



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 087**

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

Applicant: Bayer CropScience Pty Ltd

December 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance into the environment in respect of application DIR 087 from Bayer CropScience Pty Ltd (Bayer)

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public¹.

The application

Bayer applied for a licence for dealings involving the intentional release of genetically modified (GM) cotton on a limited scale and under controlled conditions. The cotton plants have been genetically modified for insect resistance and herbicide tolerance. The trial would take place at one site in the local government area of Narrabri, NSW on a maximum total area of 0.36 ha over the summer growing season 2008-2009.

The GM cotton contains three introduced genes encoding proteins expected to confer insect resistance and herbicide tolerance. All three genes have been isolated from bacteria.

The purpose of the trial is to conduct research to evaluate the agronomic performance of the GM cotton plants and to assess the efficacy of the insecticidal protein combination against cotton bollworm. Cotton seed will also be collected and used for further research and development (subject to additional approvals) The GM cotton will not be used for human food or animal feed.

Bayer proposed a number of controls to restrict the dissemination or persistence of the GM cotton and the introduced genetic materials into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including details of the GM cotton breeding program, expression levels of the three introduced genes, toxicity of the insecticidal proteins against target organisms, details of the plasmid vector and construct used for the *cry2Ae* transformation event and some details of the *cry2Ae* gene, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP.

A **hazard** identification process was used in the first instance to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Eight events were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the eight events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton into the environment are considered to be **negligible**. Hence, the Acting Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

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. As none of the eight events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

The licence conditions require Bayer to **limit** the release to a total area of 0.36 ha at one site over the summer cotton growing season 2008-2009. The **control** measures to restrict the dissemination and persistence of the GMO include containment provisions at the trial site, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of GM cotton on a maximum total area of 0.36 ha over one growing season in the NSW local government area of

Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic materials in the environment and to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

Table of Contents

EXECUTIVE SUMMARY	I
INTRODUCTION	I
THE APPLICATION	I
CONFIDENTIAL COMMERCIAL INFORMATION.....	I
RISK ASSESSMENT.....	II
RISK MANAGEMENT	II
CONCLUSIONS OF THE RARMP	II
TABLE OF CONTENTS	IV
ABBREVIATIONS	VI
TECHNICAL SUMMARY	1
INTRODUCTION	1
THE APPLICATION	1
CONFIDENTIAL COMMERCIAL INFORMATION.....	1
RISK ASSESSMENT.....	2
RISK MANAGEMENT	3
LICENCE CONDITIONS TO MANAGE THIS LIMITED AND CONTROLLED RELEASE.....	3
OTHER REGULATORY CONSIDERATIONS	4
IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES	4
SUITABILITY OF THE APPLICANT	5
CONCLUSIONS OF THE RARMP	5
CHAPTER 1 RISK ASSESSMENT CONTEXT	6
SECTION 1 BACKGROUND	6
SECTION 2 THE LEGISLATIVE REQUIREMENTS	7
SECTION 3 THE PROPOSED DEALINGS.....	7
3.1 The proposed activities	8
3.2 The proposed limits of the dealings (size, location and duration).....	8
3.3 The proposed controls to restrict the dissemination or persistence of the GMO and its genetic material in the environment.....	8
SECTION 4 THE PARENT ORGANISM.....	8
SECTION 5 THE GMO, NATURE AND EFFECT OF THE GENETIC MODIFICATION.....	9
5.1 Introduction to the GMO.....	9
5.2 The introduced genes and their encoded proteins	10
5.3 The regulatory sequences	14
5.4 Method of genetic modification	15
5.5 Characterisation of the GMO.....	15
SECTION 6 THE RECEIVING ENVIRONMENT.....	16
6.1 Relevant abiotic factors.....	16
6.2 Relevant biotic factors.....	17
6.3 Relevant agricultural practices	17
6.4 Presence of related plants in the receiving environment	17
6.5 Presence of the introduced or similar genes and encoded proteins in the environment	17
SECTION 7 AUSTRALIAN AND INTERNATIONAL APPROVALS	18
7.1 Australian approvals of the GM cotton	18
7.2 International approvals.....	19
CHAPTER 2 RISK ASSESSMENT	20
SECTION 1 INTRODUCTION	20
SECTION 2 HAZARD CHARACTERISATION AND THE IDENTIFICATION OF RISK	21
2.1 Production of a substance toxic/allergenic to people or toxic to other organisms.....	23
2.2 Spread and persistence of the GM cotton in the environment.....	25
2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	27
2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms.....	28
2.5 Unintended changes in biochemistry, physiology or ecology	31

2.6	Unauthorised activities.....	32
SECTION 3	RISK ESTIMATE PROCESS AND ASSESSMENT OF SIGNIFICANT RISK	33
SECTION 4	UNCERTAINTY.	33
CHAPTER 3	RISK MANAGEMENT.....	35
SECTION 1	BACKGROUND	35
SECTION 2	RESPONSIBILITIES OF OTHER AUSTRALIAN REGULATORS	35
SECTION 3	RISK TREATMENT MEASURES FOR IDENTIFIED RISKS.....	36
SECTION 4	GENERAL RISK MANAGEMENT	36
4.1	Licence conditions	36
4.2	Other risk management considerations	38
SECTION 5	ISSUES TO BE ADDRESSED FOR FUTURE RELEASES	40
SECTION 6	CONCLUSIONS OF THE RARMP	40
REFERENCES	41
APPENDIX A	DEFINITIONS OF TERMS IN THE RISK ANALYSIS FRAMEWORK USED BY THE REGULATOR.....	48
APPENDIX B	SUMMARY OF ISSUES RAISED IN SUBMISSIONS RECEIVED FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES ON THE CONSULTATION RARMP FOR DIR 087.....	50
APPENDIX C	SUMMARY OF ISSUES RAISED IN SUBMISSIONS RECEIVED FROM THE PUBLIC ON THE CONSULTATION RARMP FOR DIR 087	52

Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
<i>bar</i>	Gene encoding the PAT protein from <i>Streptomyces hygroscopicus</i>
Bt	<i>Bacillus thuringiensis</i>
CCI	Confidential Commercial Information as declared under section 185 of the Act
<i>cry1Ab</i>	Gene encoding the Cry protein from <i>Bacillus thuringiensis</i>
Cry1Ab	Cry protein from <i>Bacillus thuringiensis</i>
<i>Cry2Ae</i>	Gene encoding the Cry protein from <i>Bacillus thuringiensis</i>
Cry2Ae	Cry protein from <i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare(s)
LD ₅₀	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PAT	PAT protein from <i>Streptomyces hygroscopicus</i>
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
SCSV	Subterranean clover stunt virus
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration

Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance in respect of licence application DIR 087 from Bayer CropScience Pty Ltd (Bayer).

The *Gene Technology Act 2000* (the Act), the *Gene Technology Regulations 2001* and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Gene Technology Regulator (the Regulator) before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies, authorities and the public².

The application

Bayer applied for a licence for dealings involving the intentional release of cotton (*Gossypium hirsutum* cv. Coker) that has been genetically modified for insect resistance and herbicide tolerance on a limited scale and under controlled conditions. The trial is authorised to take place at one site in the local government area of Narrabri, NSW on a maximum total area of 0.36 ha over the summer cotton growing season of 2008-2009.

The GM cotton (known as TwinLink[®]) contains three introduced genes encoding proteins expected to confer insect resistance and herbicide tolerance. These genes are *cry1Ab* and *cry2Ae* from the bacterium *Bacillus thuringiensis*, encoding Bt toxins that are active against lepidopteran insect pests, and the *bar* gene from the bacterium *Streptomyces hygroscopicus*, encoding the enzyme phosphinothricin acetyltransferase (PAT) which provides tolerance to herbicides containing glufosinate ammonium.

The purpose of the trial is to conduct research to evaluate the agronomic performance of the GM cotton plants and to assess the efficacy of the insecticidal protein combination against *Helicoverpa armigera* (cotton bollworm). Cotton seed will be collected and used for further research and development (subject to additional approvals). The GM cotton will not be used for human food or animal feed.

Bayer proposed a number of controls to restrict the dissemination or persistence of the GM cotton and the introduced genetic materials into the environment. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including details of the GM cotton breeding program, expression levels of the three introduced genes, toxicity of the insecticidal proteins against target organisms, details of the plasmid vector and construct used for the *cry2Ae* transformation event and some details of the *cry2Ae* gene, have been declared Confidential Commercial Information (CCI) under

² More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

section 185 of the Act. The confidential information has been made available to the prescribed experts and agencies that have been consulted on the RARMP for this application.

Risk assessment

The risk assessment considered information contained in the application, relevant previous approvals, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities (included in Appendix B of the RARMP) as well as the public on the application (included in Appendix C of the RARMP). No new risks to people or the environment were identified from the advice received on the consultation RARMP.

A reference document on the parent organism, *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*, was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

Eight events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

The characterisation of the eight events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- limits on the size, location and duration of the release proposed by Bayer
- suitability of controls proposed by Bayer to restrict the dissemination or persistence of the GM cotton plants and their genetic material
- limited ability and opportunity for the GM cotton to transfer the introduced genes to commercial cotton crops or other sexually related species
- limited capacity of the GM cotton to spread and persist outside the areas proposed for release
- none of the GM plant materials or products will be used in human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity to non-target organisms or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton into the environment are considered to be **negligible**.

Hence, the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

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. As none of the eight events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

The Acting Regulator has imposed a number of licence conditions to limit and control the release, including requirements to:

- conduct the release on a total area of up to 0.36 ha at one site in the NSW local government area of Narrabri, over the summer growing season 2008-2009
- surround the release site with a 20 m pollen trap
- locate the trial site at least 50 m away from natural waterways
- harvest and gin seed cotton from the release separately from any other cotton crop
- not permit any materials from the release to be used in human food, or animal feed or for the production of fabrics and/or other cotton products
- destroy all plant materials not required for further analysis or future release
- following harvest, clean the site and equipment used on the site
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- monitor the site for at least 12 months and destroy any cotton plants that may grow until no volunteers are detected for a continuous 6 month period.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, July 2007*³; *Policy on transport and supply of GMOs, July 2005*⁴). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMO for the purposes of, or in the course of, the authorised dealings.

³Guidelines for the transport of GMOs

<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>>

⁴Policy on transport and supply of GMOs

<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/policies-1>>

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁵.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM cotton proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM cotton. FSANZ approval would need to be obtained before it could be used in human food in Australia.

APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. Bayer would require a research permit from APVMA for the proposed release of the GM cotton containing the insecticidal genes. Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial. If the applicant decided to apply glufosinate ammonium a research permit would be required.

