



Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 064/2006**

**Limited and controlled release of water-efficient GM
cotton**

Applicant: Monsanto Australia Limited

October 2006

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release of cotton genetically modified to enhance water use efficiency into the Australian environment, in respect of application DIR 064/2006 from Monsanto Australia Limited (Monsanto).

The DIR 064/2006 licence permits the release of 24 genetically modified (GM) cotton lines on a limited scale and under controlled conditions.

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

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

The application

Monsanto applied for a licence to release up to 24 cotton lines modified to enhance water use efficiency into the environment under limited and controlled conditions. The release was proposed to take place on up to 10 sites of up to 2 hectares (ie a maximum total area of 20 hectares) during each of the two summer growing seasons in 2006/07 and 2007/08¹.

The release may take place in the New South Wales shires of Balranald, Bourke, Central Darling, Carathool, Coonamble, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren or Lake Tandou (an unincorporated area) and/or in the Queensland shires of Paroo, Balonne, Murilla, Tara, Chinchilla, Waggamba, Wambo, Jondaryan or Pittsworth.

The GM cotton lines contain 1 of 24 different introduced genes. Twenty three of the genes are derived from the plants thale cress, corn, soybean, rice and cotton. One gene is derived from the common gut bacterium *Escherichia coli*². The introduced genes encode proteins that are intended to enable normal plant growth with reduced amounts of water (drought tolerance) either by regulating the expression of cottons' own genes or by altering biochemical pathways in the cotton plants.

The GM cotton lines also contain either a herbicide tolerance gene (*cp4 epsps*, conferring tolerance to the herbicide glyphosate) or an antibiotic resistance gene (*nptII*, conferring resistance to the antibiotics kanamycin or neomycin) that were used as markers to select modified plants during initial research and development work in the laboratory.

The purpose of the trial is to conduct early stage ('proof of concept') research to assess the agronomic performance of the GM cotton lines in the field, including under different irrigation treatments. Seed will be collected for further studies and possible future releases of

¹ In its initial application, Monsanto proposed individual sites of up to 1 hectare (ie a maximum total area of 10 hectares) per season. The company subsequently varied its application to propose individual sites of up to 2 hectares.

² Monsanto altered its initial application, which indicated all 24 genes would be from plants, to include this gene.

lines selected for further development (subject to additional applications and approvals). No products from the release will be used for human food, animal feed or for the production of fabrics and/or other cotton commodities.

Monsanto proposed a number of measures to limit the spread and persistence of the GM cotton lines and the introduced genetic materials that were considered during the evaluation of the application.

Risk assessment

The hazard identification process considered 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.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

Eighteen events were identified and assessed whereby the proposed release of the GM cotton lines might give rise to harm to people or the environment.

These 18 events 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. In addition, consideration was given to the opportunity for gene flow to other organisms and its effects.

None of the 18 events are considered to give rise to an identified risk that requires further assessment. The principle reasons comprise:

- small scale of the trial that is limited in both area and duration
- containment and disposal measures proposed by the applicant to limit the spread and persistence of the GM plants
- none of the GM plant materials will be used for any other purpose
- widespread presence of the same or similar proteins and enzymatic products in the environment and lack of evidence of harm from these proteins and their products
- the lack of known toxicity or allergenicity of the proteins (and enzymatic products) encoded by the introduced genes
- limited capacity of the GM cotton lines to spread and persist in the areas proposed for release
- limited ability and opportunity for the GM cotton lines to transfer the introduced genes to other sexually related species.

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 is considered to be **negligible**.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the 18 events identified and 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. However, containment and disposal measures have been imposed to restrict the release to the locations, size and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require the applicant to limit the duration of the release to two summer cotton growing seasons (2006/07 and 2007/08) on a maximum total area of two hectares per site on up to ten sites per growing season; prevent the use of the GMOs, or materials from the GMOs for any other purposes; maintain physical isolation of the release sites; and conduct post-harvest monitoring to ensure all GM plants are destroyed³.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 24 cotton lines modified to enhance water use efficiency into the areas proposed in New South Wales and Queensland poses **negligible** risks to the health and safety of people and the environment.

The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the locations, size and duration requested by the applicant.

³ The licence and conditions for DIR 064/2006 are available on the OGTR website (<<http://www.ogtr.gov.au/gmorec/ir.htm#table>>, following the path to DIR 064/2006).

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AQIS	Australian Quarantine and Inspection Service
CCI	Confidential Commercial Information as declared under section 185 of the Act
CSIRO	Commonwealth Scientific and Industrial Research Organisation
C-terminal	Carboxy-terminal end of a protein
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EMBL	European Molecular Biology Laboratory
FAO	Food and Agricultural Organization of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
kDa	kilodalton
LD ₅₀	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
m	Metre
mRNA	Messenger ribonucleic acid
µg	Microgram
ng	Nanogram
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OECD	Organisation for Economic Co-Operation and Development
OGTR	Office of the Gene Technology Regulator
RARMP	Risk Assessment and Management Plan
RNA	Ribonucleic acid
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration
US FDA	United States Food and Drug Administration
USDA	United States Department of Agriculture
WHO	World Health Organisation

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 064/2006) to Monsanto Australia Limited (Monsanto) for dealings involving the intentional release of up to 24 genetically modified (GM) cotton lines into the Australian environment.

The DIR 064/2006 licence permits the limited and controlled release of up to 24 cotton lines genetically modified to enhance water use efficiency. The release will occur on up to 10 sites of up to 2 hectares (ie a maximum total area of 20 hectares) during each of the two summer growing seasons in 2006/07 and 2007/08 in a number of shires in New South Wales and Queensland.

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

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Management Plans (RARMPs) that form the basis of her decisions⁴.

The RARMP for DIR 064/2006 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the dealings proposed by the applicant were taken into account by the Regulator in deciding to issue a licence and the licence conditions that have been imposed.

⁴ More information on the assessment of licence applications and copies of the Risk Analysis Framework are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>> and <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>, respectively.

Application

Title:	Limited and controlled release of water-efficient GM cotton*
Applicant:	Monsanto Australia Limited
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	<i>Gossypium hirsutum</i> L.
Modified traits:	Water use efficiency, herbicide tolerance, antibiotic resistance
Identity of the genes responsible for the modified traits:	<ul style="list-style-type: none"> • Each cotton line contains 1 of 24 different genes for water use efficiency derived from various plants including <i>Arabidopsis thaliana</i>, <i>Zea mays</i>, <i>Glycine max</i>, <i>Oryza sativa</i> and <i>Gossypium hirsutum</i>, or the bacterium <i>Escherichia coli</i>; and • <i>cp4 epsps</i> gene from <i>Agrobacterium</i> sp. strain CP4 (herbicide tolerance selectable marker); or • <i>nptII</i> from the bacterium <i>E. coli</i> (antibiotic resistance selectable marker).
Proposed locations:	Up to 10 sites per season in the NSW shires of Balranald, Bourke, Central Darling, Carathool, Coonamble, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren or Lake Tandou (an unincorporated area) and/or in the QLD shires of Paroo, Balonne, Murilla, Tara, Chinchilla, Waggamba, Wambo, Jondaryan or Pittsworth
Proposed release size:	Up to 2 hectares per site and a maximum of 20 hectares in total per growing season over 2 seasons
Proposed time of release:	Summer 2006/07 and 2007/08

* The original title of the licence application submitted by Monsanto was *Field testing of drought tolerance in cotton*.

Monsanto applied for a licence to release up to 24 cotton lines genetically modified to enhance water use efficiency⁵ into the environment. The release of the GM cotton lines is intended to take place on up to 10 sites of up to 2 hectares (ie a maximum total area of 20 hectares) during each of the two summer growing seasons in 2006/07 and 2007/08⁶.

The release may take place in a number of shires in New South Wales and/or Queensland (refer to the application table).

The purpose of the trial is to conduct early stage ('proof of concept') research to assess the agronomic characteristics of the GM cotton lines in the field including water use efficiency, yield and fibre quality under different irrigation treatments. Seed will be collected for further studies and possible future releases of lines selected for further development (subject to additional applications and approvals).

The GM cotton lines were derived by transforming plants of the cotton cultivar Coker 130, which is not grown commercially in Australia. They contain 1 of 24 different introduced genes intended to improve water use efficiency in the field without unacceptable impacts on agronomic characteristics including fibre quantity. Twenty three of the genes are derived from the plants *Arabidopsis thaliana* (thale cress), *Zea mays* (corn), *Glycine max* (soybean), *Oryza sativa* (rice) and *Gossypium hirsutum* (cotton)⁷. One gene is derived from the gut bacterium *Escherichia coli*.

The introduced genes encode proteins that are intended to confer water use efficiency by modulating biochemical pathways, functioning as molecular chaperones, or regulating signal transduction and expression of endogenous genes in the cotton plants.

The GM cotton lines also contain either an *Agrobacterium* gene (*cp4 epsps*, conferring tolerance to the herbicide glyphosate) or an *E.coli* gene (*nptII*, conferring resistance to the

⁵ Water use efficiency in cotton can be defined as the measure of total yield (lint) produced per unit of water supplied to the crop.

⁶ Monsanto varied its original request for sites up to 1 hectare and a total maximum area of 10 hectares per season to ensure experimental controls and containment measures could be accommodated.

⁷ In its initial application, Monsanto indicated all 24 introduced genes were derived from plants.

antibiotics kanamycin or neomycin) that were used to select modified plants during initial research and development work in the laboratory.

The applicant proposed measures to limit the spread and persistence of the GM cotton lines in the environment. These were taken into account in establishing the risk assessment context for the proposed release, and their suitability for limiting the release to the locations, size and duration proposed by the applicant was considered as part of the risk assessment process. No products from the GM cotton plants will be used for human food, animal feed or for the production of fabrics and/or other cotton commodities.

Some details including the names of the introduced genes, their encoded proteins and functions, and details of the gene constructs, including plasmid maps, and certain regulatory sequences, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was also made available to the prescribed expert groups and agencies that were consulted on this application.

Risk assessment

The risk assessment considered information contained in the application, previous GM cotton assessments, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received during consultation with a wide range of prescribed experts, agencies and authorities on the application (summarised in Appendix B of the RARMP). No further issues were raised in the comments received on the consultation version of the RARMP (see Appendix D).

Advice received from members of the public on the application and consultation on the RARMP, and how it was considered, is summarised in Appendices C and E, respectively.

A reference document, *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002), 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 first considered what harm to the health and safety of people or the environment could arise due to gene technology (hazard identification), and how it could happen during this release of GMOs, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The hazard identification process considered the circumstances by which the health and safety of people or the environment may be adversely affected by exposure to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

Eighteen events were identified and assessed whereby the proposed release of the GM cotton lines might give rise to harm to people or the environment.

These 18 events included consideration of whether 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. In addition, consideration was given to the opportunity for gene flow to other organisms and its effects.

None of the 18 events are considered to give rise to an identified risk that requires further assessment. The principle reasons comprise:

- small scale of the trial that is limited in both area and duration
- containment and disposal measures proposed by the applicant to limit the spread and persistence of the GM plants
- none of the GM plant materials will be used for any other purpose
- widespread presence of the same or similar proteins and enzymatic products in the environment and lack of evidence of harm from these proteins and their products
- the lack of known toxicity or allergenicity of the proteins (and enzymatic products) encoded by the introduced genes
- limited capacity of the GM cotton lines to spread and persist in the areas proposed for release
- limited ability and opportunity for the GM cotton lines to transfer the introduced genes to other sexually related species.

Therefore, as no risks to the health and safety of people or the environment were identified from the proposed limited and controlled release of GM cotton lines the level of risk is considered to be **negligible**.

Risk management

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

As none of the 18 events that were characterised in the risk assessment process are considered to give rise to an identified risk that requires further assessment, the level of risk to human health and safety and the environment from the proposed release of GM cotton lines is considered to be **negligible** (ie insubstantial with no present need to invoke actions for their mitigation).

However, containment and disposal measures have been imposed to restrict the release to the locations, size and duration requested by the applicant, as these were important parameters in establishing the context for assessing the risks.

Licence conditions

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

- implement measures to control the spread and persistence of the GMOs in the environment
- comply with the Regulator's guidelines and policies for the transport, supply, storage and disposal of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*)
- develop compliance and contingency plans and establish related reporting structures
- inform anyone covered by the licence of their obligations.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework. The Regulator sought input on the preparation of the RARMP from 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), National Health and Medical Research Council (NHMRC) and Australian Quarantine Inspection Service (AQIS). Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies⁸.

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 lines proposed for release to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM cotton lines for use in human food. However, FSANZ approval would need to be obtained before they could be used in this way.

AQIS is responsible for monitoring imports to prevent the introduction of exotic pests, weeds and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs. Additionally, as the importation of the water-efficient GM cotton seed constituted a dealing under the *Gene Technology Act 2000*, the importer required an authorisation under this Act for the import to lawfully proceed. Seed from the water-efficient GM cotton lines has been imported by Monsanto under an AQIS seed import permit.

Identification of issues to be addressed for future releases

In view of the very early stage of this research, the risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release of any of these GM cotton lines. This would include:

- molecular characterisation of the introduced genetic materials in the GM cotton lines, genotypic stability, and expression levels of the introduced genes in the GM cotton lines
- data on the potential toxicity of plant material from the GM cotton lines including levels of endogenous toxins
- data on the allergenicity of the proteins encoded by the introduced genes for water use efficiency, and plant material from the GM cotton lines
- biochemical, physiological and agronomic characteristics of the GM cotton lines indicative of weediness including measurement of tolerance to environmental stresses (eg drought, cold and salt) and/or reproductive capacity (eg seed number and window of flowering).

