</p>	HR	OSA. Changed herbicide use and the development of herbicide resistant weeds are part of the APVMA's assessment of an application by Monsanto to change the registration of glyphosate to include use on Roundup Ready or Roundup Ready Flex cotton in Australia.
		The Regulator needs to consider the potential future risk of gene stacking in northern Australia between Bollgard II®/ RoundupReady® and Liberty Link® Cotton with regard to herbicide resistance.	S, HR	The RARMP concluded that there is negligible risk to people or the environment from outcrossing with Liberty Link® Cotton (see Ch 4, IR 6). Herbicide use and the development of herbicide resistant weeds are subject to assessment by the APVMA.
		The absence of scientifically published data about weed resistance in Australia, in particular in northern Australia, represents a further critical knowledge gap.	HR	OSA. Herbicide use and the development of herbicide resistant weeds are subject to assessment by the APVMA.
		<p><b>Roundup Ready® herbicide use issues</b></p> <p>The behaviour of Roundup Ready® Herbicide with higher temperatures and humidity levels in northern Australia has not been comprehensively explored, however one could speculate that efficacy would be affected due to a greater rate of degradation under higher temperatures. There could also be interaction between metabolites of glyphosate and elevated temperature and humidity which could have unknown effects.</p>	HU,	OSA. Herbicide use is subject to assessment by the APVMA.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		There is limited information to assess the potential impact of Roundup Ready® Herbicide on native species, microorganisms, soil biochemistry and so on in northern Australia to inform whether the herbicide could be used appropriately and effectively.	HU	OSA Herbicide use is subject to assessment by the APVMA.
		For growers, there continue to be issues with re-growth and volunteers “ <i>as is the potential for resistance to glyphosate in the medium to longer term</i> ” (Cotton Consultants Association, 2005b, p.27). Further the use of Roundup Ready Flex® will require a change in weed management and different strategies to managing changing weed spectrums in different regions (Cotton Consultants Association, 2005). How has the Regulator assessed managing these risks?	W, HR	Herbicides are not used to control established cotton plants (both GM and non-GM) and cultivation is the most effective option (See Ch 2, Event 23, and Ch4, IR 2). The development of herbicide resistant weeds is briefly discussed in Ch 2, Event 34. Herbicide use is subject to assessment by the APVMA.
		The production of herbicide tolerant GM plants has resulted in an increase in herbicides on some GM crops around the world.	HU	OSA. Herbicide use is subject to assessment by the APVMA.
		The APVMA should be fully informed of the OGTR’s views on issues relating to Roundup Ready® herbicide to ensure comprehensive assessment.	RA	The OGTR and APVMA have consulted, both formally and informally, regarding the assessment of the GM cotton lines.
		<p><b>Compliance with licence conditions, liabilities and monitoring</b></p> <p>Concerned that the OGTR may not set sufficiently stringent conditions on the commercial use of the GM cottons to ensure protection to human health and safety and the environment. Also concerned about ensuring compliance with licence conditions including appropriate levels of monitoring to encourage compliance and pursue enforcement of breaches. There are no reported monitoring activities by the OGTR in the relevant Quarterly Reports since the commercial release of Bollgard II®/Roundup Ready® cotton in southern Australia. Further Monsanto Australia Ltd states in the DIR066/2006 licence application that they do “...<i>not propose to undertake any monitoring during or after the release is complete</i>” (2006, p.94). It is critical that compliance activities are undertaken by the OGTR as the responsible Commonwealth agency, independent of the Companies selling the seed or the growers growing the product.</p>	LC, H, EN, RM	Noted. The RARMP concludes that risks to human health and safety or the environment from this release are negligible. Therefore no specific management conditions have been imposed. Commercial licences contain conditions that enable oversight of releases to be maintained. To date, there has been no information in Annual Reports or other advice provided to the Regulator to indicate that monitoring of the commercial releases of the GM cotton lines in southern Australia is required.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
		<p>Requests the Regulator to provide details as to how the OGTR is going to effectively manage commercial release activities to ensure compliance with licence conditions and the ongoing protection of the environment and human health from any adverse impacts?</p> <p>There are only common law actions of negligence and nuisance if the release of Bollgard II<sup>®</sup>/Roundup Ready Flex<sup>®</sup> impacts on a third party landholder/occupier. If the environment is impacted upon there are even more remote opportunities for recourse to the law, for example through the State or Territory environmental legislation or the <i>Environment Protection and Biodiversity Conservation Act 1999</i> (Cth).</p>	LC, H, EN, RM	The company must submit a compliance plan which details how licence conditions relating to applicant suitability, auditing and reporting requirements will be met. There is a statutory requirement under the <i>Gene Technology Act 2000</i> to report any unintended effects.