AQIS is responsible for monitoring imports to prevent the introduction of exotic pests and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs, or products known to be mixed with any amount of GM material. As the importation would constitute a dealing under the Act, the importer requires an authorisation under this Act for the import to lawfully proceed. Seeds from TwinLink[®] cotton have been imported by CSIRO under an AQIS seed import permit.

No other approvals are required.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of any of the GM cotton, or to justify a reduction in containment conditions. This would include:

- characterisation of the genetic material inserted into the plants, including gene copy number and genotypic stability
- additional data on the potential toxicity of plant materials from the GM cotton, specifically in regard to the Cry2Ae protein alone and in combination with Cry1Ab
- additional data on the allergenicity of proteins encoded by the introduced genes for insect resistance, specifically that relating to Cry2Ae

⁵ 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/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

- characteristics indicative of weediness including measurement of altered reproductive capacity, and disease and insect resistance.

Suitability of the applicant

The Acting Regulator determined, at the commencement of the assessment process for this application, that Bayer is suitable to hold a DIR licence under the requirement of section 58 of the Act. The Acting Regulator is satisfied that Bayer remains suitable as no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has confirmed its ability to comply with the licence conditions.

Conclusions of the RARMP

The risk assessment concludes that this proposed limited and controlled release of GM cotton plants on a maximum total area of 0.36 ha over one growing season in the NSW local government area of Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the dissemination and persistence of the GMO and its genetic material in the environment and to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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 legislation⁶, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

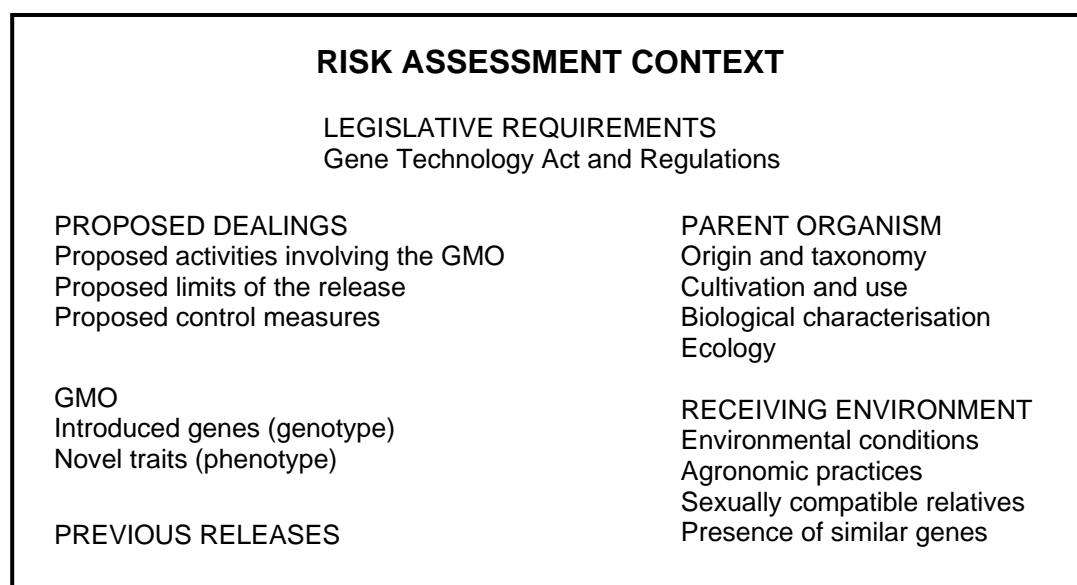


Figure 1. Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:
- the proposed dealings (Section 3.1)
 - the limits proposed by the applicant (Section 3.2)
 - the controls proposed by the applicant (Section 3.3)
 - characteristics of the parent organism (Section 4)
 - the nature and effect of the genetic modification (Section 5)
 - the environmental conditions in the location where the release would occur (Sections 6.1 and 6.2)
 - relevant agricultural practices (Section 6.3)
 - the presence of related plants in the environment (Section 6.4)
 - the presence of the introduced or similar genes in the environment (Section 6.5)

⁶ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>> and in the *Risk Analysis Framework* (OGTR 2007) <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

- any previous releases of these or other GMOs relevant to this application (Section 7).

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom she must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Acting Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMO or its genetic material in the environment. Those controls and limits are such that the Acting Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application qualifies as a limited and controlled release and the Acting Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities and their consideration is summarised in Appendix B. Two submissions were received from the public and their consideration is summarised in Appendix C.

6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

7. Bayer CropScience Pty Ltd (Bayer) proposes to release cotton plants (known as TwinLink[®]) that have been genetically modified for insect resistance and herbicide tolerance into the environment under limited and controlled conditions.

8. Some details, including details of the GM cotton breeding program, expression levels of the three introduced genes, toxicity of the insecticidal proteins against target organisms, details of the plasmid vector and construct used for the *cry2Ae* transformation event and some details of the *cry2Ae* gene, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the Risk Assessment and Risk Management Plan (RARMP) for this application.

3.1 The proposed activities

9. The applicant has stated that the principal purpose of the proposed release is to conduct experiments to evaluate the agronomic performance of the GM cotton plants and to assess the efficacy of the insecticidal protein combination against *Helicoverpa armigera* (cotton bollworm). TwinLink[®] plants and non-GM cotton plants of the parental Coker varieties would be exposed to natural and artificially induced infestations of the target insect pest and compared with plants where insect predation is controlled using selective insecticides. Cotton seed would also be collected and used for further research and development (subject to additional approvals). The GM cotton will not be used for human food or animal feed.

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

10. The applicant proposes to limit the release to one site in the local government area of Narrabri, NSW on a total area of 0.36 ha. Comparative non-GM parental plots would be planted alongside the GM cotton plants and the site surrounded by a 20 metre pollen trap. The release is proposed to occur over one cotton growing season between 2008 and 2009.

3.3 The proposed controls to restrict the dissemination or persistence of the GMO and its genetic material in the environment

11. The trial would be carried out on well-established farmland under the supervision of a project supervisor. The supervisor would be responsible for overseeing the management and training of third party contractors by qualified staff based at Bayer and only trained and authorised staff would be permitted access to the proposed location

12. The applicant proposed a number of controls to restrict the dissemination or persistence of the GM cotton and the introduced genetic material in the environment including:

- locating the trial site at least 50 metres away from natural waterways
- surrounding the site with a 20 metre pollen trap
- harvesting and ginning seed cotton from the release separately from any other cotton crop
- not permitting any materials from the release to be used in human food, or animal feed or for the production of fabrics and/or other cotton products
- destroying all plant materials not required for further analysis or future release
- cleaning the site after harvest by slashing and incorporating all plant material into the soil
- monitoring the trial site for at least 12 months and destroying any cotton volunteers
- transporting GM seed and plant materials in accordance with OGTR transportation guidelines.

13. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for restricting the proposed release is evaluated in Chapter 3, Section 4.1.1.

Section 4 The parent organism

14. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in NSW and southern and central Queensland. The cultivars Coker 312 and 315 were used as a starting point for this research as Coker cultivars are amenable to genetic modification in the laboratory. They are not grown

commercially in Australia. Further detailed information about the parent organism is contained in a reference document, *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* that was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR website <<http://www.ogtr.gov.au>> under Publications/Risk Assessment References. The document is also available on request by contacting the OGTR by email <ogtr@health.gov.au> or telephone 1800181030.

Section 5 The GMO, nature and effect of the genetic modification

5.1 Introduction to the GMO

15. The GM cotton proposed for release contains three genes encoding proteins expected to enhance insect resistance and herbicide tolerance (Table 1). Two of the genes, *cry1Ab* and *cry2Ae*, are modified *cry* genes derived from the bacterium *Bacillus thuringiensis* and encode Bt toxins which are active against lepidoteran insect pests. The third gene, *bar*, is derived from the bacterium *Streptomyces hygroscopicus* and encodes the enzyme phosphinothricin acetyltransferase (PAT) which provides tolerance to herbicides containing glufosinate ammonium.

16. The TwinLink[®] plants were generated by conventional crossing of event T304-40 (containing *cry1Ab* and *bar*), with event GHB119 (*cry2Ae* and *bar*), followed by backcrossing to homogeneity. Event T304-40 is in a Coker 315 genetic background, and event GHB119 is in a Coker 312 genetic background. TwinLink[®] cotton is thus the combination of events T304-40 and GHB119 in a Coker 312/315 background. The GM cotton plants contain two copies of the *bar* gene, which was used as a selectable marker throughout the development of TwinLink[®].

Table 1. The genes used to genetically modify cotton

Gene	Accession No (GenBank)	Protein produced	Comment	Source	Intended purpose
<i>Cry1Ab</i>	X04698	Bt toxin	Cry genes encode crystalline insecticidal proteins, highly specific to their target insects and used for insect pest control	<i>B.thuringiensis</i>	Insect resistance
<i>Cry2Ae</i>	AAQ52362	Bt toxin	Cry genes encode crystalline insecticidal proteins, highly specific to their target insects and used for insect pest control	<i>B.thuringiensis</i>	Insect resistance
<i>bar</i>		PAT (phosphinothricin acetyltransferase)	Marker gene widely used in plant genetic modification; the encoded enzyme, PAT, confers tolerance to phosphinothricin herbicides	<i>Streptomyces hygroscopicus</i>	Herbicide tolerance

17. The construct used to generate transformation event T304-40 is shown in Table 2. Details of the plasmid vector and construct used for event GHB119, containing *cry2Ae* and *bar*, have been declared CCI

Table 2. Gene construct used to generate event T304-40

Vector	Promoter	Gene of interest	Terminator	Event
pTDL008	Ps7s7	<i>Cry1Ab</i>	me1	T304-40
	35S (P335S3)	<i>Bar</i>	nos	

5.2 The introduced genes and their encoded proteins

5.2.1 *Cry genes expected to confer insect resistance and their encoded proteins*

18. The GM cotton plants contain two insecticidal genes, *cry1Ab* and *cry2Ae*, both derived from a common soil bacterium *Bacillus thuringiensis* (Bt). The revised *Cry* gene nomenclature is used here, wherein delta-endotoxins are classified into classes according to amino acid sequence similarities (Crickmore et al. 1998). These classes are comprised of several subclasses which are themselves subdivided into subfamilies or variants based on amino acid sequence homology.