Conclusions of the RARMP

The risk assessment concludes that this proposed limited and controlled release of up to 24 cotton lines modified to enhance water use efficiency into the areas proposed in New South Wales and Queensland poses **negligible** risks to the health and safety of people and the environment.

⁸ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

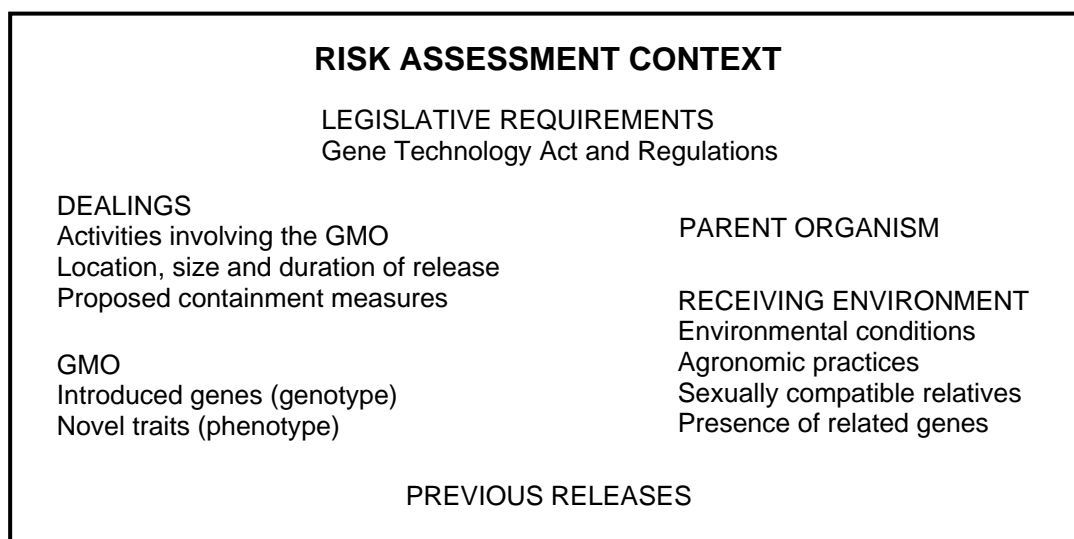
The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, conditions have been imposed to contain the release to the locations, size and duration requested by the applicant.

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 legislation⁹, details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

Figure 1 Components of the risk context considered during the preparation of the Risk Assessment



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

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

- the location, size and duration of the trial requested by the applicant
- proposed dealings
- containment measures for the GMOs proposed by the applicant
- characteristics of the parent organism
- the nature and effect of the genetic modification
- the environmental conditions in the location(s) where the release would occur
- relevant agricultural practices
- presence of related plants in the environment
- presence of the introduced or similar genes in the environment

⁹ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/ir/process.htm>> and in the *Risk Analysis Framework* (OGTR 2005) <<http://www.gov.au/pdf/raffinal2.2pdf>>.

- any previous releases of these or other GMOs relevant to this application
4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, prescribed Australian Government agencies, the Minister for Environment and Heritage and the local council(s) where the release would take place were consulted on matters relevant to the preparation of the RARMP. This advice, and where it was taken into account in the RARMP, is summarised in Appendix B. A submission from the public was also received. This advice is summarised in Appendix C.
5. In accordance with section 52 of the Act, the Regulator notified the public when the consultation version of the RARMP had been prepared and invited written submissions. Advice on the RARMP was also sought from the same experts, agencies and authorities as before. None of the latter raised any issues relating to risks to human health and safety and the environment that required further consideration (Appendix D). Issues raised by members of public and how they were addressed in the RARMP are summarised in Appendix E.

Section 2 The application

6. Monsanto proposes to release up to 24 GM cotton lines into the environment under limited and controlled conditions. The cotton lines have been modified to enhance water use efficiency.

2.1 The proposed size, location and duration

The release is proposed to take place on up to 10 sites per season on a maximum area of 2 ha per site during each of the two summer growing seasons in 2006/07 and 2007/08 in New South Wales and Queensland.

2.2 The proposed dealings

7. The purpose of the proposed trial is to conduct early stage 'proof of concept' research to assess some aspects of the agronomic characteristics of the GM cotton lines (water use efficiency, yield and fibre quality) under optimum watering and water stress treatments. Seeds would also be collected and stored for further studies including possible future releases of lines selected for further development (subject to additional applications and approvals).

2.3 The proposed measures to limit the spread and persistence of the GMOs

8. The applicant proposes measures to limit the spread and persistence of the GM cotton lines into the environment. These are taken into account in the risk assessment context (this chapter) and their suitability is evaluated in the following chapter for limiting the release to the locations, size and duration proposed by the applicant.
9. The applicant proposes the following containment measures:
- locating the proposed trial sites at least 50 m away from natural waterways
 - surrounding the trial sites by a 20 m pollen trap of non-GM cotton or GM cotton that the Regulator has approved for commercial release (Bollgard II[®], Roundup Ready[®] or Roundup Ready Flex[®] cotton), or a 50 metre isolation zone where there are no cotton crops or naturalised cotton populations within 450 metres of the trial sites
 - treatment of all plants including non-GM cotton grown at the trial sites, which includes the plants in the pollen trap, as if they were the GM cotton plants proposed for release in relation to harvesting and post harvest destruction

- harvesting all cotton plant materials (GM and non-GM) separately from other commercial cotton crops
- ginning of all harvested seed cotton from the proposed trial (GM and non-GM) in a certified PC2 facility or a facility approved in writing by the Regulator
- storage of plant materials from the proposed trial (required for future study or release) in certified PC2 facility or a facility approved in writing by the Regulator
- destruction of GM plant materials used in laboratory analysis and not required for future study or release
- after harvest destruction of all cotton plant materials, in and adjacent (eg equipment cleaning areas) to the trial sites, including the pollen trap, by stalk pulling, uprooting by ploughing, burning, treatment with herbicide, or hand weeding
- post harvest monitoring of trial sites for 12 months and destruction of any cotton volunteers
- transportation of GM cotton seed and plant materials in accordance with OGTR transportation guidelines.

10. No products from the release would be used for human food, animal feed or for the production of fabrics and/or other cotton commodities.

Section 3 The parent organism

11. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in New South Wales, and southern and central Queensland. However, the cultivar which has been transformed, Coker 130, is not grown here commercially. More detailed information on cotton can be found in the document, *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia*, which was produced to inform the risk assessment process for licence applications involving GM cotton plants (OGTR 2002). This document is available at <<http://www.ogtr.gov.au>>.

Section 4 The GMOs, nature and effect of the genetic modification

4.1 Introduction to the GMOs

12. Up to 24 GM cotton lines are proposed for release. Each line contains 1 of 24 different introduced genes. Twenty three of the genes are derived from the plants *Arabidopsis thaliana* (thale cress), *Zea mays* (corn), *Glycine max* (soybean), *Oryza sativa* (rice) and *G. hirsutum* (cotton). One gene is derived from the common gut bacterium *Escherichia coli*. The introduced genes encode proteins that are intended to confer enhanced water use efficiency (drought tolerance) by modulating biochemical pathways, functioning as molecular chaperones, or regulating signal transduction and expression of endogenous genes in the cotton plants.

13. The GM cotton lines contain the herbicide tolerance selectable marker gene *cp4 epsps* or the antibiotic resistance selectable marker gene *nptII*. The *cp4 epsps* gene, which encodes the 5-enolpyruvylshikimate-3-phosphate synthase enzyme, is from the common soil bacterium *Agrobacterium* sp. strain CP4 and confers tolerance to the herbicide glyphosate. The *nptII* gene, encoding the neomycin phosphotransferase type II enzyme, was originally derived from the bacterium *E. coli* and confers kanamycin and neomycin resistance on the GM plant. The *cp4 epsps* and *nptII* genes were used during the initial stage of selection of GM plants in the laboratory. The applicant does not intend to use these markers during the field trial.

14. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genes are also present in the GM cotton lines. These are derived from the plants *A. thaliana*, *Petunia x hybrida* and *Pisum sativum* (field pea), *G. max* (soybean), the plant viruses Cauliflower Mosaic Virus (CaMV) and Figwort Mosaic Virus (FMV), and the bacteria *Agrobacterium tumefaciens* and *E. coli*. Although *A. tumefaciens*, CaMV and FMV are plant pathogens, and *E. coli* is a facultative human pathogen, the regulatory sequences comprise only a small part of their total respective genomes, and are not infectious or capable of causing diseases.

15. Some details including the names of the introduced genes, their encoded proteins and functions, and details of the gene constructs, including plasmid maps and certain regulatory sequences, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and agencies that were consulted on this application.

4.2 Introduction to plant molecular responses to drought stress

16. Drought stress is an abiotic stress; a nonliving factor that causes harmful effects to plants. Other types of primary abiotic stresses include salinity, cold, heat and chemical pollution. Plants respond to different abiotic stresses often through an interconnecting series of signalling and transcription controls that ultimately aim to increase the plant's ability to tolerate the initial stress through different response mechanisms that include biochemical and physiological processes (Figure 2).

17. At a molecular level there are three broad categories into which plant genes can be classified depending on their role in the response to drought stress (Wang et al. 2003; Vinocur & Altman 2005). These are:

- Signal sensing, perception and transduction
- Transcriptional control
- Stress tolerance response mechanisms in terms of end functions including detoxification, osmoprotection, chaperone functions, and water and ion movement.

18. Little is known about how plants directly sense and perceive drought stress but it is thought specific receptors are involved (Xiong & Zhu 2001; Zhu 2002). Once sensed, a drought stress signal may be relayed to eventually regulate the transcription of numerous genes involved in the stress response. The transduction of the stress signal may be accomplished through various signalling molecules such as Ca^{2+} , phospholipids, reactive oxygen species, sugars and plant hormones, or via protein messengers such as mitogen activated protein kinases (MAPKs) and serine/threonine phosphatases (Xiong & Zhu 2001; Ramanjulu & Bartels 2002; Zhu 2002; Chaves & Oliveira 2004; Chinnusamy et al. 2004).

19. Drought stress signals activate genes that control the transcription of genes involved in the stress tolerance response mechanisms for cell protection. The majority of these can be categorised as transcription factors (Wang et al. 2003; Shinozaki et al. 2003), but an example of other genes involved in transcription control include those encoding proteins involved in chromatin remodelling (Singh 1998). Certain regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity) (Seki et al. 2002).

20. A transcription factor is any protein required for recognition, by RNA polymerases, of specific regulatory sequences in genes (eg a promoter) (Lewin 1994a). A particular transcription factor may affect the initiation of transcription for many genes, such as those involved in of abiotic stress reactions (Shinozaki et al. 2003; Chinnusamy et al. 2004;

Chinnusamy et al. 2006). The activity of a regulatory transcription factor may be controlled by its rate of synthesis, covalent modification (eg phosphorylation), binding to a ligand (eg hormone), or inhibitors that can sequester or affect the transcription factor's ability to bind to DNA (Lewin 1994b). Approximately 7.4% (~2000) of the expressed genes in the experimental model plant *A. thaliana* encode transcription factors (Iida et al. 2005).

21. Plants use four main classes of cellular response to drought stress in terms of end function. They are as follows:

- Molecular chaperones such as heat-shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins (Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). Chaperones and LEA proteins can provide protection and ensure proper folding and function of macromolecules such as enzymes during periods of stress.
- Metabolite production such as certain amino acids (eg proline), glycine-betaines, polyamines (eg spermine and spermidine), sugars and sugar alcohols (eg raffinose trehalose) (Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). These metabolites are thought to act primarily as osmoprotectants but may also have other secondary functions such as gene regulation, which may serve to provide further protection from drought stress.
- Detoxification enzymes (eg superoxide dismutases and catalases) for the production of antioxidants and deactivation of reactive oxygen species (eg hydrogen peroxide and hydroxyl radicals) (Wang et al. 2003; Chaves & Oliveira 2004; Vinocur & Altman 2005). Reactive oxygen species are generated as a result of various abiotic stresses and can irreversibly damage cellular components such as membranes and photosynthetic machinery.
- Adjustment of cellular water and ion content through regulation of membrane-intrinsic proteins. Examples of these proteins include water channel proteins and ion transporters (Ramanjulu & Bartels 2002; Wang et al. 2003; Chaves & Oliveira 2004). The regulation may be in the form of the protein's synthesis and/or via modifications (eg phosphorylation) and interactions with other proteins (eg 14-3-3 protein binding).