		<p><b>Integrated assessment of environmental risks and impacts of GMOs, and recommendations for intergovernmental action</b></p> <p>Requests consideration of a more integrated approach to assessing environmental risks of GM crops and their associated herbicide use with APVMA and OGTR.</p> <p>The current approach of the OGTR to avoid consideration of matters relating to the use of chemicals associated with particular genetically manipulated crops is an unreasonable case of 'hair splitting'. The OGTR focuses on the risks associated with the genetic manipulation of an organism, however it is as a result of that genetic manipulation, in this case resistance to glyphosate herbicides, that the herbicide will in fact be enabled to be used. Recommends an integrated assessment of environmental risks and impacts of GM crops as well as intergovernmental action between the OGTR and the APVMA, Department of Environment and Heritage, and the States and Territories. Recommends that the Gene Technology Ministerial Committee takes a more proactive role in the release and management of GMOs.</p>		<p>As explained in the RARMP (Technical Summary Section 3.2 and Ch 5 Section 2), the OGTR does not operate in isolation. It is part of an integrated legislative framework for gene technology regulation. The Act establishes the Regulator to identify and manage risks that may be posed by the development and use of GMOs. However, other agencies also have responsibility for regulating products irrespective of whether they are GMOs. This arrangement not only maximises the use of resources and avoids regulatory duplication, it also accommodates the fact that traits, such as herbicide tolerance, can be introduced by other forms of genetic manipulation that are classified as conventional breeding such as the use of chemical mutagenesis and radiation.</p> <p>The Act requires the Regulator to consult twice with these agencies, as well as the Minister for Environment and Heritage and the States and Territories as part of the assessment process for all DIR applications. The <i>Gene Technology (Consequential Amendments) Act 2000</i> requires reciprocal consultation by other regulatory agencies during their assessments of products that are, or contain a product from, a GMO. For more information refer Appendices B &amp; C of <i>Risk Analysis Framework</i>. There has been extensive consultation between OGTR, FSANZ and APVMA on the assessment of applications relating to these GM cotton lines</p>
41	A	<p>Supports the application as believe access to this technology would greatly benefit, not just other farmers but the cotton industry as a whole and also our environment.</p>	B	Noted. Production benefits of gene technology are outside the scope of assessments conducted under the Act.
		<p>This technology allows us to become more environmentally friendly and reduce our reliance on conventional chemistry, thus reducing resistance from pests.</p>	AG	Noted. Environmental benefits are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
42	I	Supports the application as feels cotton could have a great benefit in the Dry Tropics region of Qld.	None	Noted.
		Some areas of Qld are suitable for cotton growing and growers would appreciate a viable alternative to horticulture and sugar cane.	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
43	A	Supports the application as current cotton licence conditions allow some growers in the region to plant GM cotton, but not all as some are above 22° South. Bollgard II is vital for growers wanting to grow cotton in the warmer tropical regions and so having this technology gives growers another cropping option and improve the chance of remaining viable.	EC, AG	Noted. Production benefits and economic issues are outside the scope of assessments conducted under the Act.
44	A	Supports the application as the introduction of GM technology has meant greater certainty of yields and therefore more sustainable farming practice. No longer have chemical drift problems from one crop to another. GM technology has been embraced by all farmers in the area with no adverse affects of failure under extreme conditions.	AG	Noted. Production and environmental benefits are outside the scope of assessments conducted under the Act.