19. Bt produces insecticidal delta-endotoxins (Bt toxins) in the form of crystalline proteins that become active when ingested by insects and are highly specific to a few species in particular orders of insects (Coleoptera, Diptera, Lepidoptera, Hymenoptera and nematodes) (de Maagd et al. 2001). Over 380 *cry* genes encoding delta-endotoxins have been cloned and sequenced (Crickmore et al. 2008) and members of the *Cry* family are widely used for insect control. Their mode of action has been well characterised, principally in lepidopteran insects (see review, (Bravo et al. 2007) and section 5.2.2 below). When expressed, *cry1A* and *cry2A* genes produce insecticidal crystal proteins that are specific to lepidoptera (*cry1A*) or to lepidoptera and diptera species (*cry2A*).

20. The *cry1Ab* gene in TwinLink[®] cotton was isolated from the *B. thuringiensis* subspecies berliner 1715 (Hofte et al. 1986) and the gene sequence has been modified for expression in plants. The sequence of the expressed *Cry1Ab* protein has been compared with the sequences of *Cry1Ab* proteins used in commercial bio-pesticides such as Dipel[®] and in commercialised GM corn events such as Bt11, which have been approved world-wide by various agencies. The deduced amino acid sequence is identical to the native protein except that it is truncated at the C-terminal end and an alanine has been inserted at the N-terminal end. The truncated *Cry1Ab* contains the region responsible for insecticidal activity and is expected to be further processed in the insect gut.

21. The *cry2Ae* gene has been isolated more recently, from *B. thuringiensis*. The gene is similar to other *cry2A* genes used in commercial Bt products and has been modified for expression in plants.

5.2.2 *Toxicity/allergenicity of the Cry proteins encoded by the introduced genes*

22. *Bacillus thuringiensis* (Bt) is found in soil and plant communities worldwide and strains have been isolated from habitats including soil, insects, stored-product dust and deciduous and coniferous leaves (Schnepf et al. 1998 and references therein). Individual strains of Bt may produce up to six different *Cry* proteins.

23. Microbial preparations of Bt have also been used for decades as a pesticide, being first commercialised as insecticidal products in France in the late 1930s. Since then there have been numerous commercial releases of Bt insecticidal products and of crops genetically modified to express delta-endotoxins for insect resistance (Sanchis & Bourguet 2008). On this basis people and other organisms have a long history of exposure to Bt toxins.

Toxicity to target organisms and interactions between Bt toxins

24. As outlined above (Section 5.2.1), individual *Cry* proteins have a narrow spectrum of action against specific insect pests within the coleoptera, diptera and lepidoptera. 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 (Hofmann et al. 1988; Van Rie et al. 1989; English & Slatin 1992; Knowles & Dow 1993).

25. The Cry1A proteins are a closely related group and their toxicity is highly specific to lepidopteran insects (Macintosh et al. 1990). Cry 2A proteins have dual toxicity to lepidopteran and dipteran insect species. Purified toxins belonging to the Cry 1A and Cry2A delta-endotoxins show different, but overlapping, toxicity spectra against lepidopteran insects and appear to have differing modes of action. In competition experiments measuring binding of labelled Cry1Ab or Cry2Ab proteins to membrane vesicles from larval midgut of *H. armigera*, Cry2Ab did not compete for either Cry1Ac or Cry1Ab binding sites (Estela et al. 2004).

26. Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab are among the most toxic Cry proteins against *H. armigera*, *H. zea*, *H. punctigera* and *Heliothis virescens* (Liao et al. 2002). *H. armigera* and *H. punctigera* are major pests of cultivated cotton in Australia (Widner & Whiteley 1990; Dankocsik et al. 1990; Macintosh et al. 1990). While the toxicity profile for Cry1Ab is thus well-described, there is no published information relating to toxicity of purified Cry2Ae against target species. However, based on data for other Cry2A proteins (such as Cry2Ab) it would be expected to show a similar activity spectrum.

27. Prior to the development of GM plants containing individual *cry* genes, most Bt toxicity studies used bacterial formulations or isolated protein fractions. A number of these studies showed synergistic effects between the Cry proteins ie some combinations showed greater activity than would be expected from the activity of the individual fractions (Schnepf et al. 1998; Glare & O'Callaghan 2000). Additive and antagonistic effects have also been noted for some Cry protein combinations (del Rincon-Castro et al. 1999). The mechanism of such interactions is unclear, but a number of factors appear to be involved, including the particular protein combinations and the target insects.

28. For GM plants expressing more than one *cry* gene, synergistic, additive or antagonistic interactions between the expressed toxins might also be expected after ingestion by susceptible insect species. There have been some reports of such effects, but the results have been difficult to interpret and inconclusive.

Toxicity to non-target mammals, birds and fish

29. It is unlikely that mammals are susceptible to Cry proteins. This is partly because the alkaline conditions required to activate the toxin do not exist in the guts of mammals and because the toxic effects of Cry proteins are mediated through binding to receptors in the mid gut of target insects, which are not present in mammals, birds and fish.

30. The toxicological database on *B.thuringiensis* shows no mammalian health effects attributable to delta-endotoxins. In particular, the European Food Safety Authority (EFSA) evaluated the food safety of delta-endotoxins expressed in maize plants, such as Cry1Ab in Bt11 (EFSA 2005) and purified Cry proteins, including Cry1Ab, have been assessed in feeding assays. These studies and acute oral toxicity studies (Mendelsohn et al. 2003; OECD 2007) also confirm the low toxicity of the Cry proteins studied. In tests examining the toxicity of Cry1Ab on mammalian cells, it was found that Cry1Ab has little acute toxicity to bovine hepatocytes (Shimada et al. 2003). No specific studies have been reported for the Cry2Ae toxin, but based on homology with other Cry2A proteins and its target specificity it would be expected to show similar low toxicity.

Toxicity to non-target arthropods

31. A number of large-scale studies on the effects of Bt crops (including those containing Cry1Ab) have been published in recent years. A Bt crops reassessment study carried out by the US EPA in 2001 indicated that there was no difference in the total number of insects, or the numbers of insects of specific orders, between GM crop plots and either isogenic or wild-type control plots. Toxicity data for Bt crops (including Cry1Ab corn) confirmed that the only susceptible non-target species were amongst the Lepidoptera (US EPA 2001; Mendelsohn et al. 2003).

32. Numerous individual studies have also been conducted to assess possible lethal effects of Cry proteins on non-target invertebrates. Recent meta-analyses using data from these studies found no uniform negative or positive effects of the toxins, depending on what pest management practices were compared. One of these analyses, using data from 42 field experiments, identified an increase in total abundance of non-target invertebrates in Bt cotton and maize fields as compared with non-GM fields managed with pesticides. However, in comparison with insecticide-free control fields, certain non-target taxa were less abundant in Bt fields (Marvier et al. 2007). Meta-analysis of a modified public database showed no differences in abundance of functional guilds of non-target arthropods when sprayed Bt crops were compared with sprayed non-Bt controls (Wolfenbarger et al. 2008).

33. Effects of Cry toxins on important pollinators such as honeybees have also been investigated. Cotton pollen is an important food source for bees and the possibility of lethal and sublethal effects has been raised. However, a meta-analysis of studies that assessed the potential effects of Cry proteins (including Cry1Ab) on honey bees supported a conclusion that the proteins had no adverse effect (Duan et al. 2008). No similar studies relating to Cry2Ae have been published.

Toxicity to soil microorganisms

34. GM crops containing cry genes release the insecticidal proteins to soil via plant residues and root exudates, giving rise to potential effects on soil microorganisms.

35. (Icoz & Stotzky 2008) conducted a four year field study in which three Bt corn varieties that express Cry1Ab protein (events Bt11 and MON810) were compared with near-isogenic non-Bt corn varieties and evaluated for their effects on microbial diversity and on the activity of enzymes involved in degradation of plant biomass. After four years there were found to be no consistent statistically significant differences in the numbers of different groups of microorganisms, the activities of the enzymes and the pH between soils planted with Bt and non-Bt corn.

36. In soils where Bt cotton expressing Cry1Ac was grown and compared to isogenic non-Bt lines for biological and biochemical indicators, data showed some positive and no negative effects of Bt cotton on studied indicators. It was concluded that there was no risk to soil ecosystem functions (Sarkar et al. 2008).

Sublethal effects on non-target organisms

37. There have been few studies addressing the potential for sublethal physiological and behavioural effects of Cry toxins on non-target organisms. Such effects may include feeding and mating behaviours and general fitness.

38. Honeybees have been identified as a particular cause for concern with respect to sublethal effects and when Cry1Ab toxin was included in artificial feeding syrup at concentrations of 5000 ppb (Ramirez-Romero et al. 2008) effects on honeybee feeding behaviours and learning performance were observed. The toxin levels used in the study were

based on concentrations in the pollen of non-commercial maize plants in which *cry* was under the control of a pollen specific promoter (Fearing et al. 1997). Such concentrations have not been found to be present in the pollen of commercial GM cotton plants (Mendelsohn et al. 2003). Furthermore, after modelling for pollen ingestion under natural conditions, no risk was identified by the authors.

Allergenicity

39. Previous assessment of GM cotton containing the *cry1Ab* and *bar* genes (DIR016/2002) concluded that the GMO was unlikely to prove more allergenic to humans or animals than non-GM cotton. Cry1Ab does not display characteristics common to food allergens including: presence as a major component of food, being a derivative of a known allergenic source, glycosylation, or resistance to degradation by heat, acid and proteases of the digestive system.

40. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The amino acid sequence of the Cry1Ab protein was compared with sequences of proteins in publicly available databases. No similarities with known allergens or mammalian toxins were observed based on a criterion of 35% identity over 80 amino acids. No similarities between the Cry1Ab protein and known allergens were identified based on 100% identity over an eight amino acid segment (US EPA 2007). Similar analysis has not been performed with Cry2Ae but based on the lack of allergenic potential identified for other Cry2A proteins such as Cry2Ab, Cry2Ae would be predicted to have similar lack of allergenicity.

41. In summary, a comprehensive search of the scientific literature has yielded no information to suggest that any of the encoded Cry proteins are toxic or allergenic to people, or toxic to organisms other than the intended target species.

5.2.3 The herbicide tolerance marker gene (*bar*) and the encoded protein

42. The GM cotton proposed for release also contains two copies of the *bar* herbicide tolerance marker gene that was isolated from *S. hygroscopicus*, a common saprophytic, soil-borne microorganism (Thompson et al. 1987). The *bar* gene encodes the PAT protein, which confers tolerance to glufosinate ammonium, the active component in a number of herbicides. Glufosinate ammonium was used for selection in laboratory cultures during the initial stage of selection of GM plants and applied to whole plants in the field. Thus no other selectable marker was required.