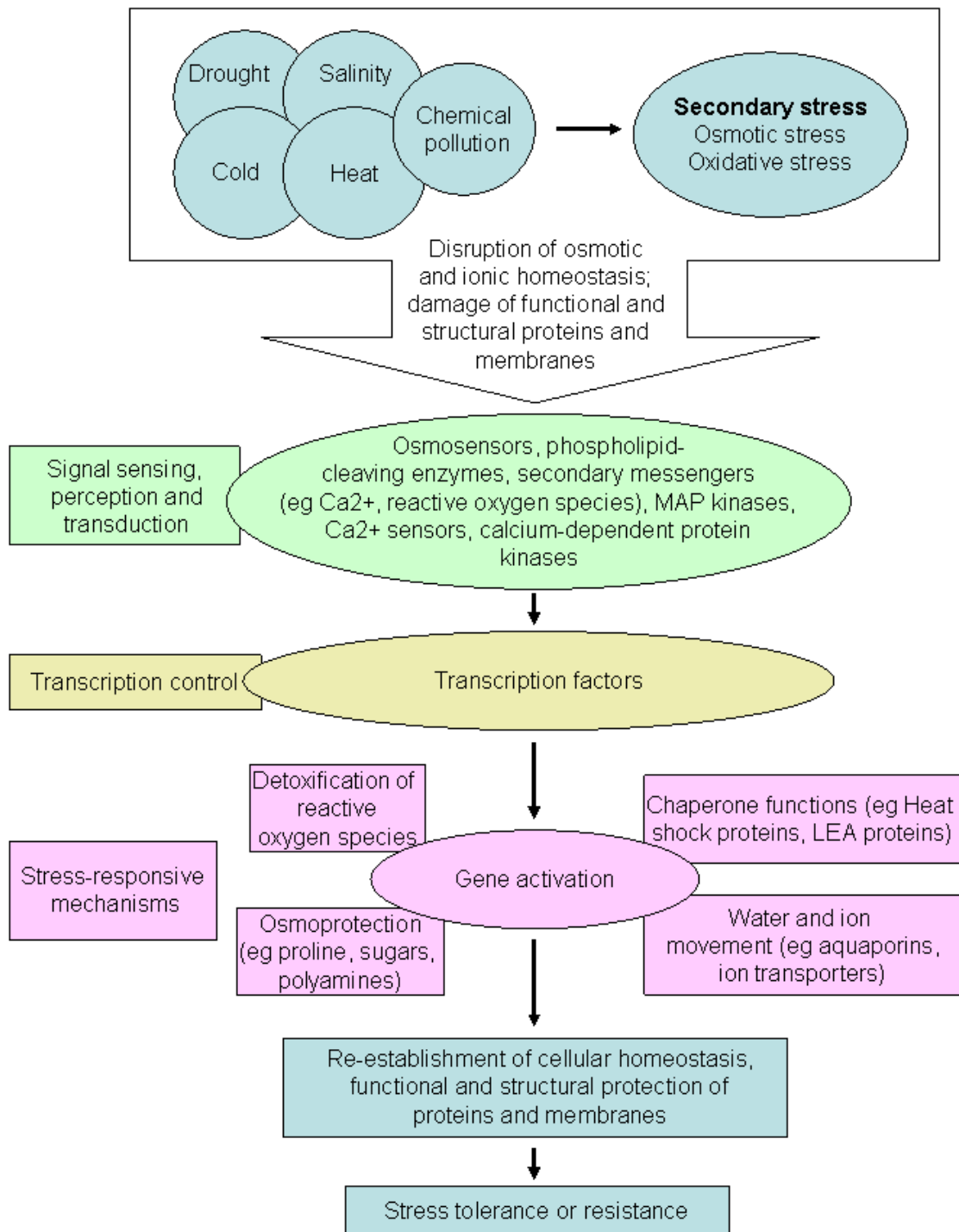


Figure 2 The abiotic stress tolerance response process in plants. Redrawn and modified from *Current Opinion in Biotechnology*, Volume 16, Vinocur, B. and Altman, A., Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations, Page no. 124, Copyright (2005), with permission from Elsevier. Abbreviations: MAP, mitogen activated protein; LEA, late embryogenesis abundant.

4.3 The introduced genes and regulatory sequences, and the gene products

22. Each GM cotton line contains 1 of 24 different introduced gene constructs for water use efficiency, which contain genes 1 to 24, respectively (details declared as CCI have been removed). The GM cotton line containing gene number 1 (of construct 1) is referred to as line 1 and so forth. The applicant states the introduced genes have demonstrated the capacity to produce a water-efficient phenotype in cotton and/or other plants. Published references relating to four of the introduced genes and their impact on enhanced drought tolerance in plants including cotton have been provided by the applicant (details relating to declared CCI have been removed). Some of the introduced genes, or their homologs, are also known to confer other agronomic effects in plants (details relating to declared CCI have been removed). The majority of the introduced genes for water use efficiency encode transcription factors. The GM cotton lines also contain the herbicide tolerance selectable marker gene *cp4 epsps* (lines 1 to 14) or the antibiotic resistance selectable marker gene *nptII* (lines 15 to 24) (for details see Sections 4.3.3 and 4.3.4).

23. The expression of the introduced genes for water use efficiency in the GM cotton lines is controlled by one of three different promoter types derived from CaMV, *Petunia x hybrida* and *A. thaliana*. The mRNA termination region for the introduced genes is derived from *P. sativum*. Further details about the regulatory sequences that control the expression of the introduced genes for water use efficiency have been declared as CCI.

4.3.1 Toxicity of the proteins encoded by the introduced genes for water use efficiency

24. To determine if the proteins encoded by the introduced genes for water use efficiency may potentially be toxins a bioinformatics assessment was performed by the applicant. A toxin sequence database was assembled from public sequence databases, including GenBank, EMBL (release 124) and SwissProt (release 1) by retrieving protein sequence entries containing the word 'toxin'. The assembled database contained 12,771 entries. This database was queried with the sequences of the proteins encoded by the introduced genes for water use efficiency. The significance of the degree of similarity between a pair of sequences was evaluated using an *E* score, which depends on overall length of local sequence alignments, the quality (percent identity, similarity) of the overlap and the size of the database. An *E* score of 1×10^{-5} was set as an initial high cut-off value for alignment significance. None of the introduced genes were found to encode proteins of significant homology to any sequences in the toxin database.

25. A literature search did not reveal any reports that imply the proteins encoded by the genes for water use efficiency may be toxins.

4.3.2 Allergenicity of the proteins encoded by the introduced genes for water use efficiency

26. Comparisons of the deduced amino acid sequence of each gene for water use efficiency to amino acid sequences in the publicly available Food Allergy Research and Resource Program (FARRP) Protein Allergen Database <<http://www.allergenonline.com>>, as of 13th February 2006, was carried out. Firstly, no matches of at least 35% identity in a segment of 80 amino acids or more were detected (information supplied by the applicant), which is below the threshold for allergen epitope screenings suggested by the FAO/WHO (FAO 2001; Codex Alimentarius Commission 2003). Secondly, no matches to known allergens greater than eight contiguous identical amino acids were identified (information supplied by the applicant). To avoid false negatives, the threshold suggested for allergen epitope screenings suggested by the

FAO/WHO is ideally six contiguous identical amino acids (FAO 2001; Codex Alimentarius Commission 2003).

27. A literature search did not reveal any reports that imply the proteins encoded by the genes for water use efficiency may be allergens.

4.3.3 The herbicide resistance marker gene (*cp4 epsps*) and the encoded protein

28. Fourteen GM cotton lines (constructs 1 to 14) also contain the *cp4 epsps* herbicide tolerance marker gene. The *cp4 epsps* gene, which confers tolerance to glyphosate (N-phosphonomethyl glycine), was isolated from the *Agrobacterium* sp. strain CP4. This *cp4 epsps* gene encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgett et al. 1996).

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

30. Glyphosate herbicide functions by inhibiting the activity of the naturally occurring EPSPS enzyme in plants, thus blocking the biosynthesis of aromatic amino acids and eventually leading to cell death (Steinrucken & Amrhein 1980). The *cp4 epsps* gene from *Agrobacterium* is naturally insensitive to the effects of glyphosate (Padgett et al. 1993), as are a number of other microbial EPSPS enzymes (Schulz et al. 1985; Eschenburg et al. 2002), being still able to function normally in the biosynthesis of aromatic amino acids. Consequently, in GM plant cells expressing the *Agrobacterium cp4 epsps* gene, biosynthesis of aromatic amino acids is not blocked in the presence of glyphosate and the plants are not harmed by application of glyphosate.

31. In plants, the EPSPS enzyme and the site of aromatic amino acid synthesis are located in the chloroplast. Thus, the *cp4 epsps* gene in the GM cotton lines was linked to a chloroplast transit peptide (CTP) coding region from the *epsps* gene of the plant *Petunia x hybrida* to provide transport to the cotton chloroplast. The CTP targets the CP4 EPSPS enzyme to the chloroplast. In plants, EPSPS is synthesised as a preprotein (ie containing the CTP) by free cytoplasmic ribosomes. The precursor is transported into the chloroplast stroma and proteolytically processed to yield the mature enzyme (della-Cioppa et al. 1986). Once cleaved from the mature protein, chloroplast transit peptides are rapidly degraded (Bartlett et al. 1982; della-Cioppa et al. 1986).

Regulatory sequences for the expression of the *cp4 epsps* gene

32. The bacterial *cp4 epsps* gene was modified by the addition of the 35S RNA transcript promoter from the FMV (Richins et al. 1987) and elements from *tsfl* gene (encoding the elongation factor EF-1 alpha) from the *A. thaliana* (Axelos et al. 1989) to allow effective expression in plant cells.

33. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the *cp4 epsps* gene in the GM cotton lines is the 3' untranslated region derived from the *auxin down-regulated* gene from *G. max* (Datta et al. 1993).

Toxicity of CP4 EPSPS

34. The CP4 EPSPS protein expressed in the GM cotton plants proposed for release is the same as that present in commercially released Roundup Ready[®] cotton. Toxicity studies using the purified form of the introduced CP4 EPSPS protein present in Roundup Ready[®] cotton have been conducted. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 022/2002 and DIR 023/2002. Key results are summarised below.

35. Purified CP4 EPSPS protein, at acute doses of up to 572 mg/kg body weight, produced no adverse effects in mice (Harrison et al. 1996). This is more than a thousand times the anticipated potential consumption of CP4 EPSPS in commercial food derived from all GM food crops expressing this enzyme under development by Monsanto at that time (soybean, potato, tomato, corn) (Harrison et al. 1996). Sequence alignment with proteins in the toxin database did not show structurally relevant similarity between the CP4 EPSPS protein and any known toxic or pharmacologically active protein relevant to human health (information supplied by the applicant).

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

37. In some countries, including the USA (but not Australia), cotton seed meal is approved for use in human diets (OGTR 2002). Levels of CP4 EPSPS protein in seed from Roundup Ready Flex[®] cotton, a GM cotton variety expected to have a higher concentration of CP4 EPSPS protein than that of the water-efficient cotton lines, have been measured at 340 µg/g dry weight. A 70 kg person would need to consume approximately 117.7 kg of dried cotton seed at one sitting in order to be acutely exposed to 572 mg/kg body weight. As indicated above, no toxic effects were observed in mice at this level.

Allergenicity of CP4 EPSPS

38. The CP4 EPSPS protein is 47.6 kDa in size, which is in the typical range for allergenic proteins. It is rapidly denatured by heat, enzymatic digestion and acid in simulated mammalian digestive or gastric fluid ((Harrison et al. 1996; Canadian Food Inspection Agency 1997; ANZFA 2001f), and information provided by the applicant). The protein shows no significant sequence homology to allergens assembled in the Genpept, PIR and SwissProt protein databases (Mitsky 1993). No immunologically relevant sequences (eight contiguous amino acid identities) were detected when the amino acid sequence of the CP4 EPSPS protein was compared to the ALLERGEN3 sequence database and the protein shows no sequence similarity to any proteins known to pose human health risks (information provided by the applicant). Roundup Ready[®] soybean expressing the identical introduced CP4 EPSPS protein has been shown not to be allergenic (Batista et al. 2005).

4.3.4 The antibiotic resistance marker gene (*nptII*) and the encoded protein

39. Ten GM cotton lines (constructs 15 to 24) also contain the *nptII* antibiotic resistance marker gene. The *nptII* gene was isolated from the bacterial Tn5 transposon (from *E. coli*) (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to aminoglycoside antibiotics, eg kanamycin and neomycin. NPTII uses ATP to phosphorylate those antibiotics, thereby inactivating and preventing them from killing the NPTII-producing cells. The *nptII* gene functioned as a selectable marker during the

laboratory stages of cotton plant tissue selection following genetic modification, allowing GM cells to grow in the presence of the antibiotic while inhibiting the growth of non-GM cells. The NPTII protein is in common use as a selectable marker in the production of GM plants (Miki & McHugh 2004).

40. Other regulatory agencies, in Australia and in other countries, have previously assessed the *nptII* gene as safe for use in human food (US FDA 1998; ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; ANZFA 2001d; FSANZ 2003). In addition, a number of genetically modified food crops containing the *nptII* gene have been approved for commercial release both in Australia (DIRs 012/2002, 021/2002, 022/2002 and 059/2005) and overseas. No adverse effects on humans, animals or the environment have been reported from these releases (US FDA 1998; Flavell et al. 1992; EFB 2001).

Regulatory sequences for the expression of the nptII gene

41. The bacterial *nptII* gene was modified by the addition of regulatory sequences including the 35S promoter from the CaMV (Odell et al. 1985) and the *nopaline synthase* gene (*nos*) transcription termination region from *A. tumefaciens* to allow efficient expression in plant cells.

Toxicity of NPTII

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

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

44. The insertion of the *nptII* gene into a wide range of GMOs has not resulted in any adverse effects (Flavell et al. 1992). The *nptII* gene was introduced into mammalian cell lines with no effects on viability or growth. During gene therapy experiments, mammalian cells expressing the NPTII protein have been infused into cancer patients. Again, no adverse effects have been observed (Flavell et al. 1992).