		Comments that insect resistance is a major challenge to the industry with fewer chemical companies funding R&D and the only new products being prohibitively expensive. GM technology being the exception.	IR	Noted. The management of insect resistance is the responsibility of the APVMA.
45	I	Supports the application as have seen the results of cotton trials and have contributed to WA Dept Agriculture research program.	None	Noted.
		Accessing RR and Bollgard II cotton will enable improvement of farming practices, better risk management and would compliment existing sugar cane cropping plans. Having two viable crops in the area will enhance diversity and ensure strong, viable agriculture for future Ord River Irrigation developments.	AG, EC	Noted. Agricultural production issues and economic benefits are outside the scope of assessments conducted under the Act.
46	I	Supports the application as have benefited from 10 years of successful GM cotton and these benefits should be available to all farmers eg reduction in insecticides need to control the major pest Heliothis. Weed control has been aided by the introduction of Roundup Ready..	B, AG	Noted. Production benefits of gene technology are outside the scope of assessments conducted under the Act.
		Confident in the regulatory and scientific based assessment of new technology.	None	Noted.
		Monsanto and the cotton industry have developed a comprehensive management system to monitor all aspects of GM cotton production to ensure all regulatory guidelines are met and the potential for resistance is kept to an absolute minimum. Personal experience has shown the audit process adopted is very comprehensive and driven towards the longevity of GM crops within our industry.	AG	Noted. Agricultural production issues and resistance management are outside the scope of assessments conducted under the Act.

Sub	Type <sup>a</sup>	Summary of issues raised	Issue	Consideration of issue
47	I	Supports the application as 10 years of growing GM cotton have had the following benefits: <ul style="list-style-type: none"> <li>- Reduced chemical costs</li> <li>- Improved pest control</li> <li>- Less reliance on insecticides so improved health benefits</li> <li>- Reduced tillage</li> </ul>	B,AG	Noted. Health, environmental and production benefits of gene technology are outside the scope of assessments conducted under the Act.
48	I	Supports the application. as it will provide a diverse crop mix which is important for an economic business and necessary for good agronomic practices. GM cotton will give new opportunities for the entire region.	AG, EC	Noted. Agricultural production issues and economic issues benefits are outside the scope of assessments conducted under the Act.
		Australia has the world's best cotton farmers, producing 240% above the world average in lint yield per hectare. It is a low water and chemical usage crop, and would fit very well into our production program.	AG	Noted. Agricultural production issues are outside the scope of assessments conducted under the Act.
		Ord Land and Management Plan states on page 35, 3.4.5 strategy 2: Genetic modification is a desirable control measure in adopting and the success of Integrated Pest Management. The plan was published in 2000 and was written by all sectors of the community.	None	Noted.
49	I	Supports the application as believes GM cotton is another opportunity crop for the Ord River Irrigation Area.	None	Noted.
		Wouldn't want GM cotton if felt there would be a risk to the environment but is confident in the scientific work that has been done for GM cotton in southern Australia, and as such should have same opportunities in northern Australia.	None	Noted. The risks to the environment from this release have been assessed as negligible.
		Concerned that if GM cotton isn't licensed, then standard cotton may be grown with possible detrimental effects to the environment as seen in late 1960's	AG	Noted. Sustainability of agricultural practices are outside the scope of assessments conducted under the Act.
50	I	Supports the application as due to the enormous benefits.	B	Noted. Benefits of gene technology are outside the scope of assessments conducted under the Act.
		Believes the technology can be managed responsibly and the regulatory process and scientific assessments is taken extremely seriously to help ensure continued access and the longevity of the technology	None	Noted.
		It cannot be understated how much impact gene technology, especially Bollgard II <sup>®</sup> and Roundup Ready <sup>®</sup> crops has had on reducing the environmental footprint of cotton production not only on the Darling Downs, but in every other cotton producing area. Bollgard II <sup>®</sup> crops form the cornerstone of the production systems in conjunction with Integrated Pest Management systems.	AG	Noted. Environmental benefits are outside the scope of assessments conducted under the Act.