43. Glufosinate ammonium is widely used as a broad-spectrum herbicide and is registered for use in many countries. Herbicides containing glufosinate ammonium as the active constituent are currently registered in Australia by the Australian Pesticides and Veterinary Medicines Authority (APVMA) as Basta[®] for horticultural use and non-agricultural use, Finale[®] for home garden and non-agricultural use, and Liberty[®] for use on GM InVigor[®] hybrid canola and GM Liberty Link[®] Cotton varieties.

44. The presence of two copies of the *bar* gene is likely to result in higher levels of PAT production in TwinLink[®] plants compared to T304-40 or GHB119 plants. Other regulatory agencies, both in Australia and in other countries, have previously assessed the *bar* gene, or related *pat* gene encoding the same PAT enzyme, as safe for use in human food. In addition, a number of GM crops, including food crops, containing the *bar* gene have been approved for commercial release both in Australia (DIR 021/2002 and DIR 062/2005) and overseas. No adverse effects on humans, animals or the environment have been reported from any releases.

45. For more detailed information on the *bar* gene and the encoded protein refer to the RARMP prepared for DIR 062/2005 (Liberty Link[®] Cotton) available at <<http://www.ogtr.gov.au>>

5.2.4 Toxicity/allergenicity of PAT

46. PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria as well as in other GM crops approved for commercial release. The PAT protein expressed in the GM cotton plants proposed for release is the same as that present in commercially approved InVigor[®] hybrid canola (DIR 021/2002) and Liberty Link[®] Cotton (DIR 062/2005). Extensive toxicity studies using the purified form of the PAT protein have been conducted and have shown that the PAT protein is not likely to be toxic or allergenic to humans. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 021/2002 and DIR 062/2005.

47. Food Standards Australia New Zealand (FSANZ) has approved the use of food derived from other GM plants containing either the *bar* or *pat* gene, including GM cotton, corn, canola, rice and soybean, concluding that the PAT protein is not toxic (eg ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; FSANZ 2003; FSANZ 2005; FSANZ 2008). The studies submitted in support of the food uses for this protein indicate that it has none of the properties associated with protein toxins or allergens.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the introduced genes

48. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. Two independent transformation events were used in a conventional cross, then backcrossed to generate the proposed release. Information on the promoters and terminators for one of the events (T304-40) is summarised in Table 3. Information on regulatory sequences for the other event (GHB119) has been declared CCI.

Transformation event T304-40:

49. The promoter of the *cryIAb* gene (Ps7s7) is derived from the Subterranean clover stunt virus (SCSV) genome segment 7 (Boevink et al. 1995), with polyadenylation signals from the chloroplastic NADP-malic enzyme (3'me1) of the C4 plant *Flaveria bidentis* (Marshall et al. 1996).

50. The 35S promoter sequence (P335S3 for the *bar* gene in event T304-40) is derived from the Cauliflower mosaic virus (CaMV) 35S transcript, as described by (Odell et al. 1985). CaMV is a double stranded DNA virus with a host range restricted primarily to cruciferous plants. The 35S promoter is considered a model plant nuclear promoter system and is widely used in plant biotechnology for the high expression of transgenes.

51. The mRNA termination region for the *bar* gene is derived from the *nopaline synthase* gene (*nos*) from pTiT37 of *A. tumefaciens*. This sequence is widely used in constructs for plant genetic modification (Reiting et al. 2007).

52. While some of the regulatory sequences are derived from plant pathogens (*A. tumefaciens*, CaMV and SCSV), the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants.

Table 3. Details of the promoters and terminators used for T304-40

Promoter abbreviation	Source/Full name	Commentary	Reference
Ps7s7	Subterranean clover stunt virus	Constitutive promoter derived from the SCSV genome segment 7	(Schunmann et al. 2003)
35S (P335S3)	Cauliflower mosaic virus (CaMV)/35S	Constitutive promoter widely used in plant genetic modification	(Odell et al. 1985)
Terminator abbreviation	Source/Full name	Commentary	Reference
nos	Agrobacterium/nopaline synthase	Terminator widely used in plant genetic modification	(Depicker et al. 1982)
Me1	<i>F. bidentis</i> /NADP malic enzyme		(Schunmann et al. 2003)

5.4 Method of genetic modification

53. Disarmed binary plasmid vectors containing the constructs (see Table 2) were transformed into hypervirulent disarmed *A. tumefaciens* strain EHA101 (Hood et al. 1986). GM cotton lines T304-40 (containing *cry1Ab* and *bar*) and GHB119 (*cry2Ae* and *bar*) were generated by *Agrobacterium*-mediated transformation of the readily transformed cotton cultivars Coker 312 and Coker 315, respectively, using standard protocols (Firoozabady et al. 1987; Cousins et al. 1991; Umbeck 1991). This method of transformation has been discussed in previous RARMPs (for example, DIR 070/2006). The TwinLink[®] cotton plants were then obtained by conventional crossing of T304-40 and GHB119 followed by backcrossing to homogeneity.

5.5 Characterisation of the GMO

5.5.1 Stability and molecular characterisation

54. The applicant states that the genotype of TwinLink[®] cotton in the Coker background is stable and the transgenes have been maintained as single dominant Mendelian traits over a number of generations of self-crosses and crossings. The binary vector plasmid constructs used to generate the T304-40 and GHB119 events have been fully sequenced. For each construct, the insertion of a single copy at a single locus within the TwinLink[®] cotton genome has been confirmed by Southern blot analysis and segregation data from the breeding programme. Southern blot and PCR also confirmed that the *Agrobacterium* vector is not present in the genome of the T304-40 and GHB119 transformants.

55. *Agrobacterium* inserts introduced genes into plant genomic DNA via illegitimate recombination⁷ and the different gene constructs are expected to be inserted at random. The exact locations of the inserted genes within the cotton genome is not known but is currently being characterised to develop an event-specific PCR protocol. The chromosomal location has not yet been identified due to the cotton genome being poorly characterised. However, since the cotton plants are to be used for field experiments only and not for commercial use, it is not essential to know the exact location of the insertions within the genome.

5.5.2 Characterisation of the phenotype of the GMO

56. The effect of expressing the three introduced genes is expected to lead to resistance of the cotton plants to lepidopteran insect pests (specifically *Helicoverpa* spp) and to post-emergence tolerance to herbicides that are based on the active ingredient glufosinate

⁷ Illegitimate recombination is a term used to describe the recombination that occurs between DNA sequences that contain no or very little homology. It results in the random insertion of foreign DNA into the host genome.

ammonium. The purpose of the proposed trial is to evaluate the agronomic performance of the GM cotton plants and to assess the efficacy of the insecticidal protein combination against *Helicoverpa armigera*. The applicant has provided the following information as to characteristics of the individual events and TwinLink[®] cotton plants.

57. According to the applicant, there have been no deleterious effects on morphology, seed set or seedling vigour of the individual events T304-40 and GHB119 when compared to the parental Coker varieties in preliminary glasshouse trials. Field trials in the US have identified no obvious secondary effects resulting in agronomic penalties as a result of the transformation events. TwinLink[®] plants have been grown in the field in small scale preliminary insecticidal efficacy testing for three years in the US (USDA 05-035-12n (2005), 06-047-04n (2006), 07-044-104n, 07-059-101n and 07-065-119n (2007) and in one glasshouse trial in Australia (NLRD 2136/2006). The plants have performed in a similar manner to non-GM varieties, with growth and yield characteristics within the range expected for commercial varieties of cotton.

58. Apart from the proteins produced as a result of expression of the introduced genes, no new products are expected to be produced. There may, however, be unintended effects due to random insertion of the introduced genes (Chapter 2, Event 6)

Section 6 The receiving environment

59. The receiving environment forms part of the context in which the risks associated with the dealings involving the GMO are assessed. This includes the geographic region where the release would occur and any relevant biotic/abiotic properties of the location; the intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007).

6.1 Relevant abiotic factors

60. The abiotic factors relevant to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*.

61. The size, location and duration of the release are outlined in Chapter 1, Section 3.2. The proposed dealings involve planting in a field on well-established farmland at the centre of the cotton cropping system of the Namoi Valley, NSW. This is a cotton growing region and has a typical climate for summer cotton growing regions in Australia, with warm summers and higher summer than winter rainfall (see Table 4).

Table 4. Climatic data for Narrabri, NSW

	Narrabri Post Office
Average daily max/min temperature (summer)	33.3 °C /18.7 °C
Average daily max/min temperature (winter)	18.9 °C /4.5 °C
Average monthly rainfall (summer)	73.2 mm
Average monthly rainfall (winter)	45.7 mm

Source: <<http://www.bom.gov.au>>.

62. The closest population centres are Wee Waa (population 2000) and Narrabri, (population 7500).

6.2 Relevant biotic factors

63. The biotic factors pertaining to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008). Of relevance to this proposed release are the following points:

- the proposed site is located within a major cotton growing region
- there are plots growing both GM and non-GM cotton at other sites near the proposed field trial, which are located at a minimum distance of 50 m from the trial site
- invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products.

6.3 Relevant agricultural practices

6.3.1 General information

64. The applicant intends to grow the GM cotton lines following standard agricultural protocols. The cotton plants at the release site would therefore receive applications of fertilisers, herbicides, and other agronomic management practices similar to commercial (non-GM) cotton. These are outlined in Sections 2 and 6 of *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* (OGTR 2008).

65. The applicant also intends to monitor the performance of the GM cotton lines relative to non-GM parental varieties under conditions of natural and artificially induced infestations of *Helicoverpa armigera*. These plots will be compared with plots where natural lepidopteran insect predation will be controlled using selective insecticides.

6.4 Presence of related plants in the receiving environment

66. Cotton (*G. hirsutum*) is grown in the shire of Narrabri and includes herbicide tolerant and/or insect resistant GM cotton plants that have previously been approved for commercial release (DIR 062/2005 and DIR 066/2006). As a result of these commercial releases GM cotton plants are widespread in the agricultural environment, comprising about 92% of commercially grown cotton crops in the 2006/2007 growing season (see DIR 074/2007 RARMP).

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

67. *Bacillus thuringiensis* is an aerobic gram-positive endospore-forming bacterium that is ubiquitous in the environment. The delta-endotoxins produced by *B. thuringiensis* are encoded by *cry* genes and over 380 of these genes have been cloned and sequenced (Crickmore et al. 2008). Naturally occurring Bt strains typically contain one to six *cry* genes (Masson et al. 1998). The genes and their encoded proteins are thus widespread in the soil environment and have also been found associated with plant products and insects (Schnepf et al. 1998). Commercial GM cotton plants such as Bollgard II[®] (*cry1Ac* and *cry2Ab*) also contribute to the presence of *cry* genes in agricultural areas of Australia.