45. The NPTII protein produced in GM tomatoes has been fed to rodents and reported to be rapidly inactivated and degraded (Calgene 1990). An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). A similar study in mice also reported no adverse effects of NPTII at 5000 mg/kg of body weight (Fuchs et al. 1993b).

Allergenicity of NPTII

46. The NPTII protein is approximately 29 kDa in size, which is within the typical size range of allergenic proteins. However, it does not possess glycosylation sites, is not stable in the mammalian digestive system and is heat labile, decreasing the probability that it is allergenic (US FDA 1998; Fuchs et al. 1993a; FDA 1994; ANZFA 2001e; ANZFA 2001f). Fuchs et al. reported that no NPTII was detected 10 seconds after addition of simulated gastric

fluid as measured by both western blot and enzymatic activity (Fuchs et al. 1993b). Protein sequence comparisons using sequences from four separate protein databases (EMBL, GenBank, PIR29 and Swiss-Prot) indicated that NPTII does not have significant sequence identity to any known protein food allergens (Fuchs & Astwood 1996).

47. The FDA has evaluated data submitted for deliberate releases of GMOs expressing the NPTII protein and concluded that NPTII does not have any of the characteristics associated with allergenic proteins (US FDA 1998). The UK Royal Society have concluded that there is at present no evidence that available GM foods cause allergic reactions, and that the risks posed by GM plants are in principle no greater than those posed by conventional breeding or by plants introduced from other areas of the world (The Royal Society 2002).

4.4 Method of genetic modification

48. The 24 water-efficient GM cotton lines were each generated by *Agrobacterium*-mediated transformation using standard protocols (Umbeck 1991).

49. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens* through the mediation of genes from the virulence region of Ti plasmids.

50. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmid vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984).

51. The GM cotton lines were generated by *Agrobacterium*-mediated transformation using standard protocols (Umbeck 1991). Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA) located between specific border sequences on a resident plasmid from *A. tumefaciens* through the mediation of genes from the virulence region of Ti plasmids. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing genes and regulatory sequences for their expression into plants. Unintended changes in phenotype can occur upon transformation, or because of the tissue culture process used in the transformation method, and are similar to those in conventional breeding and mutation breeding.

52. A disarmed binary plasmid vector was used to introduce the 24 different gene constructs containing either the *cp4 epsps* or *nptII* gene (see Sections 4.3.3 and 4.3.4) into cotton cultivar Coker 130 using standard *Agrobacterium* transformation protocols. Coker 130 was used as it is readily transformed. However, this cultivar is not grown commercially in Australia. Following the transformation process and plant regeneration, screening was performed in the presence of glyphosate (*cp4 epsps* gene containing constructs), or kanamycin or neomycin (*nptII* gene containing constructs). Subsequently, cotton plants containing the introduced gene constructs were obtained that were tolerant to glyphosate, or kanamycin and neomycin.

53. The 24 GM cotton lines were generated from independent transformation events, and therefore the introduced genes are expected to be located at different sites in the cotton genome in each line. The modified traits have not been introduced into any of the Australian elite cotton cultivars.

4.5 Characterisation of the GMOs

4.5.1 Stability and molecular characterisation

54. The applicant states that all 24 GM cotton lines are in early development stage and have not been tested for genotypic stability. In addition, the applicant states that all 24 GM cotton lines form part of a 'proof of concept' field trial. Detailed molecular characterisation using standard molecular biology techniques would take place once the best performing GM cotton lines have been identified under field conditions. Those GM cotton lines would then be selected for molecular characterisation. Such data would be required for possible future applications involving large scale or commercial releases of these GM cotton lines. However, this information is not required for assessing the risks of this proposed release because of the containment measures proposed by the applicant to limit the spread and persistence of the GM cotton lines, and the trial is limited in size, duration and location.

4.5.2 Characterisation of the phenotype of the GMOs

55. One of the aims of the proposed trial is to compare agronomic performance of GM cotton lines and non-GM parent under field conditions. The intended effect is improved water use efficiency in the field without unacceptable impacts on agronomic characteristics including fibre quantity. The applicant states that all of the 24 introduced genes for water use efficiency have been shown in cotton and/or other plants to confer tolerance to confer water use efficiency. Published references relating to four of the introduced genes and their impact on enhanced drought tolerance in plants including cotton have been provided by the applicant (details relating to declared CCI have been removed). Some of the genes have conferred enhanced tolerances to one or more abiotic stresses in test plant(s) (details relating to declared CCI have been removed). Only one of GM cotton lines (line 1) has undergone preliminary field testing (see Section 6.2) for water use efficiency (drought tolerance). However, the applicant states the data collected on water use efficiency is inconclusive in this instance. Data on the tolerances of the GM cotton lines to drought stress and other abiotic stresses would be required for possible future applications involving large scale or commercial releases of these GM cotton lines. However, this information is not required for assessing the risks of this proposed release because of the containment measures proposed by the applicant to limit the spread and persistence of the GM cotton lines, and the trial is limited in size, duration and location.

Toxicity and allergenicity of the water-efficient GM cotton

56. Data on the toxicity and allergenicity of the GM cotton lines would be required for possible future applications involving large scale or commercial releases of these GM cotton lines. However, this information is not required for assessing the risks of this proposed release because of the containment measures proposed by the applicant to limit the spread and persistence of the GM cotton lines, and the trial is limited in size, duration and location.

Section 5 The receiving environment

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

5.1 Relevant abiotic factors

58. To meet the water demand of cotton (approximately 7 ML/ha of irrigated water utilised per crop) for good economic returns the majority of Australia's crop is grown under irrigation, mostly in the Murray-Darling Basin (Anthony 2003). Alternatively, cotton may be grown as an unirrigated crop known as dryland cotton. In some years up to 20% of the total cotton production area consists of dryland cotton although this has accounted for less than 10% of total production (Australian Cotton Cooperative Research Centre 2002). When cotton is grown as an unirrigated crop the biggest climatic factor influencing cotton yield is rainfall (Ford 2002). In Australia, the majority of dryland production occurs in areas that have a moderate to high variability in rainfall during January to March, the crucial period of the growing season determining yield quantity and quality (Gibb & Constable 1995; Ford 2002). In this period cotton has a daily water use of up to 8 to 10 mm (Gibb & Constable 1995). Therefore, in Australia it is advised that dryland cotton production should ideally be planted when subsoil moisture is adequate to reduce crop failure risk (Australian Cotton Cooperative Research Centre 2002).

59. Water use efficiency in cotton can be simply defined as the measure of total yield (lint) produced per unit of water supplied to the crop (Gibb & Constable 1995). Because of the reliance on rainfall that is highly variable, dryland production has the biggest potential to benefit through improved water use efficiency of cotton plants (Cooperative Research Centre for Sustainable Cotton Production 1995; Australian Cotton Cooperative Research Centre 2002). However, irrigated cotton may also benefit from improved water use efficiency particularly in drought years where less water is available for irrigation (Cooperative Research Centre for Sustainable Cotton Production 1995), if water allocations were to be reduced because of environmental demands or the cost of water were to rise. Overall, the improvements in the water use efficiency of cotton could potentially increase the area currently dedicated to both dryland and irrigated cotton production.

60. The size, locations and duration of the proposed limited and controlled release of water-efficient GM cotton are outlined in Section 2.1. A list of all the areas where the trial could take place is provided in Table 1.

61. Selected climatic data representing several geographically distinct commercial cotton growing regions where the trial is proposed to take place is given in Table 2. These areas have typical climates for summer cotton growing regions in Australia, with warm summers and mostly higher summer than winter rainfall.

Table 1 Shires proposed by the applicant for release of water-efficient GM cotton

New South Wales			Queensland	
Balranald	Lachlan	Unincorporated area	Balonne	Tara
Bourke	Moree Plains	(Lake Tandou)	Chinchilla	Waggamba
Carathool	Narrabri		Jondaryan	Wambo
Central Darling	Narromine		Murilla	
Coonamble	Walgett		Paroo	
Hay	Warren		Pittsworth	

Table 2 Climatic data for sites representative of proposed water-efficient GM cotton trial areas

Representative site (within area)	Av. daily max/min temperature (summer)	Av. daily max/min temperature (winter)	Av. monthly rainfall (summer)	Av. monthly rainfall (winter)
Bourke NSW (Bourke)	35.6/20.3	19.0/5.6	38.8 mm	23.6 mm
Hay NSW (Hay)	32.2/15.9	16.0/4.2	27.3 mm	32.9 mm
Menindee NSW (Lake Tandou)	33.5°C/17.7°C	17.8°C/4.7°C	21.9 mm	19.2 mm
Moree NSW (Moree Plains)	34.4°C/18.7°C	19.5°C/4.3°C	64.1 mm	73.2 mm
Warren NSW (Warren)	33/17.9	17/3.4	56.8 mm	30.3 mm
Cunnamulla QLD (Paroo)	35.3/21.5	19.8/6.5	45.3 mm	22.1 mm
Pittsworth QLD (Pittsworth)	29.6°C/16.6°C	17.5°C /5.7°C	89.2 mm	37.6 mm

Source: <<http://www.bom.gov.au>>. Abbreviations: Av., Average

5.2 Relevant agricultural practices

62. The applicant intends to trial the GM cotton alongside the non-GM parent Coker 130 under both optimal and water stress treatments. Fertilizers, insecticides and other *Cotton Industry Good Management Practices* would be applied, similar to those used for commercially grown non-GM cotton. Therefore, the management of the GM cotton plants is expected to be the same as for non-GM commercial cotton occurring in the areas surrounding the proposed trial sites with the exception of some altered irrigation regimes.

5.3 Presence of related plants in the receiving environment

63. In the areas proposed for release, *G. hirsutum* is the most common species of cotton commercially grown. Small quantities of *G. barbadense* (Pima cotton) are also commercially grown. Herbarium records for *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in central and south eastern Queensland (OGTR 2002). The presence of remnants of some of these populations has not been confirmed.

64. A number of GM cotton (*G. hirsutum*) lines have previously been approved for commercial release as follows:

- insect resistant Bollgard II[®] GM cotton and the glyphosate tolerant Roundup Ready[®] GM cotton and conventional crosses between them (authorised under DIR 12/2002 and 23/2002 licences)
- glyphosate tolerant Roundup Ready Flex[®] MON 88913 (referred to as Roundup Ready Flex[®]) and glyphosate tolerant/ insect resistant (Roundup Ready Flex[®]/ Bollgard II[®]) cotton (authorised under DIR 059/2005 licence)
- glufosinate ammonium tolerant (Liberty Link[®] Cotton) cotton (authorised under DIR 062/2005 licence).

65. These approvals include releases in the areas proposed in the current application. As a result of the commercial release of several GM cotton lines in southern Australia (south of latitude 22°S) GM cotton plants are widespread in the agricultural environment comprising about 90% of commercially grown cotton crops.

5.4 Presence of the introduced genes or similar genes in the environment

66. Twenty three of the introduced genes for water use efficiency are derived from either the crop plants *G. hirsutum*, *G. max*, *O. sativa* and *Z. mays*, or the well characterised plant *A. thaliana*, which is widely used in experimental studies. The introduced genes for water use efficiency are highly homologous and most likely orthologous, to genes in most, if not all, crop and other plants. Therefore, it is expected humans routinely encounter, through contact with plants and food, the introduced genes and their gene products, or their homologs. Further details relating to declared CCI have been removed.

67. One of the introduced genes for water use efficiency (details relating to declared CCI have been removed) is derived from the bacterium *E. coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected humans routinely encounter, through contact with plants and food, the encoded protein, or its homologs.

68. EPSPS enzymes are present in all plants, bacteria and fungi. The CP4 EPSPS enzyme is derived from the common soil bacterium *Agrobacterium* spp. strain CP4 (Barry et al. 1992; Padgett et al. 1996), which is found in soil and on plants. The difference between the natural plant enzymes and the bacterially derived CP4 EPSPS is in the amino acid sequence, not in the biochemical function. Other EPSPS enzymes from both plant and microbial sources also vary to a similar degree in amino acid sequence (Felsot 2000a; Padgett et al. 1996).

69. The NPTII protein is widespread in the environment since it is naturally produced by the common gut bacterium *E. coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997).

Section 6 Australian and international approvals

6.1 Australian approvals of the GM cotton lines

6.1.1 Previous releases approved by GMAC or the Regulator

70. There has been no release of these GM cotton lines in Australia.

6.1.2 Approvals by other Australian government agencies

71. 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) and Food Standard Australia New Zealand (FSANZ). This is discussed further in Chapter 3, Risk Management.

72. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests, weeds and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs. Additionally, as the importation of the water-efficient GM cotton seed constituted a dealing under the *Gene Technology Act 2000*, the importer required an authorisation under this Act for the import to lawfully proceed. Seed from the water-efficient GM cotton lines has been imported by Monsanto under an AQIS seed import permit.

73. 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 lines proposed for release to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM cotton lines for use in human food. However, FSANZ approval would need to be obtained before they could be used in this way.

6.2 International approvals

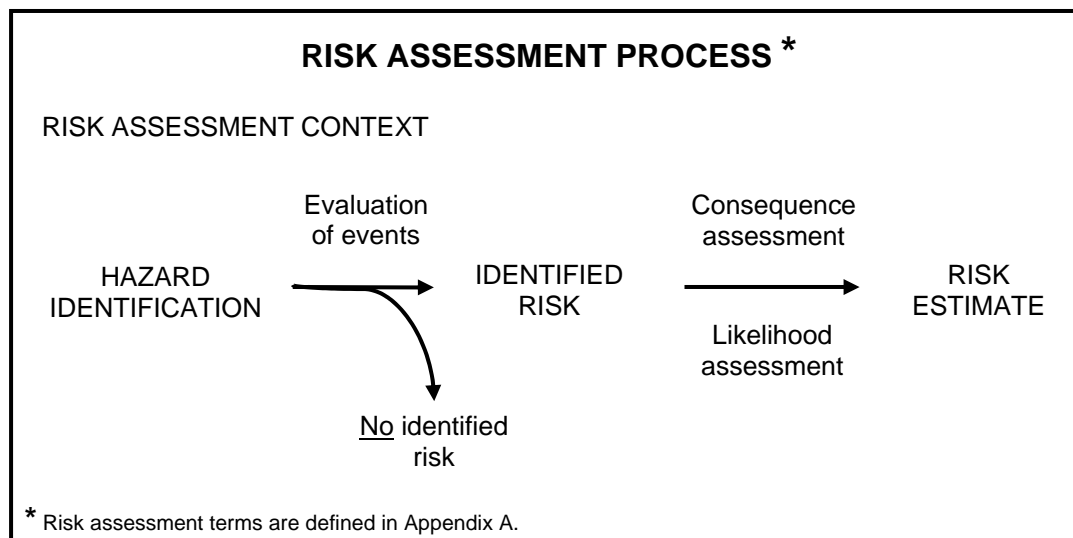
74. Field trials with GM cotton containing gene 1 were conducted in the USA in 2005 under the USDA permit number 05-278-02n (information supplied by the applicant).

Chapter 2 Risk assessment

Section 1 Introduction

75. 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 3) considers risks from the proposed dealings with the GMO that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

Figure 3 The risk assessment process.



76. 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 this GMO into the environment.

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

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

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

80. 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

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

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

83. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2005). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. The risk assessment process focuses on measurable criteria for determining harm.

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

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMO;
- the size, duration and regions of the release;
- containment measures proposed by the applicant;
- characteristics of the non-GM parent (OGTR 2002);
- routes of exposure to the GMOs, the introduced gene(s) and its product(s);
- potential effects of the introduced gene(s) and its product(s) expressed in the GMO;
- potential exposure to the introduced gene(s) and its product(s) from other sources in the environment;
- properties of the biotic and abiotic environment at the site(s) of release;
- agronomic management practices for the GMO.

85. There have been no releases of these GMOs in Australia.

86. Events that are discussed in detail later in this Section are summarised below in Table 3. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. Eighteen events were characterised, none of which were considered to lead to an identified risk that required further assessment.

87. The prevalence of the *cp4 epsps* and *nptII* genes in the environment and the lack of evidence for toxicity or allergenicity of the CP4 EPSPS and NPTII proteins are discussed in Chapter 1, Sections 4.3.3 to 4.3.4. In addition, the potential effects of the *cp4 epsps* or *nptII* genes and their products were considered in detail in previous assessments for DIRs 055/2004, 056/2004, 059/2005 and 060/2005, some of which are for commercial releases. RARMPs for those DIR licences are available from the OGTR or from the website <<http://www.ogtr.gov.au>>. Risks arising from the expression of *cp4 epsps* or *nptII* genes in GM cotton are considered to be negligible. Therefore, the potential effects of *cp4 epsps* and *nptII* genes will not be further assessed for this application.

Table 3 Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for water use efficiency

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 to people	1. Ingestion of GM plant materials containing proteins encoded by the introduced genes.	Toxicity for people	No	<ul style="list-style-type: none"> The introduced genes are widespread in the environment. There are no reports of toxicity associated with the encoded water use efficiency proteins. None of the GM plant materials from the proposed release would be used as human food.
	2. Contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes.	Toxicity for people	No	<ul style="list-style-type: none"> The introduced genes are widespread in the environment. There are no reports of toxicity associated with the encoded water use efficiency proteins. Contact with, or inhalation of GM plant materials would be limited to workers associated with the field trial. Contact would be further limited by the small size and short duration of the proposed field trial.
Section 2.2 Production of a substance allergenic to people	3. Use of GM plant materials, containing proteins encoded by the introduced genes, in food.	Allergic reactions in people	No	<ul style="list-style-type: none"> None of the water use efficiency proteins have been listed in a publicly available allergen database. None of the GM plant materials from the proposed release would be used as human food.
	4. Contact with GM plant materials (including pollen) containing proteins encoded by the introduced genes.	Allergic reactions in people	No	<ul style="list-style-type: none"> None of the water use efficiency proteins encoded by the introduced genes have been listed in a publicly available allergen database. None of the GM plant materials from the proposed release would be used as human food, animal feed or in the production of fabrics and/or other cotton products. Cotton pollen is heavy and not easily dispersed by wind. Contact with, or inhalation of GM plant materials would be limited to workers associated with the trial. Contact would be further limited by the small size and short duration of the proposed field trial.
Section 2.3 Production of a substance toxic to organisms other than people	5. Direct or indirect ingestion of GM plant materials, containing proteins encoded by the introduced genes, by vertebrates, invertebrates and microorganisms.	Toxicity for vertebrates, invertebrates and microorganisms	No	<ul style="list-style-type: none"> Vertebrates generally do not feed on cotton plants as they find them unpalatable. Exposure of vertebrates, invertebrates and microorganisms to the GM cotton lines would be limited by the small size and short duration of the proposed release. None of the GM plant materials from the proposed release would be used as animal feed. Vertebrates, invertebrates and microorganisms are already widely exposed to the same or similar proteins.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.4 Spread and persistence of the GM cotton in the environment	6. Expression of the introduced genes improving the survival of cotton volunteers through enhanced water use efficiency (drought stress tolerance)	Weediness	No	<ul style="list-style-type: none"> Many factors limit the spread and persistence of cotton in the areas proposed for release, other than water availability, including temperature (particularly frost), nutrient levels, roadside management practices, short growing seasons, soil type, competition from other plants and disease. The release would be of small size, short duration and the applicant proposes measures to limit the spread and persistence of the GM cotton lines to the proposed release sites.
	7. Expression of the introduced genes improving the survival of cotton volunteers through enhancement of: <ul style="list-style-type: none"> abiotic stress tolerances other than drought stress tolerance biotic stress tolerance 	Weediness	No	<ul style="list-style-type: none"> Many factors limit the spread and persistence of cotton in the areas proposed for release, other than water availability, including temperature (particularly frost), nutrient levels, roadside management practices, short growing seasons, soil type, competition from other plants and disease. The release would be of small size, short duration and the applicant proposes measures to limit the spread and persistence of the GM cotton lines to the proposed release sites.
	8. Exposure of vertebrates (including people), invertebrates and microorganisms to GM cotton volunteers expressing the introduced genes	Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> The release would be of small size and short duration, and the applicant proposes measures to limit the spread and persistence of the GM cotton lines. The potential effects on people are discussed in Sections 2.1 and 2.2. The potential effects on organisms other than people are discussed in Section 2.3.
	9. Dispersal of GM seed or GM plant research materials during transport, storage, equipment use or flooding.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> The applicant proposes to transport and store all GM plant materials including seeds according to OGTR guidelines, to clean equipment on site and to destroy all plant material remaining on the sites at the end of each growing season. Additional research would be carried out in approved facilities and any GM plant waste materials would be destroyed. The sites would be located above flood level and at least 50 m away from natural waterways to limit dispersal via flooding.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Gene flow by vertical gene transfer (ie gene transfer to sexually compatible plants)	10. Expression of the introduced genes in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (including commercially released GM cotton lines).	Weediness	No	<ul style="list-style-type: none"> • Cotton is primarily a self-pollinating plant, so gene transfer to other cotton plants is expected to occur at low frequencies. • Outcrossing to other <i>G. hirsutum</i> and <i>G. barbadense</i> including commercial cotton crops would be limited due to the small size, short duration and containment measures proposed by the applicant. • Additionally, agricultural practices proposed by the applicant include the use of insecticides, which would further limit pollen dispersal. • Factors limiting the spread and persistence of cotton are discussed in Section 2.4.
	11. Exposure of vertebrates (including people), invertebrates and microorganisms to other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (including commercially released GM cotton lines), expressing the introduced genes.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for invertebrates and microorganisms.	No	<ul style="list-style-type: none"> • The limited potential for gene flow to other cotton is discussed in Event 10. • The potential effects on people are discussed in Sections 2.1 and 2.2. • The potential effects on organisms other than people are discussed in Section 2.3.
	12. Presence of the introduced regulatory sequences in <i>G. hirsutum</i> or <i>G. barbadense</i> plants as a result of gene transfer.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> • Outcrossing to other <i>G. hirsutum</i> and <i>G. barbadense</i> including commercial cotton crops would be limited due to the small size, short duration and containment measures proposed by the applicant. • The introduced regulatory sequences are not known to behave any differently than endogenous regulatory sequences in plants.
	13. Gene transfer to native <i>Gossypium</i> species.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> • Well established genetic incompatibility prevents vertical gene transfer to native <i>Gossypium</i> species.
Section 2.6 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	14. Presence of the introduced genes, or regulatory sequences, in other organisms as a result of gene transfer.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms, Increased pathogenicity	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. • Gene transfer from plants to bacteria has not been demonstrated under natural conditions.
Section 2.7 Unintended changes in toxicity or allergenicity	15. Altered levels of innate toxic or allergenic compounds as a result of the expression, or random insertion into the cotton genome, of the introduced genes.	Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms, Weediness	No	<ul style="list-style-type: none"> • Unintended adverse effects, if any, would be limited by the small scale and short duration of the proposed release. • Information on toxicity and allergenicity of the GM cotton plants would be a prerequisite for a large scale or commercial release.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.8 Unintended changes in biochemistry, physiology or ecology	16. Altered biochemistry, physiology or ecology of the GM cotton lines resulting from expression of the introduced genes.	Weediness, Toxicity for, or allergic reactions in, vertebrates (including people), Toxicity for invertebrates and microorganisms	No	<ul style="list-style-type: none"> Unintended adverse effects, if any, would be limited by the small scale and short duration of the proposed release. The applicant proposes to measure the agronomic performance of the GM cotton lines; adverse effects, if any, would be detected during the trial.
Section 2.9 Secondary impacts	17. Adverse impact on populations of organisms that interact with organisms affected by the GM cotton lines.	Toxicity for vertebrates and invertebrates	No	<ul style="list-style-type: none"> The release would be of small size and short duration. Effects, if any, would be confined to the areas of the proposed release.
Section 2.10 Unauthorised activities	18. Use of GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.9	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 to people

88. Toxicity is the cascade of reactions resulting from exposure to a dose of a chemical that is sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000b). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992). Toxicity may occur through ingestion, contact or inhalation. The level of toxicity is often expressed as the LD₅₀. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

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

Event 1: Ingestion of GM plant materials containing the proteins encoded by the introduced genes

90. The GM cotton lines proposed for release differ from non-GM cotton in that they contain two introduced genes; one encoding a protein intended to confer water use efficiency and another encoding either the CP4 EPSPS or NPTII protein. As explained in paragraph 87, only the proteins encoded by the introduced genes for water use efficiency will be considered further. As the proposal is a 'proof of concept' field trial, no toxicity measurements have been made of the proteins encoded by the introduced genes or of the GM cotton lines. However, the introduced genes for water use efficiency present in the GM cotton lines are the same or similar to those present in many crop plants (eg corn, soybean and rice) or microorganisms (eg *E. coli*). People are exposed to the proteins (and enzymatic products) encoded by the introduced genes for water use efficiency, or similar proteins, through normal diet or the environment (see Chapter 1, Section 5.4). There are no reports of toxicity associated with the proteins encoded by the introduced genes (for details see Chapter 1, Section 4.3.1).

91. The applicant does not intend to use GM plant materials (including cotton seed oil or linters) from the proposed release in human food or as animal feed, but to destroy plants

remaining on the trial sites after collecting samples for analysis and harvesting seed. Ingestion of materials from the GM cotton line containing the introduced proteins is not expected to occur from the proposed release.

92. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment and FSANZ approval would be required before products from the GM cotton line could be used in human food.

93. Therefore, **no risk is identified**. The potential for toxicity for people as a result of ingestion of the introduced proteins will not be assessed further.

Event 2: Contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes

94. Dermal and inhalation toxicity studies have not been conducted with the proteins (or their products) encoded by the introduced genes for water use efficiency. However, on the basis of the presence of these or similar proteins in many common plant and bacterial species, they are also expected to be of very low acute dermal and inhalation toxicity. Dermal and inhalation contact will occur via pollen or when the plant cells have been damaged or broken. Workers may come into contact with the protein (and products) encoded by the introduced genes during handling and/or processing the GM cotton. It is highly unlikely people living nearby could come into contact with GM cotton pollen as it is relatively large and heavy, and not easily dispersed by wind (OGTR 2002).

95. Since the proposed release is of small size and short duration, the frequency and duration of contact with the proteins (and enzymatic products) encoded by the introduced genes is expected to be very limited. In addition, the applicant does not intend to use any cotton plant materials from the proposed release in human food, animal feed or for the production of fabrics and/or other cotton commodities.

96. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, GM plant materials containing the proteins encoded by the introduced genes for water use efficiency will not be further assessed.