68. The PAT protein is also widespread in the environment through the presence of the bacteria from which it is derived. PAT proteins are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). These species of *Streptomyces* are saprophytic, soil-borne bacteria and are not considered pathogens of plants, humans or other animals (OECD 1999). A search of the GenBank database reveals that other

genes encoding PAT or similar enzymes are present in a wide variety of bacteria. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals. Different versions of PAT protein have also been expressed in other GM crop plants trialled (DIRs 010/2001, 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003) or commercially approved (canola DIR 021/2003 and cotton DIR 062/2005) in Australia.

69. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the introduced genes and their resulting gene products, or their homologues, through contact with plants, food derived from plants and microorganisms. This information forms the baseline data for assessing the risks from exposure to these proteins as a result of the trial of the GM cotton.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM cotton

7.1.1 Previous releases approved by the Gene Technology Regulator or authorised by the Genetic Manipulation Advisory Committee

70. There has been no previous release of this GM cotton in Australia.

71. However, under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) as well as under the current regulatory system, there have been numerous field trials of GM cotton plants containing the *bar* gene and the same or similar *cry* genes.

72. CSIRO has conducted a trial of GM cotton containing the *cryIAb* and *bar* genes (DIR 016/2002) as well as three trials of Liberty Link[®] cotton, which contains the *bar* gene only: DIR 015/2002, DIR 038/2003 and DIR 056/2004. An insect resistant GM cotton containing a modified *cryIAb* gene has also been trialled by Deltapine (DIR 065/2006).

73. In addition, licences have been issued which authorise release of GM cotton containing other *cry* genes (and/or the *bar* gene). Licence DIR 056/2004 authorises the field trial of both GM herbicide tolerant (LLCotton25) and herbicide tolerant/insect resistant (LLCotton25/Bollgard II[®]) cottons. DIR 062/2005 authorised a commercial release of Liberty Link[®] and Liberty Link[®]/Bollgard II[®] Cotton. Additional GM cottons containing either the *bar* herbicide tolerance gene (DIR 036/2003), or the related *pat* gene together with *cryIAc* and *cryIFa* (DIR 040/2003 and DIR 044/2003) have also been trialled in Australia.

74. DIR 066/2006 authorises the commercial release of herbicide tolerant and/or insect resistant cotton and incorporates DIR 059/2005, DIR 023/2002, DIR 022/2002 and DIR 012/2002 (Roundup Ready[®], Roundup Ready Flex[®], Bollgard II[®], Roundup Ready Flex[®]/Bollgard II[®], Roundup Ready[®]/Bollgard II[®]). Bollgard II[®] contains the *cryIAc* and *cry2Ab* genes.

75. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

7.1.2 Approvals by other Australian government agencies

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

77. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM cotton lines in human food; accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from these GM cotton lines could be used in food.

78. APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. Bayer would require a research permit from APVMA for the proposed release of the GM cotton containing the insecticidal genes. Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial. If the applicant decided to apply glufosinate ammonium a research permit would be required.

79. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs, or products known to be mixed with any amount of GM material. As the importation would constitute a dealing under the Act, the importer requires an authorisation under this Act for the import to lawfully proceed. Seeds from TwinLink[®] cotton have been imported by CSIRO under an AQIS seed import permit.

7.2 International approvals

80. Plants derived from the T304-40 and GHB119 events and TwinLink[®] plants have been grown in the field in small scale preliminary trials in the US. These trials have been authorised by the USDA: 05-035-12n (2005), 06-047-04n (2006), 07-044-104n, 07-059-101n and 07-065-119n (2007)

81. There have been no environmental releases or food approvals for TwinLink[®] cotton. However, GM cotton plants containing other *cry* genes and/or the *bar* gene, such as Bollgard II[®] and LibertyLink[®] cotton, have been approved in a number of countries for commercial release and/or use in food (Refer to DIRs 062/2005 and 066/2006 for details).

Chapter 2 Risk assessment

Section 1 Introduction

82. 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) considers risks from the proposed dealings with the 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. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

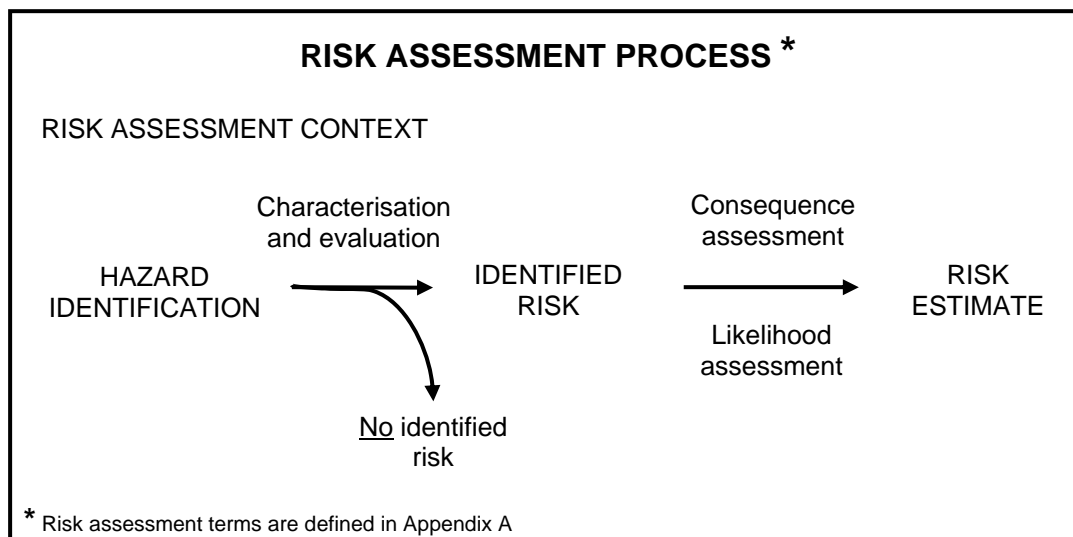


Figure 2. The risk assessment process

83. 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 a release of these GMOs into the environment.

84. 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 2007).

85. 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 GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

86. 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 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

87. 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 and the identification of risk

88. Each event compiled during hazard identification is 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.

89. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2007). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

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

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the non-GM parents
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic factors at the site of release
- agronomic management practices for the GMOs.

91. The eight events that were characterised are discussed in detail later in this Section. They are summarised in Table 5 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

92. The GM cotton plants contain two *cry* genes coding for insecticidal delta-endotoxins and the *bar* gene encoding the PAT protein, which has been shown to confer tolerance to the herbicide glufosinate ammonium.

93. The prevalence of the *cry* genes in the environment and the lack of evidence for toxicity or allergenicity of the encoded proteins to humans and animals have been discussed in Chapter 1, Section 5.2.2. *CryIAb* is contained in a number of commercially released GM crops and has thus been the subject of assessment by regulatory bodies overseas (see RARMP for DIR 065/2006).

94. The *bar* gene, and its product, PAT, has already been considered in detail in the RARMP prepared for DIR 062/2005 (Liberty Link[®] Cotton) as well as by other regulators. No risk was identified to people or the environment, with respect to toxicity or allergenicity of the protein or the end products.

95. GM cotton plants containing both *cryIAb* and *bar* have been considered previously in the RARMP for DIR 016/2002 and no adverse outcome was predicted. Event T304-40, which

is used to generate the TwinLink[®] cotton plants, has been licensed by the US EPA for experimental use (264-EPU-140).

96. No harm has been identified as a consequence of any of these releases. Therefore, potential effects of the *cry1Ab* and *bar* genes will not be assessed in detail for this application, unless specific effects are identified resulting from their expression in conjunction with *cry2Ae*.

Table 5. Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for insect resistance and herbicide tolerance.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing proteins encoded by the introduced genes or their end products.	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The encoded proteins are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other non-target organisms. The limited scale, short duration and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence of the GM cotton in the environment	2. Expression of the introduced genes improving the survival of GM cotton plants.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Cultivated cotton is not considered to be weedy and the genetic modifications are not expected to change the weediness characteristic of the GMOs. Resistance to lepidoptera and tolerance to herbicide is unlikely to increase weediness as other abiotic factors limit the spread and persistence of cotton. The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Cotton seeds have limited dispersal characteristics, which are not expected to be changed in the GMOs. The proposed limits and controls would minimise dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes and regulatory sequences in other GM and non-GM cotton plants.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> Cotton is predominately self-pollinating and outcrossing is limited. The applicant proposed a number of controls, including a 20 m pollen trap which would minimise gene flow via pollen.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Events 1 – 3 did not constitute risks which required further investigation.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry, physiology or ecology of the GM cotton resulting from expression, or random insertion, of the introduced genes.	Weediness; increased allergic reactions in people or toxicity to people and other non-target organisms	No	<ul style="list-style-type: none"> Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	7. Altered biochemistry or ecology of the surrounding environment as a result of growing the GM cotton.	Adverse change in numbers and/or species diversity of soil biota.	No	<ul style="list-style-type: none"> • Cry proteins are already present in soil as <i>B.thuringiensis</i> is a common soil microorganism. • The proposed limits and controls would minimise any adverse effects.
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> • The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

97. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

98. Allergenicity is the potential of a protein to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

99. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for insect resistance and herbicide tolerance. Workers cultivating the GM cotton would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM cotton plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM cotton plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1: Exposure to GM plant materials containing proteins encoded by the introduced genes or their end products

100. Expression of the introduced genes for insect resistance or herbicide tolerance could potentially result in the production of novel toxic or allergenic compounds in the GM cotton, or alter the expression of endogenous cotton proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

101. Cotton tissues, particularly seeds, contain anti-nutritional and toxic factors, the most studied being gossypol and cyclopropanoid fatty acids (OGTR 2008 and references therein). As a result, cotton can be toxic to animals if ingested in excessive quantities. Levels of these natural defensive chemicals have not been measured in TwinLink[®] cotton. However, compositional components of cotton seed including gossypol and tannin have been measured in Bollgard[®] (containing Cry1Ac) and Bollgard II[®] (containing Cry1Ac and Cry2Ab), and found to be comparable to other non-GM cotton seeds (Tang et al. 2006).

102. The GM cotton differs from non-GM cotton in that it expresses additional proteins: Cry1Ab, Cry2Ae and PAT. No information was found to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms (Chapter 1,

Section 5.2.2) or could affect the production of endogenous cotton allergens, and therefore exposure to the GM plant materials is not expected to adversely affect the health of humans or other organisms.

103. Toxicity studies for the Cry2Ae protein expressed in the GM cotton proposed for release are in progress and are not available at this early stage of research. Cry2Ae belongs to the same subclass of delta-endotoxins as Cry2Ab, which is present in Bollgard II[®] cotton and has not been demonstrated to show any off-target toxic effects, specifically mammalian toxicity. Thus, the Cry2Ae GM cotton lines are not expected to be toxic to people or other non-target organisms. However, toxicity studies on the Cry2Ae protein would be required if approval was sought for commercial release of the GMO in Australia or for use of the GM cotton as food or animal feed (see discussion in Section 7.1.2).

104. The stacking of two *cry* genes in TwinLink[®] cotton raises the possibility of additive, antagonistic or synergistic interactions between Cry1Ab and Cry2Ae (Chapter 1, Section 5.2.2). Additive or synergistic effects may result in greater toxicity of the GM cotton to target and possibly non-target organisms than would otherwise be expected. A comparison of protein expression and toxicity data for TwinLink[®] cotton and the parental lines would be required to establish this. For this limited and controlled release, adverse outcomes, if any, occurring as a result of a possible additive or synergistic effect would be minimised by the limits and controls as outlined below.

105. There is a further opportunity for stacking of different *cry* genes if the pollen trap were planted with GM cotton approved for commercial release. Progeny resulting from crosses between TwinLink[®] plants and pollen trap plants containing one or more different *cry* genes may have increased toxicity to a wider range of susceptible insect species. However, for this limited and controlled release, any affected species are likely to be target pests. Furthermore, since pollen trap plants are treated as GMOs, they are destroyed at the conclusion of the trial.

106. Potential risks associated with the *bar* gene and its encoded protein have been comprehensively assessed in the RARMP for DIR 062/2005 and no adverse effects were identified with respect to toxicity or allergenicity of the PAT enzyme. The mechanism by which PAT detoxifies glufosinate ammonium was also outlined (DIR 062/2005) and the major metabolites in GM plants were found to have toxicities that were comparable to or less than that of the parent compound (OECD 1999; OECD 2002). Thus, while TwinLink[®] cotton contains two copies of the *bar* gene and will potentially possess higher PAT activity than GM plants containing only one copy, the metabolic end products of the glufosinate ammonium detoxification pathway are of very low toxicity. The applicant does not intend to apply glufosinate ammonium during the trial, so the potential for increased levels of metabolites are not a consideration for this proposed release.

107. The proposed limits and controls of the trial (Chapter 1, Section 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. The short duration (one growing seasons) and small size (0.36 ha) of the trial will limit the potential for exposure of humans and other organisms to the GM plant tissues. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff associated with the field trial. There is limited potential for exposure of the public to plant materials via ingestion, skin contact or inhalation as no plant material will be used as human food, animal feed or plant products.

108. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms as a result of consumption of, contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes, or their end products as a result of the genetic modification is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM cotton in the environment

109. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM cotton plants in particular, is provided in the review document *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008). In summary, cotton lacks most characteristics that are common to many weeds, such as the ability to produce a persisting seed bank, rapid growth to flowering, continuous seed production, very high seed output, high seed dispersal and long-distance seed dispersal.

110. Cotton has been grown for centuries throughout the world without any reports that it is a serious weed, and is likewise not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2002; Groves et al. 2003). The weed status of cotton has also been considered extensively in RARMPs produced during the assessment of a variety of GM cotton lines including recent commercial approvals DIR 062/2005 and 066/2006.

111. Scenarios that could lead to increased spread and persistence of the GM cotton include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins and their end products.

Event 2: Expression of the introduced genes improving the survival of the GM cotton plants

112. If the GM cotton plants were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials and the encoded proteins has been considered in Event 1 and was not considered an identified risk.

113. If the expression of the introduced genes for insect resistance and herbicide tolerance were to provide the GM cotton plants with a significant selective advantage over non-GM cotton plants and they were able to establish and persist in favourable non-agricultural environments this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM cotton plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM cotton.

114. The GM cotton expresses two genes expected to confer insect resistance to the plants as well as a gene conferring herbicide tolerance. The impact of the gene combination on survival of the GM cotton has not been characterised under field conditions. However, the applicant states that there are no observable changes in phenotype for GM cotton grown in glasshouse experiments.

115. TwinLink[®] cotton expresses two *cry* genes encoding insecticidal toxins. This could confer a selective advantage on the GM plants in regions where lepidopteran insect predation limits one or more of the key life stages of cotton insect resistance and lead to weediness. This would particularly be the case if additive or synergistic effects between the expressed Cry proteins led to increased toxicity of the GM plants to a wider range of susceptible pest species. Cry 1A and Cry2A proteins are thought to have different modes of action (Chapter 1, Section 5.2.2), so TwinLink[®] cotton may affect an expanded range of susceptible insects. If the pollen trap were planted with GM cotton, generation of plants carrying multiple *cry* genes could further increase the potential for weediness of GM cotton plants in circumstances where insect predation by susceptible pest species was the major limiting factor.

116. However, spread and persistence of the GM cotton plants would still be limited by a number of other abiotic factors including water and nutrient availability, temperature and soil type (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008).

117. Expression of the *bar* gene is not expected to confer an advantage on the plants unless they were subject to treatment with herbicide containing glufosinate ammonium. Cotton is not regarded as a weed in Australia and is not controlled by glufosinate ammonium on the farm or in the natural environment. Glufosinate ammonium has limited effectiveness in controlling cotton volunteers even at the seedling stage and other herbicides are available. Cultivation/mechanical removal is the best option to remove established cotton plants.

118. The applicant has proposed limits and controls to minimise the possibility of persistence of the GM cotton (Chapter 1, Section 3.2 and 3.3). They include post-harvest monitoring of the proposed site for 12 months and destruction of any volunteers.

119. Therefore, expression of the introduced genes for insect resistance and herbicide tolerance is not expected to provide the GM cotton plants with a significant selective advantage over non-GM cotton plants unless the major factor limiting survival was lepidopteran insect predation, and/or under conditions of glufosinate ammonium herbicide application.

120. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for insect resistance and herbicide tolerance improving the survival of the GM cotton is **not an identified risk** and will not be assessed further.

Event 3: Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions

121. If the GM cotton was to be dispersed from the release site it could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of exposure to GM cotton materials have been assessed in Event 1 and were not an identified risk. The introduced genes improving survival of the GM cotton in the environment was assessed in Event 2 and was also found not to be an identified risk.

122. In a natural situation cotton does not reproduce vegetatively (Sheelavantar et al. 1975; OGTR 2008), and therefore dispersal of GM cotton materials other than seed would be highly unlikely to result in the establishment of the GM cotton plants in the environment. Seed production, dispersal and digestibility characteristics are not expected to be altered in the TwinLink[®] cotton compared to non-GM cotton.

123. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content. They are therefore unlikely to carry bolls any great distance from the cotton fields. Similarly, there is no evidence of avian species transporting cotton seed (OGTR 2008). Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

124. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal (Chapter 1, Section 3.2 and 3.3). The proposed release site is located at least 50 m away from natural waterways and the applicant proposes regular monitoring for volunteers in the field in which the cotton is planted, as well as any connecting irrigation channels.

125. Finally all GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise the opportunity to disperse the GM material.

126. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials through various means including animals and extreme weather conditions is **not an identified risk** and will not be assessed further.

2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

127. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

128. Baseline information on vertical gene transfer associated with non-GM cotton plants is provided in *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008). In summary, cotton is predominantly self-pollinating and outcrossing is rare, although cross-pollination can occur at low levels over short distances. The only sexually compatible species present in Australia that could receive genes from the GM cotton are *G. hirsutum* (including both cultivated GM and non-GM, and naturalised cotton) and *G. barbadense*.

129. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distances from cultivated cotton fields. Furthermore, there is well established genetic incompatibility between native *Gossypium* species and cultivated cotton; the likelihood of fertile hybrids occurring between cultivated cotton and native *Gossypium* species is very low (OGTR 2008).

Event 4: Expression of the introduced genes and regulatory sequences in other GM and non-GM cotton plants

130. Transfer and expression of the introduced genes for insect resistance and herbicide tolerance in other GM or non-GM *G. hirsutum* or *G. barbadense* plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

131. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM cotton plants by the introduced genes or regulatory sequences. This will also apply if the introduced genes are expressed in other cotton plants such as non-GM *G. barbadense*, or in other commercially approved GM cottons.

132. In the event that genes for insect resistance were transferred to feral or cultivated cotton, the plants would have a survival advantage only in regions where lepidopteran insect pests may limit their growth or regulate their populations. However, as discussed in Event 2, the distribution of cotton is determined by soil type, soil moisture and temperature, rather than by insect pressure (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008).

133. Therefore, as is the case for the GM cotton plants, expression of the introduced genes in *G. hirsutum* would also result in plants limited by these factors. The expression of the introduced genes in the sexually compatible species *G. barbadense* is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM cotton and *G. barbadense* would be expected to limit the spread and persistence of any hybrids between the GM cotton and *G. barbadense*.

134. Transfer of the herbicide tolerance gene to non-GM cotton would confer an advantage only under circumstances where glufosinate ammonium was being applied. As indicated in Event 2, cotton is not regarded as a weed in Australia and is not controlled by glufosinate ammonium on the farm or in the natural environment.

135. A number of insect resistant and/or herbicide tolerant GM cotton lines are currently approved for commercial release in Australia and may be grown in the areas proposed for this release. These GM cotton lines were comprehensively assessed (most recently in the RARMPs for DIR 062/2005 and 066/2006) prior to release and comprised more than 90% of the commercial cotton crop in 2006-07. If crossing occurred between these GM plants and TwinLink[®] plants there could be further stacking of insect resistance and herbicide tolerance genes which would result in cotton plants producing several different Cry toxins and possibly higher levels of PAT protein.

136. Nonetheless, expression of multiple introduced genes for herbicide tolerance and insect resistance in the commercial GM cotton lines is not expected to increase their spread and persistence as they would still be limited by the abiotic factors discussed above and could be controlled by the use of alternative herbicides and/or cultivation.

137. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. However, even if it did occur, the chance of an adverse effect to people or the environment is highly unlikely.

138. Dispersal characteristics, as well as allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM cotton plants by the introduced genes or regulatory sequences (see Events 1 and 3). As discussed in the *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008) cotton is predominantly self-pollinating, with pollen that is large, sticky and heavy and not easily dispersed by wind. Cotton gene flow studies consistently show that outcrossing is localised around the pollen source and decreases significantly with distance. Furthermore, as discussed above, outcrossing will only be successful between the GM cotton plants and other *G. hirsutum* or *G. barbadense* plants due to genetic incompatibility with other *Gossypium* species. It is unlikely that constitutive expression of the introduced genes in the GM cotton will alter pollen characteristics and/or genetic compatibility relative to cotton plants containing the endogenous homologues.