2.2 Production of a substance allergenic to people

97. The possibility that exposure of people to proteins encoded by the introduced genes for water use efficiency in the GM cotton plants may result in an allergic reaction is considered. Routes of exposure to the proteins encoded by the introduced genes could include consumption of food containing cotton products or dermal contact with materials from cotton plants such as fibres or pollen.

Event 3: Use of GM plant materials, containing proteins encoded by the introduced genes, in food

98. The proteins encoded by the introduced genes for water use efficiency have none of the characteristics of typical allergens (see Chapter 1, Section 4.3.2) and no other similar proteins have been reported as allergens.

99. None of the GM cotton materials from the proposed release would be used in human food or animal feed. Thus, the potential for allergic reactions in people resulting from exposure to food is unlikely. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. FSANZ approval would be required before products from the GM cotton lines could be used in human food.

100. Therefore, **no risk is identified** and the potential for production of a substance allergenic to people will not be further assessed.

Event 4: Contact with GM plant materials (including pollen) containing proteins encoded by the introduced genes

101. As mentioned in Event 2, there would be some contact between a small number of people and GM cotton materials during the release. There are no reports of proteins encoded by the introduced genes for water use efficiency causing allergic reactions in people, nor do the proteins encoded by the introduced genes have the characteristics associated with known allergens (see Chapter 1, Section 4.3.2). As a result, it is unlikely that contact with GM plant materials would cause allergic reactions in people due to the presence of proteins encoded by the genes for water use efficiency.

102. Human contact with the GM plant materials containing the proteins (and enzymatic products) encoded by the introduced genes would be limited because cotton pollen is heavy and not easily dispersed by wind; the small scale and short duration of the proposed release; and none of the materials would be used in human food, animal feed or in the production of fabrics and/or other cotton products.

103. Therefore, **no risk is identified**. The potential for allergic reactions in people, resulting from the use or contact with GM plant materials (including pollen) containing the introduced proteins, will not be further assessed.

2.3 Production of a substance toxic to organisms other than people

104. A range of organisms may be exposed directly or indirectly to the proteins (and enzymatic products) encoded by the introduced genes for water use efficiency. 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 or degrade it (vertebrates, insects, fungi, Oomycetes and/or bacteria).

Event 5: Direct or indirect ingestion of GM plant materials, containing proteins encoded by the introduced genes, by vertebrates, invertebrates and microorganisms

105. Vertebrates such as livestock and wildlife (including mammals) may be exposed to the GM cotton lines containing the proteins (and enzymatic products) encoded by the introduced genes for water use efficiency through direct feeding or indirectly through consumption of other organisms which have fed on the GM cotton plants.

106. Cotton tissue (from either GM or non-GM plants), particularly the seeds, can be toxic to vertebrates if ingested in large quantities because of the presence of natural toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids).

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

108. Invertebrates, including beneficial insects, could be exposed directly to the GM cotton lines containing the proteins (and enzymatic products) encoded by the introduced genes through feeding on the plants, seeds or pollen, or via the soil when cotton tissues decompose. Exposure of soil invertebrates to the proteins (and enzymatic products) encoded by the introduced genes could also occur as a result of root exudations. Exposure could also occur indirectly, through consumption of other organisms that have fed on the GM cotton plants.

109. Microorganisms, particularly soil micro-organisms, will be exposed to the GM cotton lines containing the proteins (and enzymatic products) encoded by the introduced genes during growth and decomposition of plant material, possibly as a result of root exudations. Root exudation has been observed in GM corn (Saxena et al. 1999; Stotzky 2000) and GM cotton (Gupta et al. 2002). Root breakage could also lead to the release of the introduced proteins into the soil.

110. Contact with or ingestion by vertebrates, invertebrates and microorganisms of GM plant materials containing the proteins (and enzymatic products) encoded by the introduced genes would be limited because of the small scale and short duration of the proposed release. In addition, after harvest the applicant proposes to destroy GM cotton materials produced, apart from retaining some plant materials for research purposes, and none of the materials would be used as animal feed. There are no reports of toxicity associated with the proteins (and enzymatic products) encoded by the introduced genes (see Chapter 1, Section 4.3.1). Furthermore, vertebrates, invertebrates and micro-organisms would already be exposed to the introduced proteins (and enzymatic products) or very similar proteins in the environment (see Chapter 1, Section 5.4).

111. Therefore, **no risk is identified**. The potential for toxicity for organisms other than people as a result of direct or indirect ingestion of the GM plant material containing the proteins encoded by the introduced genes will not be assessed further.

2.4 Spread and persistence of the GM cotton in the environment

112. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton being considered in this risk assessment. Attributes of non-GM cotton associated with potential weediness are discussed in the document *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002). This document concludes that non-GM cotton is not a serious weed in Australia. Firstly, cotton does not have certain characteristics typically associated with problematic weeds such as prolonged seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance dispersal of seeds. Secondly, abiotic and biotic factors including temperature (particularly frost), soil moisture, nutrient levels and roadside management practices limit the establishment and/or persistence of cotton outside of agricultural and other disturbed environments. Additional limiting abiotic and biotic factors that determine whether cotton will persist in the environment proposed for release include short growing seasons, soil type, competition from other plants (Farrell & Roberts 2002). Disease, such as fungal wilts, is also expected to play a role in limiting spread and persistence. The relative impact of each of these factors is dependent on whether the cotton plants are in the northern or southern areas of Australia. For example, frost is a major limiting factor in southern areas of Australia. Whereas, the distribution of pathogenic organisms is variable and susceptibility is determined by the cultivar. The reliable availability of water is a limiting factor everywhere in Australia.

113. An important indicator of potential weediness of a particular plant is its history of weediness in any part of the world and its taxonomic relationship to declared weeds

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

114. The weed status of cotton has also been considered previously in many of the RARMPs produced during the assessment of a variety of GM cotton lines (eg DIRs 012/2002, 022/2002, 023/2002, 055/2004, 056/2006 and 059/2005). In addition to the information in the Biology and Ecology document (OGTR 2002), these RARMPs have considered new data that has been collected during the assessment of application for previous releases of GM cotton lines in Australia.

Event 6: Expression of the introduced genes improving the survival of cotton volunteers through enhanced water use efficiency (drought stress tolerance)

115. The GM cotton lines contain introduced gene constructs for water use efficiency, which if successful would confer enhanced drought stress tolerance. The applicant states the introduced genes have demonstrated the capacity to produce a water-efficient phenotype in cotton and/or other plants. In an environment in which water availability was the main factor limiting the spread and persistence of cotton, expression of the genes for water use efficiency could result in weediness of the GM cotton lines.

116. The applicant states that this is a 'proof of concept' field trial. Therefore, the ability of the GM cotton plants to withstand drought stress throughout different stages of their lifecycle, as compared to commercially available cotton cultivars including those with high water use efficiencies, is unknown. Data on the enhancement of water use efficiencies of the GM cotton lines would be required for possible future applications involving large scale or commercial releases of these GM cotton lines. However, the data are not required for the proposed release because the trial is limited in size, duration and location.

117. As discussed above, multiple factors limit the distribution of cotton in the areas proposed for release include water availability, temperature (particularly frost), nutrient levels, roadside management practices, short growing seasons, soil type, competition from other plants and disease.

118. In addition, the release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM cotton line proposed for release (see Chapter 1, Section 2.3).

119. Therefore, **no risk is identified** and the potential for the spread and persistence (weediness) of cotton as a result of the expression of the introduced genes for water use efficiency will not be further assessed.

Event 7: Expression of the introduced genes improving the survival of cotton volunteers through enhancement of abiotic stress tolerances (other than drought stress tolerance) or biotic stress tolerances

120. The GM cotton lines express genes that encode proteins that may enhance water use efficiency. It is possible that the encoded proteins could confer tolerances to other environmental stresses, other than drought tolerance, which could lead to increased spread and persistence of the GM cotton plants.

121. Other enhanced abiotic stress tolerances may improve the GM cotton lines' ability to survive, or have improved performance, under lower and higher growing temperatures. It is also possible the plants could tolerate higher soil salinity, or have increased seed dormancy, viability, and/or improved germination seedling germination rates under stress (other than drought stress).

122. Some homologs to the introduced genes for water use efficiency are known to confer enhanced abiotic stress tolerances other than drought stress to some transgenic plants (details relating to declared CCI have been removed). However, only one of the actual genes (number 5) is known to enhance another abiotic stress tolerance other than drought stress tolerance (details relating to declared CCI have been removed). It should be noted that when a gene is expressed in different plant species the same effect(s) on phenotype does not always eventuate (Oh et al. 2005). Therefore, gene number 5 may not confer enhanced stress tolerance, other than drought stress tolerance, to the GM cotton plants.

123. Tolerance to chemicals such as herbicides is another form of abiotic stress tolerance. Some of the GM cotton plants (lines 1 to 14) contain the *cp4 epsps* herbicide tolerance gene from *Agrobacterium* sp. strain CP4. This gene was used selectable marker gene throughout the early stages of the genetic modification process in the laboratory for the GM cotton plants (see Chapter 1, Section 4.4). As discussed in paragraph 87, the potential effects of the *cp4 epsps* gene and encoded protein were considered in detail in previous DIR applications including some for commercial releases. RARMPs for those DIR applications are available from the OGTR or from the website <<http://www.ogtr.gov.au>>. Risks arising from the expression of the *cp4 epsps* gene in GM cotton were estimated as negligible.

124. The applicant states that this is a 'proof of concept' field trial. Therefore, the ability of the GM cotton plants to withstand abiotic stress tolerances throughout different stages of their lifecycle as compared to commercially available cotton cultivars is unknown.

Biotic stress tolerance

125. The genes for water use efficiency could potentially enhance resistance of the GM cotton lines to microorganisms. This could lead to a spread and persistence of the GM cotton lines if diseases were the main limiting factors.

126. A homolog of gene number 8, when expressed as an introduced gene (details relating to declared CCI have been removed), has the ability to enhance tolerance to fungal wilts, which are known diseases of cotton. However, the exact mechanism for the resistance is not known and it may not eventuate in the GM cotton line containing gene number 8. Furthermore, in the case of fungal wilt there are already some non-GM cotton varieties resistant to the disease (Allen et al. 2006). These varieties have been grown for many years without becoming weedy.

127. The introduced genes for water use efficiency, or homologs other than the aforementioned (homolog of gene number 8), are not known to confer enhanced biotic tolerances (details relating to declared CCI have been removed).

128. The applicant states that this is a 'proof of concept' field trial. Therefore, the ability of the GM cotton plants to withstand biotic stress tolerances throughout different stages of their lifecycle as compared to commercially available cotton cultivars is unknown.

Conclusion

129. As discussed above, multiple factors limit the distribution of cotton in the areas proposed for release include water availability, temperature (particularly frost), nutrient levels, roadside management practices, short growing seasons, soil type, competition from other plants and disease.

130. In addition, the release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM cotton lines proposed for release (see Chapter 1, Section 2.3).

131. Therefore, **no risk is identified** and the potential for the spread and persistence (weediness) of cotton as a result of the expression of the introduced genes for water use efficiency will not be further assessed.

Uncertainty

132. Currently, there is no direct evidence that some of the GM cotton lines may have enhanced tolerances to a number of abiotic and biotic stresses as compared to their non-GM parent. However, in the possible, yet highly unlikely, instance where the plants may have enhanced tolerances to several environmental stresses, the GM plants may be less fit as compared to other commercially available cotton varieties because of the potential metabolic/physiological burdens. For example, the cotton may have stunted growth, produce less seeds, and have a decreased ability to tolerate competition from other plants.

133. Data on abiotic/biotic tolerances, additional to that for enhanced drought stress tolerance, of the GM cotton lines would be required for possible future applications involving large scale or commercial releases of these GM cotton lines. However, the data are not required for the proposed release because the trial would be limited in size, duration and location.

Event 8: Exposure of vertebrates (including people), invertebrates and microorganisms to GM cotton volunteers expressing the introduced genes

134. The potential of increased spread and persistence of the GM cotton lines in the environment was addressed in Events 6 and 7 no risk was identified. However, in the highly unlikely instance of this occurring, spread and persistence of the GM cotton plants in the environment could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the proteins (and enzymatic products) encoded by the introduced genes for water use efficiency.

135. An adverse outcome could occur, if the proteins (and enzymatic products) encoded by the introduced genes for water use efficiency were toxic or allergenic for people. The potential for the proteins causing toxic or allergic reactions in people was assessed in Sections 2.1 and 2.2 and no risk was identified.

136. Organisms other than people may be exposed directly, through feeding on the GM cotton plants or indirectly through eating organisms that have fed on or degrade the GM cotton plants as a result of spread and persistence of the GM cotton in the environment. These organisms include vertebrates, invertebrates and microorganisms. The potential for toxicity of proteins encoded by the water use efficiency genes to organisms other than people was considered in Section 2.3 and no risk was identified.

137. In addition, the chain of events that would lead to increased exposure of vertebrates, invertebrates and microorganisms depends on the least likely event to occur, ie an increase in the spread and persistence of the GM cotton lines proposed for release.

138. The release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM cotton lines in the environment (see Chapter 1, Section 2.3).