139. The applicant has proposed a number of measures to limit and restrict the potential for pollen flow and gene transfer to sexually compatible plants (Chapter 1, Section 3.2 and 3.3). These include a 20 m zone surrounding the trial site to act as a pollen trap. The applicant states that the trial site is located at least 50 m from any other cotton plants. The applicant also proposes to perform post harvest monitoring of the site for twelve months or until the site has been clear of volunteers for one growing season; and to destroy any volunteer plants found. These proposed controls would reduce the already low likelihood of gene flow from the GMOs to other cotton resulting in expression of the introduced genes.

140. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in other cotton plants or other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

141. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by horizontal gene transfer (HGT). HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer

a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

142. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

143. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 5.2 and 5.3. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

144. Possible adverse outcomes from the proposed dealings with the GM cotton and/or its products that might arise as a result of horizontal gene transfer include adverse reactions, such as allergenicity/toxicity or increased spread and persistence of the organism that has acquired the introduced genetic elements.

Event 5: Presence of the introduced genes, or the introduced regulatory sequences, in unrelated organisms as a result of gene transfer

145. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material.

HGT from GM cotton plants to bacteria

146. Bacteria are afforded many opportunities to encounter DNA from GM plants. These include, exposure to GM plant material in the soil or aquatic environments where GM plant material is present, through a bacterial species natural interactions with the GM plants as commensals, symbionts or parasites, or through the interactions of GM plant material and gut bacteria in herbivores (Keese 2008). Few examples of HGT to bacteria from eukaryotes resulting in an evolutionary advantage exist (Andersson 2005) and limited transfer and persistence of DNA from plants to bacteria has been shown in experimental and laboratory studies (Nielsen et al. 1998).

147. Bacteria that occur naturally in an environment are the best source for genes that may cause an adverse effect as a result of HGT (Keese 2008). It is suggested that bacterial genes are the only genes in GM plants likely to transfer successfully to bacteria (Pontiroli et al. 2007). For example, antibiotic resistance genes, which occur naturally in a number of bacterial species and are commonly used in the process to generate GM plants. However, these genes are often abundant in the environment and more readily transferable by conjugation and transduction from other bacteria (Keese 2008).

HGT from GM cotton plants to animals

148. DNA entry across the gastrointestinal tract is the most likely route of HGT from GM plants to animals (Keese 2008). This will occur for invertebrates and vertebrates that feed on GM plants, animals that feed on herbivores, or plant pollinators. The potential for transient gene transfer into somatic cells has been shown, but gene transfer to the germ line cells of animals has not been detected (Van Den Eede et al. 2004). The analysis of genomic sequences have shown only rare examples of HGT from plants to animals (Lambert et al. 1999; Bird & Koltai 2000).

HGT from GM cotton plants to viruses

149. While plant viruses have the capacity to acquire new genetic material as a result of recombination events with the genetic material from the plants they infect or other pathogens infecting the plant, the vast majority of recombination events that occur involve other viral sequences (Keese 2008). The genome size of plant viruses is small and only rare examples of host plant sequences have been found in the genomes of viruses (Khatchikian et al. 1989; Mayo & Jolly 1991; Agranovsky et al. 1991; Meyers et al. 1991; Masuta et al. 1992). This suggests that the HGT from a GM plant to viruses is likely to be restricted to GM plants transformed with viral sequences and the viruses that naturally infect that plant species. Examples of HGT resulting from recombination between a virus and a homologous viral gene introduced into a GM plant have been documented. However, in most cases a selective advantage to the virus was favoured by the use of a defective virus as the infecting agent for which recombination with the introduced genetic material in the GM plant would restore full infectivity (Keese 2008).

150. There are potentially far greater background levels of HGT to plant viruses from non-GM donor sources due to co-infections in plants by two or more viruses and from a broad range of viral sequences that occur naturally in plant genomes (Bejarano et al. 1996; Ashby et al. 1997; Harper et al. 1999; Harper et al. 2002; Peterson-Burch & Voytas 2002).

HGT from GM cotton plants to other eukaryotes

151. Algae, fungi and a range of protists are other potential eukaryotic HGT recipients of the introduced genetic material. However, HGT from plants to these organisms is exceedingly rare. Opportunities for these organisms to obtain genes with related sequences or functions to the introduced genes are more likely to occur by mutation or HGT from non-GM donor organisms (Keese 2008).

Nature of introduced genetic material

152. Conclusions reached for Events 1-4 associated with the expression of the introduced genes or end products did not represent an identified risk to people, animals or the environment. Furthermore, the gene sequences expressed from the introduced genetic material are not expected to assist the process of HGT by facilitating gene movement across cell membranes or recombination with a host genome. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

153. The introduced genes are widespread amongst common soil microorganisms. In light of the discussion above, it is far more likely that horizontal gene transfer will occur from naturally occurring *B. thuringiensis* to other soil microorganisms than from the GM cotton plants to bacteria. Furthermore, the introduced *cry* genes in the GM cotton plants have been modified for plant codon usage so in the unlikely event that gene transfer were to occur, only low levels of gene expression in bacteria would be expected.

154. A key consideration in the risk assessment process should be the safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced gene sequences or their end products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Events 1-4 associated with the expression of the introduced genes or end products did not represent an identified risk.

155. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unintended changes in biochemistry, physiology or ecology

156. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy⁸ (Haslberger 2003). 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 pleiotropic 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 the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

157. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, 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).

Event 6: Changes to biochemistry, physiology or ecology of the GM cotton resulting from expression or random insertion of the introduced genes

158. Considerations relevant to altered biochemistry, physiology and ecology in relation to expression of all three of the introduced genes have already been discussed in Events 1 to 3, and were not considered identified risks.

159. Unintended changes in gene expression are also possible, which could lead to alterations in the biochemistry, the physiology or the ecology of the GM cotton. Such changes could occur either as a result of the introduced genes or of the transformation process itself.

160. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). No obvious visible phenotypic difference from non-GM cotton has been observed in the glasshouse for the GM cotton. (Chapter 1, Section 5.5.2). In addition, during this limited and controlled release the applicant proposes to measure the agronomic performance of the GM cotton lines, and any unintended effects are likely be detected during the trial.

161. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Section 3.2 and 3.3. In particular, the proposed release is limited in size, duration and location and none of the GM plant materials

⁸ Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

are intended for use in human food or animal feed or for the production of fabrics and/or other cotton products

162. **Conclusion:** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

Event 7: Altered biochemistry or ecology of the surrounding environment as a result of growing the GM cotton

163. The potential effects of Bt toxins on the surrounding environment have been extensively reviewed (Shelton et al. 2002; Nester et al. 2002; Mendelsohn et al. 2003; OECD 2007). Toxicity of Cry proteins to non-target organisms has been assessed in Event 1 and no risk was identified. However, it is also possible that expression of the introduced genes may have sublethal effects on non-target organisms within the agricultural environment.

164. Effects on honeybee feeding behaviours and learning performance have been identified at high concentrations of Cry toxin (Chapter 1, Section 5.2.2), but such concentrations are unlikely to occur in the field. This is particularly the case for TwinLink[®] cotton plants, where Cry1Ab expression is controlled by a 35S promoter, which has only weak activity in pollen (Sunilkumar et al. 2002).

165. Bt toxins are exuded from the roots of GM plants so effects on rhizosphere ecology are possible. This has been reviewed widely (Icoz & Stotzky 2008) and no significant detrimental effects on non-target soil fauna such as woodlice, collembolans, mites, earthworms, nematodes and protozoa have been reported (see Chapter 1, Section 5.2.2). Where identified, such effects are transient and more likely to result from differences in geography, temperature, plant variety and soil type than from the presence of Cry proteins (Icoz and Stotzky 2008). This limited and controlled release would be unlikely to cause any changes in numbers or composition of soil biota beyond this natural variation.

166. Therefore, changes in the biochemistry or ecology of the surrounding environment which result in adverse outcomes as an indirect effect of the expression of the introduced *cry* genes for insect resistance and herbicide tolerance are considered unlikely. The likelihood will be further minimised by the limits and controls (Chapter 1, Section 3.2 and 3.3) proposed for the release. In particular, the short duration of the trial and the small site will limit any unintended effects resulting from growing the GM cotton plants.

167. **Conclusion:** The potential for an adverse outcome as a result of unintended changes in biochemistry or ecology of the surrounding environment is **not an identified risk** and will not be assessed further.

2.6 Unauthorised activities

Event 8: Use of GMOs outside the proposed licence conditions (non-compliance)

168. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM cotton lines outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

169. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

170. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

171. Eight events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

172. A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

173. The characterisation of the eight events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- the limits on the size, location and duration of the release proposed by Bayer
- suitability of controls proposed by the Bayer to restrict the dissemination or persistence of the GM cotton plants and their genetic material
- limited capacity of the GM cotton to spread and persist in the areas proposed for release
- limited ability and opportunity for the GM cotton to transfer the introduced genes to commercial cotton crops or other sexually related species
- none of the GM plant materials or products will be used in human food or animal feed
- widespread presence of the same or similar proteins encoded by, and end products produced as a result of the activity of, the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

174. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton lines into the environment are considered to be **negligible**. Hence, the Acting Regulator considers that the dealings involved in this proposed limited and controlled release of GM cotton **do not pose a significant risk** to the health and safety of people or to the environment⁹.

Section 4 Uncertainty.

175. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

⁹ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

176. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability. For field trials, some knowledge gaps are inevitable because they involve the conduct of research. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of a GMO and its genetic material in the environment, rather than necessarily to treat an identified risk.

177. For DIR 087 which involves early stage research, uncertainty exists in relation to the characterisation of:

- Event 1, regarding toxicity of Cry2Ae to non-target organisms and potential increases in allergenicity or toxicity of plant material containing the combination of proteins encoded by the introduced genes for insect resistance; and
- Event 2, associated with a potential for increased survival of the GMOs.

178. Additional data including information to address these uncertainties would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of this GM cotton.

179. Chapter 3, Section 5 discusses the additional data that may be required for future releases.

Chapter 3 Risk management

180. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

181. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence 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.

182. Section 63 of the Act 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 the licence holder to 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.

183. 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 Responsibilities of other Australian regulators

184. 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 Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁰.