139. Therefore, **no risk is identified**. The potential for toxicity or allergic reactions in people or other organisms as a result of spread and persistence of the GM cotton lines in the environment will not be further assessed

Event 9: Dispersal of GM cotton seed or GM plant research materials during transport, storage, equipment use or flooding

140. In the course of the proposed dealings the applicant proposes to transport seed to and from the release sites, cultivate GM cotton plants, store all GM cotton seed harvested from the crop and collect GM plant materials for research purposes, laboratory research or possible future release of promising lines (subject to further applications and assessment processes). Accidental spillage or dispersal of GM plant materials, especially seed, in the course of these dealings could allow the GM cotton plants to spread and persist in the environment.

141. The applicant proposes a number of containment measures to limit dispersal of the GM cotton seed (see Chapter 1, Section 2.3). Therefore, any spillage of seed during transport to and from the release sites or while in storage would be rare. Any incident involving spillage of GM seed is expected to be readily controlled through cleaning and monitoring of the site of the spill. In addition, the opportunity for any adverse outcome from any such rare occurrence is further diminished by the need for appropriate environmental conditions for germination, survival and persistence of any few escaped seeds.

142. The applicant proposes to clean equipment of any viable plant material at the sites or pollen traps immediately or as soon as possible after use.

143. The applicant proposes to destroy all GM and non-GM cotton plant materials (including materials from the non-GM pollen trap) remaining on the sites at the end of each growing season.

144. The applicant proposes to destroy GM plant materials used in laboratory analysis if not required for future study or release.

145. Severe weather conditions (eg flooding) could lead to the dispersal of GM plant materials, including seed. The sites where the proposed release would take place are expected to be located above flood level. In case of a flood, any seed or other GM plant material on the ground is therefore not expected to be dispersed beyond the area of the proposed release (including the pollen trap, which would act as a physical barrier once the plants were established). Dispersal of cotton seed would be the only way in which the GM cotton lines could spread effectively as cotton does not generally propagate vegetatively (OGTR 2002).

146. In addition, the applicant proposes sites located at least 50 m away from natural waterways (see Chapter 1 Section 2.3). Irrigation practices (*Cotton Industry Good Management Practice*) used by cotton growers in Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice would also reduce the dispersal of seed (for more information refer to DIR 059/2005).

147. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of dispersal of GM seed or other GM plant materials during transport, research storage, equipment use or flooding will not be further assessed.

2.5 Gene flow by vertical gene transfer (ie gene transfer to sexually compatible plants)

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

Event 10: Expression of the introduced genes in other *G. hirsutum* or *G. barbadense* plants (including commercially released GM cotton lines)

149. In the areas proposed for release, cotton (*G. hirsutum* and *G. barbadense*) is grown commercially, including a number of *G. hirsutum* GM cotton lines (see Chapter 1, Section 5.3). The commercially released GM cotton lines have traits for insect resistance and herbicide tolerance. The genes for water use efficiency could be transferred to these sexually compatible cotton plants resulting in an increased potential for weediness.

150. Cotton is primarily self-pollinating, but it may outcross when insect pollinators are present. Outcrossing decreases significantly with distance and it is estimated only 1 to 2% of seeds in adjacent rows are a result of cross-pollination (OGTR 2002).

151. Although commercial cotton crops may be planted adjacent to the trial sites (information provided by the applicant), outcrossing would be limited due to the containment measure of a 20 m pollen trap proposed by the applicant that would surround the GM cotton plants (see Chapter 1, Section 2.3).

152. In addition, the GM cotton lines proposed for release and the non-GM parent cotton plants within the trial sites have no traits conferring insect resistance. The applicant has indicated that this may necessitate the adoption of the heavy insecticide spraying regime required for growing non-GM cotton or non-insect resistant GM cotton. This in turn would further limit the chance of insect mediated pollen transfer to plants outside the proposed trial sites.

153. If the highly unlikely case of outcrossing to a commercially grown non-GM cotton plant were to occur, the potential for weediness would be the same as for the GM cotton line itself, which was assessed in Section 2.4 (no risks were identified). The spread and persistence of cotton in the areas proposed for release is limited by multiple factors other than water availability including temperature (particularly frost), nutrient levels, roadside management practices, short growing seasons, soil type, competition from other plants and disease (Farrell & Roberts 2002; OGTR 2002).

154. If the pollen recipient were a commercially released GM cotton plant, stacking of genes conferring herbicide tolerance and/or insect resistance with an introduced gene for water efficiency could occur. This may give any water use efficiency gene carrying plant a fitness advantage in an environment where water availability combined with herbicide tolerance and/or insect herbivory were the main limiting factors for the spread and persistence of cotton. However, as described above, multiple factors limit the spread and persistence of cotton in the areas proposed for release. If the rare event of outcrossing to commercially released GM cotton plants were to occur, the resulting seed would not be used for subsequent

plantings as farmers are required to buy certified GM cotton seed for each growing season. This would further reduce the already limited possibility of spread and persistence of the GM cotton lines proposed for release.

155. In addition to the proposed measures to limit the spread and persistence of the GM cotton lines, the applicant proposes a release of limited size and short duration.

156. The applicant proposes to conduct post harvest monitoring of the trial sites for 12 months and destroy all volunteers.

157. Therefore, **no risk is identified** and the potential of expression of the introduced genes for water use efficiency in other *G. hirsutum* or *G. barbadense* cotton plants leading to weediness will not be further assessed.

Uncertainty

158. The genes for water use efficiency could potentially affect fertility and alter flowering time of the GM cotton lines as compared to commercially grown cotton. For example, homologs of gene numbers 2, 6, 10 and 13 (details relating to declared CCI have been removed), when expressed in *A. thaliana* as introduced genes, have lead to earlier or later flowering (as compared to the non-GM parent plant). A homolog of gene 6 is also known to be involved in flower morphology and pollen development (details relating to declared CCI have been removed). It should be noted that when a gene is expressed in different plant species the same effect(s) on phenotype (eg pollen grain number) does not always eventuate (Oh et al. 2005). Therefore, the effect on flowering and fertility as a result of the introduced genes may not eventuate in the GM cotton lines.

159. The applicant is not aware of differences in the flowering window of the GM plants compared to the non-GM parent in glasshouse studies.

160. If flowering times of any of the GM cotton lines during the field trials was altered as compared to pollen trap, or the GM cotton plants were more fertile (eg higher pollen production), then the potential for pollen flow to cotton plants outside the trial site would be increased. However, in the highly unlikely instance when all of the following occur simultaneously—the GM cotton plants at the trial site are in flower, the pollen trap is not in flower and neighbouring commercial cotton plants in close vicinity are in flower—the applicant proposes two measures. Firstly, to spray the trial site with prophylactic applications of insecticide to limit pollinator numbers and hence limit pollen flow. Secondly, treat and manage the first 20 metres of any commercial crop in the immediate vicinity as a pollen trap.

161. Considering the above points, it is highly unlikely the genes for water use efficiency will be transferred to sexually compatible cotton plants ie *G. hirsutum* or *G. barbadense* cotton plants (including commercially released GM cotton lines).

162. Data on the reproductive capacity of the GM cotton lines indicative of weediness (eg seed number and window of flowering) would be required for possible future applications involving large scale or commercial releases of these GM cotton lines.

Event 11: Exposure of vertebrates (including people), invertebrates and microorganisms to other *G. hirsutum* or *G. barbadense* cotton plants (including commercially released GM cotton lines), expressing the introduced genes.

163. Transfer of the water efficiency genes to other *G. hirsutum* or *G. barbadense* cotton plants could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the effects of introduced genes for water efficiency.

164. The transfer of the introduced genes to other *G. hirsutum* or *G. barbadense* cotton plants was assessed in Event 10 and considered highly unlikely. The potential adverse outcomes of these cotton lines would be highly similar to the GM cotton lines proposed for release, which was assessed in Sections 2.1, 2.2, and 2.3.

165. In addition to the proposed measures to limit the spread and persistence of the GM cotton lines, the applicant proposes a release of limited size and short duration.

166. The applicant proposes to conduct post harvest monitoring of the GM cotton trial sites for 12 months and destroy all volunteers.

167. Therefore, **no risk is identified**. The potential for toxicity or allergenicity to vertebrates (including people), invertebrates and microorganisms, through increased exposure as a result of the introduced genes being expressed in *G. hirsutum* and *G. barbadense* cotton plants will not be assessed further.

Event 12: Presence of the introduced regulatory sequences in *G. hirsutum* or *G. barbadense* plants as a result of gene transfer

168. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

169. The transfer of genetic material to other *G. hirsutum* or *G. barbadense* cotton plants was assessed in Event 10 and considered highly unlikely.

170. The applicant proposes to conduct post harvest monitoring of the GM cotton trial sites for 12 months and destroy all volunteers.

171. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of vertical gene transfer of introduced regulatory sequences will not be assessed further.

Event 13: Gene transfer to native *Gossypium* species

172. As discussed in the *Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002), Australian flora contains 17 native *Gossypium* species, all of which are diploid (C, G or K genomes), while the cultivated cotton species are tetraploid (AD genomes). The native cotton species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids between these two species have been produced without application of plant hormones, when plants were in close proximity to each other. However, these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations.

173. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from cultivated cotton fields. Thus, gene transfer from the GM cotton lines to native cotton plants is prevented not only by genetic incompatibility but also by geographic constraints on cross pollination (OGTR 2002).

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

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

Event 14: Presence of the introduced gene, or the introduced regulatory sequences, in other organisms as a result of gene transfer

175. Transfer of the introduced regulatory sequences and/or the introduced genes for water use efficiency, antibiotic resistance (*nptII* gene) or herbicide tolerance (*cp4 epsps* gene), from the GM cotton plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) could occur only rarely without human intervention.

176. Most gene transfers have been identified through analyses of gene sequences (Worobey & Holmes 1999; Ochman et al. 2000). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence 1999). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). In contrast, transfers of plant genetic materials to other microorganisms such as bacteria, viruses or fungi have been exceedingly rare.

177. Transfer of the regulatory sequences to other organisms could alter the expression of endogenous genes in unpredictable ways. However, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in adverse unpredictable effects. As there is no difference between those two events, this does not represent a novel adverse outcome as a result of the genetic modification.

178. Horizontal gene transfer has been examined in detail in a number of other RARMPs (most recently DIR 059/2005 and DIR 060/2005), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have not only relied on the use of highly similar sequences to allow homologous recombination to occur, but also on conditions designed to enhance the selective advantage of gene transfer events (Nielsen et al. 2000; Gebhard & Smalla 1998; Mercer et al. 1999; Nielsen 1998; De Vries et al. 2001). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed limited release.

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

2.7 Unintended changes in toxicity or allergenicity

180. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy and all such multiple effects are sometimes unknown. Certain types of genes have a higher likelihood of causing pleiotropic effects. These genes include those that encode proteins that can regulate the transcription of a number of genes (eg transcription factors).

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

- altered expression of an unrelated gene at the site of insertion

- altered expression of an unrelated gene distant to the site of insertion, for example, due to 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.

182. 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 the process has little potential for unexpected outcomes that are not detected and eliminated during the routine process of selecting plants that are morphologically similar to the conventional plant except for the new properties (Bradford et al. 2005). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

183. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropic effects (Haslberger 2003). Most often the important nutrients, toxicants, and other components are present in the new plant variety at levels that are within the range expected for commercial varieties.

Event 15: Altered levels of innate toxic or allergenic compounds as a result of the expression, or random insertion into the cotton genome, of the introduced genes

184. Cotton tissue (from either GM or non-GM plants), particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional compounds including gossypol and cyclopropenoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids). Further discussion regarding the toxicity and allergenicity of non-GM cotton is provided in *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002) and the *Consensus document on compositional considerations for new varieties of cotton (Gossypium hirsutum and Gossypium barbadense): Key food and feed nutrients and anti-nutrients* (OECD 2004). There is potential for the GM cotton plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the expression of the genes for water use efficiency. Additionally, random insertion of the gene constructs during the genetic modification process could potentially result in increased toxic or allergenic compounds in the GM cotton lines.

185. Thus far, exposure to plant materials (including pollen) from the GM cotton lines has been limited to a few workers maintaining these plants in the glasshouse or limited field trial in the USA. The applicant has reported no adverse outcomes from exposure to the GM cotton plant materials, suggesting that expression of the introduced genes has not altered the toxicity or allergenicity endogenous to non-GM cotton. Data on toxicity of plant material and/or levels of endogenous toxins would be required for risk assessments of applications for large scale or commercial release of these GM cotton lines. Further information is not required for assessing the risks of this proposed release because the trial is limited in size, duration and location and none of the GM cotton plant materials are intended for use in human food, animal feed or for the production of fabrics and/or other cotton commodities.

186. Therefore, **no risk is identified** and the potential for changes in levels of innate toxic or allergenic compounds as a result of random insertion of the gene construct into the GM cotton lines will not be further assessed.

2.8 Unintended changes in biochemistry, physiology or ecology

Event 16: Altered biochemistry, physiology or ecology of the GM cotton lines resulting from expression of the introduced genes

187. Biochemical, physiological or ecological changes to the GM cotton lines proposed for release could occur either as a result of the expression of the introduced genes for water use efficiency or of the transformation process itself. Apart from any observed adverse outcomes, of which the applicant has reported none, this information is not required for assessing the risks of this proposed release because the trial is limited in size, duration and location. Furthermore, none of the GM cotton plant materials are intended for use in human food, animal feed or for the production of fabrics and/or other cotton commodities.

188. The potential adverse outcomes (weediness, toxicity, and allergic reactions) resulting from altered biochemistry, physiology or ecology of the GM cotton lines proposed for release were assessed in Sections 2.1 to 2.7. No risks were identified.

189. The expression of the introduced genes for water use efficiency in the GM cotton plants could result in unintended pleiotropic effects, and the uncertainty of potential effects is discussed in Section 2.7.

190. During this limited and controlled release, the applicant proposes to measure the agronomic performance of the GM cotton lines, and adverse effects, if any, would most likely be detected during the trial. More data on the potential pleiotropic effects of the genetic modifications on GM cotton lines selected for further development, and how these may affect potential weediness, toxicity and allergenicity, would be required before any future application for a large scale or commercial releases of these GMOs could be assessed.

191. Therefore, **no risk is identified**. The potential for weediness and/or toxicity and/or allergenicity to people and other organisms, as a result of unintended changes in biochemistry, physiology or ecology will not be further assessed.

2.9 Secondary impacts

Event 17: Adverse impact on populations of organisms that interact with organisms affected by the GM cotton lines

192. If there are unintended pleiotropic effects resulting in an increased level of endogenous toxins widespread and long-term use of the GM cotton lines expressing the introduced genes for water use efficiency could adversely affect some organisms that interact with affected organisms in the environment including the food web. This could result in reduced and altered biodiversity. However, as the proposed trial would be of small size and short duration, there would be limited opportunity for the GM cotton lines expressing the introduced genes for water use efficiency to affect the wider environment including the food web.

193. Therefore, **no risk is identified** and the potential for an adverse impact on populations of organisms that interact with affected organisms, as a result of the expression of the introduced genes for water use efficiency in the GM cotton lines, will not be further assessed.

2.10 Unauthorised activities

Event 18: Use of GMOs outside the proposed licence conditions (non-compliance)

194. 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.

195. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unauthorised activities will not be further assessed.

Section 3 Risk estimate process for identified risks

196. The hazard identification process considered 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.

197. Eighteen events were identified and assessed whereby the proposed release of the GM cotton lines might give rise to harm to people or the environment.

198. These 18 events 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, produce unintended changes in the biochemistry, physiology or ecology of the GM cotton lines, or alter characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the opportunity for gene flow to other organisms and its effects.

199. None of the 18 events are considered to give rise to an identified risk that requires further assessment. The principle reasons include:

- small scale of the trial that is limited in both area and duration
- containment and disposal measures proposed by the applicant to limit the spread and persistence of the GM plants
- none of the GM plant materials will be used for any other purpose
- widespread presence of the same or similar proteins and enzymatic products in the environment and lack of evidence of harm from these proteins and their products
- the lack of known toxicity or allergenicity of the proteins (and enzymatic products) encoded by the introduced genes
- limited capacity of the GM cotton lines to spread and persist in the areas proposed for release
- limited ability and opportunity for the GM cotton lines to transfer the introduced genes to other sexually related species.

200. Therefore, as no risks to the health and safety of people or the environment were identified from the proposed limited and controlled release of GM cotton lines the level of risk is considered to be **negligible**.

Chapter 3 Risk management

201. 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 release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures were used to inform the decision-making process and determine licence conditions that have been imposed by the Regulator under the Act.

Section 1 Background

202. 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.

203. All licences are required to be subject to three conditions prescribed in the Act. In summary, section 63 requires that each licence holder inform relevant people of their obligations under the licence, section 64 requires that licence holders provide access to premises by authorised persons, and section 65 requires that in certain circumstances the licence holder is to provide information to the Regulator (see Chapter 3, Section 4.2). These provisions are reproduced in full in each licence.

204. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Matters to which conditions may relate are listed in section 62 of the Act. For example, licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Other Australian regulators

205. Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, NICNAS, NHMRC and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁰.

206. 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.

207. 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 lines proposed for release to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM cotton lines for use in human food. However, FSANZ approval would need to be obtained before they could be used in this way.

208. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests, weeds and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs. Additionally, as the importation of the water-efficient GM cotton seed

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 (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

constituted a dealing under the *Gene Technology Act 2000*, the importer required an authorisation under this Act for the import to lawfully proceed. Seed from the water-efficient GM cotton lines has been imported by Monsanto under an AQIS seed import permit.

Section 3 Risk treatment measures for identified risks

209. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed release. The *Risk Analysis Framework* (OGTR 2005), which guides the risk assessment and risk management process, defines **negligible** risks as insubstantial with no present need to invoke actions for their mitigation.

210. These events were considered in the context of the proposed release on up to 10 sites on a maximum area of 2 ha per site during each of the two summer growing seasons in 2006/07 and 2007/8, the receiving environment, and the containment measures and agricultural practices proposed by the applicant (see Chapter 1, Section 5).

Section 4 General risk management

211. Containment measures consistent with the risk assessment context have been imposed to limit the release to the locations, size and duration requested by the applicant, which are summarised below.

4.1 Summary of licence conditions

4.1.1 Measures to limit and control the proposed release

212. A number of licence conditions have been imposed to limit the spread and persistence of the GMOs:

- surround each release site with a pollen trap
- locate the release sites at least 50 m away from natural waterways
- harvest and gin the cotton seed from the release separately from any other cotton crop
- prohibit the use of cotton seed or other materials from the release for any other purpose
- destroy all plant materials remaining at the trial sites after harvest
- clean the sites and any equipment used on the sites
- conduct regular inspections of the release sites and other areas following harvest for at least 12 months and until the last six consecutive months have passed without any volunteer cotton plants.

4.1.2 Measures to control other activities associated with the release

213. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). 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.

4.2 Other risk management considerations

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

- identification of the persons or classes of persons covered by the licence
- applicant suitability

- contingency and compliance plans
- 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 release sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

215. 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.

216. Before making the decision to issue a licence for this application (DIR 064/2006), the Regulator determined that Monsanto Australia Limited (Monsanto) is suitable to hold a licence.

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

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

4.2.2 Compliance and contingency plans

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

220. Monsanto is also required to submit contingency plan to the Regulator within 30 days of the issue date of the licence. This plan must detail measures to be undertaken in the event of any unintended presence of the GM cotton lines outside of the permitted areas. Additionally the contingency plan must describe how to prevent the dissemination of the GM cotton lines, or material from the GM cotton lines, outside of the permitted areas in the event of flooding or failure of the pollen trap to flower at the same time as the GM cotton lines.

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

4.2.3 Reporting structures

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

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

- any unintended effects of the release

223. 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.

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

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of final destroying and cleaning at the end of the trial

Section 5 Monitoring and Compliance

225. A range of monitoring and compliance activities are undertaken on behalf of the Regulator (OGTR 2005) to check compliance with licence conditions. 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.

226. 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.

227. In cases of non-compliance with licence conditions, the Regulator may also 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 6 Issues to be addressed for future releases

228. In view of the very early stage of research involved in the proposed release, the risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment conditions or a commercial release of any of these GM cotton lines. This would include:

- molecular characterisation of the introduced genetic materials in the GM cotton lines, genotypic stability, and expression levels of the introduced genes in the GM cotton lines
- data on the potential toxicity of plant material from the GM cotton lines including levels of endogenous toxins
- data on the allergenicity of the proteins encoded by the introduced genes for water use efficiency, and plant material from the GM cotton lines
- biochemical, physiological and agronomic characteristics of the GM cotton lines indicative of weediness including measurement of tolerance to environmental stresses (eg drought, cold and salt) and/or reproductive capacity (eg seed number and window of flowering).

Section 7 Conclusions of the RARMP

229. The risk assessment concludes that this limited and controlled release of up to 24 GM cotton lines into the areas proposed for release in New South Wales and Queensland poses **negligible** risks to the health and safety of people and the environment.

230. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the locations, size and duration requested by the applicant.

Section 8 DIR 064/2006 Licence

231. The licence DIR 064/2006 is available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>), following the path to DIR 064/2006).

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Appendix A Definitions of risk analysis terms used by the Regulator

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

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

*Event**

occurrence of a particular set of circumstances

*Hazard**

source of potential harm

Hazard identification

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

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

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

Risk

the chance of something happening that will have an undesired impact

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

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

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

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

Risk analysis

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

Risk analysis framework

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

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

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

Risk Context

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

Risk estimate

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

Risk evaluation

the process of determining risks that require treatment

Risk management

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

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

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

States

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

Uncertainty

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

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹¹ on application DIR 064/2006

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

Issues raised relating to the Risk Assessment and where they have been considered:

- Risk of enhanced spread and persistence (weediness) of the GM cotton (Chapter 2, Events 6 and 7)
- Risks arising from any gene flow to other commercial cotton crops and related species (Chapter 2, Events 10 and 13)
- Risks associated with the toxicity and/or allergenicity of the proteins (and enzymatic products) encoded by the introduced genes (Chapter 2, Events 1 to 5)
- Risks arising from the dissemination of the GM cotton material beyond the intended areas (Chapter 2, Event 10)
- Risks resulting from potential pleiotropic effects of the introduced genes (Chapter 2, Events 15 and 16).

Issues raised relating to the Risk Management Plan (considered in Chapters 3 and 4):

- Adequate containment measures
- Adequate transport procedures for the GM cotton
- Adequate post-harvest monitoring and practices
- Adequate disposal of GM plant materials not required for further research or approved plantings.

¹¹ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and Local councils where the release may occur.

Appendix C Summary of submissions received from the public on application DIR 064/2006

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

As summarised in the table below, one submission was received from the public that raised a number of issues.

Issues raised: **E:** economic issues; **EN:** environmental risks; **H:** human health and safety; **OGTR:** Office of the Gene Technology Regulator evaluation process.

Other abbreviations: **GM:** genetically modified; **OSA:** outside scope of the assessment; **RARMP:** Risk Assessment and Risk Management Plan.

Issue	Summary or issues raised	Consideration in RARMP	Comment
H, EN	Monsanto should be prosecuted for crimes against humanity and the environment, not given licences to contaminate our environment.	Ch 2	The introduced genes are widespread in the environment and the encoded proteins and their products are not known to be toxic or allergenic to organisms (including people). Exposure to the proteins encoded by the introduced genes would be negligible as the proposed trial would be a limited and controlled release.
E	GM crops are profit-driven.		OSA. The Act requires the Regulator to assess risks to human health and safety and the environment; economic issues are excluded from consideration.
OGTR	Approval of releases of GM crops by the OGTR is a mere formality and ignores public interest.		OSA. The case by case assessment process takes into account issues relating to risks to human health and safety or the environment raised in submissions from the public, experts, agencies and authorities. Assessment of applications is based on scientific evidence.

Appendix D Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹² on the consultation RARMP for DIR 064/2006

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

¹² GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and Local councils where the release may occur.

Appendix E Summary of submissions received from the public on the consultation RARMP for DIR 064/2006

The Regulator received three submissions from the public on the consultation RARMP, for the proposed release.

All issues relating to risks to the health and safety of people and the environment were considered following the case-by-case assessment as required by the legislation and in the context of the currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. These are summarised, in order of receipt of submission, in the table below.

Issues raised: EN: environmental risks.

Other abbreviations: Ch: chapter; GM: genetically modified; GMO: genetically modified organism; L: Licence; OSA: outside scope of the assessment; RARMP: Risk Assessment and Risk Management Plan.

Sub. No.	Issue	Summary or issues raised	Consideration in RARMP	Comment
1	EN	Against the release for the following reasons: Inherent instability of GMOs	Ch 1, 2 & 3	This trial is at the proof of concept stage. If any lines demonstrate commercial potential their genetic stability will be tested over several generations.
	EN	Inability to control living organisms Inability to recall escapees Inability to de-contaminate areas of release	Ch 2, L	This trial is a limited and controlled release. Licence conditions have been imposed to restrict the release in location, size and duration and to enforce containment and disposal measures. Refer to licence conditions for details.
2		Supports issuing of the licence. Believes that the cotton lines must be tested in Australian conditions to determine their potential economic benefits to the industry and consequently the national economy.		Noted. OSA.
		States that benefits are likely to arise from the potential improvements in productivity that could emerge from their commercial use due to: expansion of the rainfed production area and increased cotton yields in the existing rainfed areas through more efficient use of available moisture, and possible reduced applications of water in the irrigated production areas.		OSA.
		Supports the granting of a licence because reducing the water dependence of irrigated cotton is environmentally sound and clearly in the public interest. Recommends that the commercial introduction of approved new cotton events such as this becomes part of the national water policy framework.		OSA.

Sub. No.	Issue	Summary or issues raised	Consideration in RARMP	Comment
		Notes there are negligible risks to human health and safety as well as to the environment from the managed usage of the cotton lines.		Noted.
		States the water use efficiency characteristics of this suite of GM lines can only enhance previous productivity gains through GM.		OSA.
3		Strongly supports the proposed trial. Satisfied that the risk management plan sufficiently covers the minimal risks attached to this technology.		Noted.
		If the proof of concept trial proves to be valid, the concept has wide ranging implications for cropping in Australia. Believes significant gains in yield, conversion efficiency of natural resources and sustainable production for many of our crops including cotton are likely to come from genetic modification technology and in particular research conducted in this area.		OSA.
		Agrees with the risk assessment; 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 is negligible.		Noted.
		Agrees with the assessment relating to weediness in the RARMP. Believes that adding traits to the cotton genome to improve water use efficiency is unlikely to improve cottons uncultivated fitness. The likelihood of the GMO cottons in this application becoming weeds is minimal, given the limited size, short duration and required management plan to control volunteers.		Noted.