185. 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 purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

186. FSANZ is responsible for human food safety assessment, including GM food. The purpose of the proposed trial is to conduct early stage research with TwinLink[®] cotton and the

¹⁰ 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. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

applicant does not intend any material from these GM plants to be used in human food. Accordingly, the applicant has not applied to FSANZ for evaluation of the GM cotton for use in human food. FSANZ approval would need to be obtained before materials from cotton lines containing Cry1Ab and Cry2Ae proteins could be used in this way.

187. APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. Bayer would require a research permit from APVMA for the proposed release of the GM cotton containing the insecticidal genes. Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend to apply this herbicide during the trial. If the applicant decided to apply glufosinate ammonium a research permit would be required.

188. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs, or products known to be mixed with any amount of GM material. As the importation would constitute a dealing under the Act, the importer requires an authorisation under this Act for the import to lawfully proceed. Seeds from TwinLink[®] cotton have been imported by CSIRO under an AQIS seed import permit.

189. No other approvals are required.

Section 3 Risk treatment measures for identified risks

190. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM cotton. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

191. These events were considered in the context of the scale of the proposed release (a maximum total area of 0.36 hectares over one growing season) on one site in the local government area of Narrabri, NSW, the containment measures (Chapter 1, Section 3.3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

192. Licence conditions have been imposed to control the dissemination and persistence of the GMO and its genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are summarised in Sections 4.1.2 and 4.1.3.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by Bayer

193. Chapter 1, Section 3.2 and 3.3 provide details of the limits and controls proposed by Bayer in their application, and discussed in the events characterised for the release in Chapter 2. Many of these proposed control measures are considered standard GM cotton licence conditions and have been imposed by the Regulator in previous DIR licences. The

appropriateness of these controls has been assessed in detail in previous GM cotton RARMPs, most recently in DIR 073/2007 and will be discussed briefly here.

194. The proposed release would be limited to one site in the local government area of Narrabri, NSW. The trial, including planting, harvesting and post-harvest monitoring, will be carried out by staff trained by Bayer and supervised by an appointed project supervisor. All personnel have appropriate training in practices relevant to the handling and disposal of GMOs. Additionally, the duration of the proposed release will be limited to one growing season. These measures will limit the potential exposure of humans and vertebrates to the GMO (Event 1) and the potential for the GM cotton to persist or to establish outside the proposed release site (Event 2).

195. The applicant has proposed to grow experimental plots of both GM and parental non-GM cotton within the trial site. The entire site would be surrounded by a 20 m wide pollen trap to limit gene flow from the GM cotton. As discussed in the *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008), cotton is predominantly self-pollinating, with the highest level of out-crossing occurring between adjacent rows. Out-crossing is rare beyond 20 m (Llewellyn et al. 2007), and a 20 m pollen trap of non-GM cotton plants will minimise gene transfer to sexually compatible plants (Event 4).

196. The applicant has proposed a number of measures to minimise the potential dispersal and persistence of the GM cotton. The trial site will be located more than 50 m from the nearest waterway which will minimise the chance of plant material being washed away from the site (Event 3). The GM cotton will be harvested and ginned separately from other cotton crops to prevent mixing and none of the seed or GM plant material will be used in human food, or animal feed or for the production of fabrics and/or other cotton products. These measures will limit the potential exposure of humans and vertebrates to the GMO (Event 1) and the potential for the GM cotton to be dispersed outside the proposed release site (Event 3).

197. After the GM cotton has been harvested, the applicant proposes to destroy all remaining plant materials not required for further testing by slashing and incorporating the material into the soil for microbial degradation, and to clean the site and all equipment used. The site will then be monitored for 12 months and until the site has been clear of volunteers for at least six months. As discussed in the *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008), cotton seeds have low dormancy levels and do not generally form a viable seed bank. However, dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture. Licence conditions requiring the irrigation and cultivation of the site and pollen trap areas in the first spring or summer following harvest have been imposed by the Acting Regulator to promote cotton seed bank reduction and minimise the persistence of the GM cotton at the proposed release site (Event 2).

198. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the *OGTR Guidelines for the transport of GMOs* (<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>). These are standard protocols for the handling of GMOs to minimize exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer (Events 4 and 5).

4.1.2 Summary of measures imposed by the Acting Regulator to limit and control the release

199. A number of licence conditions have been imposed to limit and control the release, including requirements to:

- conduct the release on a total area of up to 0.36 hectare at one site in the NSW local government area of Narrabri, over the summer cotton growing season 2008–2009
- surround the release site with a 20 m pollen trap
- locate the trial site at least 50 m away from natural waterways
- harvest and gin seed cotton from the release separately from any other cotton crop
- not permit any materials from the release to be used in human food or animal feed
- destroy all plant materials not required for further analysis or future release
- following harvest, clean the site and equipment used on the site
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- monitor the site for at least 12 months and destroy any cotton plants that may grow until no volunteers are detected for a continuous 6 month period.

4.1.3 Measures to control other activities associated with the trial

200. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

201. Conditions applying to the conduct of experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

202. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- applicant suitability
- contingency and compliance plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

203. 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.

204. On the basis of information submitted by the applicant and records held by the OGTR, the Acting Regulator considers Bayer suitable to hold a licence.

205. The licence conditions 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.

206. Bayer must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

207. Prior to planting the GM cotton, Bayer is required to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance.

208. Bayer is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM cotton outside of the permitted areas.

209. Bayer is also required to provide a method to the Regulator for the reliable detection of the presence of the GMO and the introduced genetic materials in a recipient organism. This method is required within 30 days of the issue date of the licence.

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

210. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting structures

211. 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 trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

212. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

213. A number of written notices are also required under the licence that would assist the OGTR in designing and implementing a monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting
- expected and actual dates of harvest and cleaning after harvest.

4.2.5 Monitoring for Compliance

214. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

215. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

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

Section 5 Issues to be addressed for future releases

217. Additional information has been identified that may be required to assess an application for a large scale or commercial release of the GM cotton, or to justify a reduction in containment conditions. This would include:

- characterisation of the genetic material inserted into the plants, including gene copy number and genotypic stability
- additional data on the potential toxicity of plant materials from the GM cotton, specifically in regard to the Cry2Ae protein alone and in combination with Cry1Ab
- additional data on the allergenicity of proteins encoded by the introduced genes for insect resistance, specifically that relating to the Cry2Ae
- characteristics indicative of weediness including measurement of altered reproductive capacity, and disease and insect resistance.

Section 6 Conclusions of the RARMP

218. The risk assessment concludes that this proposed limited and controlled release of GM cotton on a maximum total area of 0.36 ha over the summer cotton growing season 2008-2009 years in the NSW local government area of Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

219. The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Appendix A Definitions of terms in the Risk Analysis Framework 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 authorities¹¹ on the consultation RARMP for DIR 087

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

Summary of issues raised	Comment
It was noted that more information should be disclosed by the applicant regarding the outcome of trials with TwinLink cotton in the United States to provide useful background information.	The TwinLink cotton trial in the United States is at an early stage and results have not yet been made available. However, the applicant states that no adverse effects have been observed to date. Chapter 3 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release any of the GMOs on a larger scale or if reduced containment measures were proposed.
The proposed licence conditions on page 3 of the consultation RARMP mention a "monitoring zone" with no definition supplied.	A monitoring zone was not imposed as a licence condition for this application. Therefore, the reference to a "monitoring zone" on pages 3 and 38 of the RARMP have been deleted.
It was considered that the proposed trial containment and licence conditions appear to be adequate to limit the risk of transgene escape.	Noted. Refer to chapter 2 of the RARMP.
Given the location of the trial, its small scale and the containment measures proposed, the risk to the environment posed by this proposed release is low and manageable. The risk assessment has adequately assessed risks and identified management strategies to limit the spread of pollen or seed beyond the location of the trial.	Noted. Refer to chapter 2 of the RARMP.
<p>Considers that insect resistance and herbicide tolerance have some potential to increase cotton weediness in natural habitats and points out that there are mechanisms by which a plant can increase in weediness and still be limited by general factors.</p> <p>Recommends that the applicant should collect data on potential fitness advantages for the GM cotton plants in non-agricultural environments as well as the effects of proteins encoded by cry2Ae</p>	The application has been assessed as a limited and controlled release. The RARMP outlines future research requirements should the applicant seek a licence for commercial release. These include characteristics indicative of weediness, specifically disease and insect resistance. Additional data on the toxicity of proteins encoded by the introduced genes for insect resistance would also be required, including that relating to Cry2Ae alone and in

¹¹ GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment, Heritage & the Arts.

Summary of issues raised	Comment
on non-target invertebrates.	combination with Cry1Ab. This future research requirement has been clarified in the RARMP (Chapter 2, Section 4 and Chapter 3 Section 5).

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 087

The Acting Regulator received two submissions from the public on the consultation RARMP. One of these submissions, summarised in the table below, raised issues relating to human health and safety and the environment. It was considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Acting Regulator's decision to issue the licence.

Position (general tone): n = neutral; x = do not support; y = support

Issues raised: H: Human health; EN: Environmental risks; UE: Unintended effects.

Other abbreviations: DIR: Dealings involving Intentional Release; GM: Genetically Modified; OGTR: Office of the Gene Technology Regulator; RARMP: Risk Assessment and Risk Management Plan

Type: I: individual; O: organisation

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	EN	The submitter is opposed to the release of GM cotton on the grounds of inadequate research results relating to gene stability in the environment.	The genotype has been shown to be stable over a number of generations of self-crossing and crosses (Chapter 2). Chapter 3 identified issues that may need to be addressed for releases on a larger scale or subject to fewer controls; these issues include genotypic stability.
			H, EN	Sensitivity of humans and other species to GM technology may only show up over time.	The current licence is for a limited and controlled release. Stringent licence conditions have been imposed to restrict the dissemination of the GM cotton and the introduced genetic material into the environment. Thus, exposure of humans and other species to the GM cotton is extremely limited and greatly reduces the likelihood of adverse effects.
			UE	The Regulator should consider indirect effects of GM technology on human health and ecological stability	Where available, evidence relating to indirect effects on human health and the environment are considered in the RARMP. No such effects were identified for the current application (Chapter 2, event 7). In addition, statutory licence conditions require the licence holder to report any unintended effects. Chapter 3 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release any of the GMOs on a larger scale or if reduced containment measures were proposed.

2	O	y	none	Supports the current regulatory process and the ability of the OGTR to protect some information as CCI. Considers that appropriate information on the events has been provided to the Regulator to allow a Risk Assessment suitable for the trial. Comments that the Risk Assessment is detailed and scientifically based.	Noted
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