



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 081/2007**

**Limited and controlled release of cotton genetically
modified for enhanced water use efficiency**

Applicant: Monsanto Australia Limited

September 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of cotton genetically modified for enhanced for enhanced water use efficiency into the environment in respect of application DIR 081/2007 from Monsanto Australia Ltd.

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

The application

Monsanto applied for a licence for dealings involving the intentional release of up to 504 genetically modified (GM) cotton lines² on a limited scale and under controlled conditions. The GM cotton lines have been modified for enhanced water use efficiency (WUE). The trial would be conducted at up to 20 sites of no more than 2 ha each, on a maximum total area of 40 ha per year between September 2008 and June 2010.

The proposed sites³ may be located in the New South Wales local government areas (LGAs) of Balranald, Bourke, Central Darling, Carathool, Coonamble, Gunnedah, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren and Lake Tandou (an unincorporated area); the Queensland LGAs of Paroo, Balonne, Dalby Regional, Goondiwindi Regional, Toowoomba Regional, Somerset Regional, Brisbane City and Lockyer Valley Regional; and the Western Australia LGA of Wyndham-East Kimberley. Glasshouses in the LGAs of Brisbane City and Toowoomba Regional would be producing the seed for planting at field sites.

The cotton lines were genetically modified using one of 56⁴ different gene constructs. All the constructs contain one gene for WUE, except for one construct which contains two different genes. The introduced genes were derived from various plants, bacteria, yeast or fungi and encode proteins that are intended to confer enhanced water use efficiency.

The GM cotton lines contain either an antibiotic resistance selectable marker gene, derived from *Escherichia coli*, or a herbicide tolerance selectable marker gene derived from

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

² The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

³ The original application indicated that the sites may be located in up to 25 different LGAs. The applicant has requested the addition of Gunnedah (NSW) to the list of LGAs, but due to council amalgamation in Queensland, the number of proposed locations has decreased from 25 to 23.

⁴ The original application was for 63 gene constructs containing 56 different genes. However, Monsanto withdrew 7 constructs leaving 56 constructs containing 50 different genes.

Agrobacterium sp. strain CP4. These genes were used as selective markers to identify transformed plants during initial development of GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments to evaluate agronomic characteristics including water use efficiency, yield and fibre quality of the GM cotton lines under optimal and water stress conditions. Seed will be collected for further studies, including possible future releases (subject to additional assessments and approvals). The GM cotton will not be used for human food or animal feed

Monsanto proposed a number of controls to restrict the dissemination or persistence of the GM cotton lines into the environment that have been considered during the evaluation of the application.

Confidential Commercial Information

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

Risk assessment

The risk assessment takes into account information in the application (including proposed containment measures), relevant previous approvals and current scientific knowledge, advice received from a wide range of experts, agencies and authorities consulted on the RARMP and submissions from the public.

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

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

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

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

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

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the seven events characterised in the risk assessment are considered to give rise to an identified risk that

requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

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

The licence conditions require Monsanto to **limit** the duration of the release to between September 2008 and June 2010 on a maximum total area of 40 ha per year at up to 20 sites. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying waste GM plant materials; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed⁵.

Conclusions of the consultation RARMP

The risk assessment concludes that this limited and controlled release of the GM cotton lines on up to 20 sites, located in various LGAs in NSW, QLD and WA, totalling no more than 40 ha per year over a two year period between 2008 and 2010 poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

⁵ The licence for DIR 081/2007 is available on the OGTR website
<<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>> via the link to DIR 081/2007

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Abbreviations (does not include gene and protein abbreviations)

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| the Act | <i>Gene Technology Act 2000</i> |
| APVMA | Australian Pesticides and Veterinary Medicines Authority |
| AQIS | Australian Quarantine and Inspection Service |
| CaMV | Cauliflower mosaic virus |
| CCI | Confidential Commercial Information |
| DIR | Dealings involving Intentional Release |
| DNA | Deoxyribonucleic Acid |
| FMV | Figwort Mosaic Virus |
| FSANZ | Food Standards Australia New Zealand |
| GM | Genetically Modified |
| GMO | Genetically Modified Organism |
| GTTAC | Gene Technology Technical Advisory Committee |
| ha | hectare(s) |
| km | kilometre(s) |
| LGA | Local government area |
| m | metre(s) |
| mRNA | Messenger Ribonucleic Acid |
| NICNAS | National Industrial Chemicals Notification and Assessment Scheme |
| OGTR | Office of the Gene Technology Regulator |
| PC2 | Physical Containment level 2 |
| RARMP | Risk Assessment and Management Plan |
| the Regulations | Gene Technology Regulations 2001 |
| the Regulator | Gene Technology Regulator |
| RNA | Ribonucleic Acid |
| TGA | Therapeutic Goods Administration |
| UTR | Untranslated region |
| USDA | United States Department of Agriculture |
| WUE | Water Use Efficiency |

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Technical Summary

Introduction

The Acting Gene Technology Regulator (the Acting Regulator) has made a decision to issue a licence (DIR 081/2007) to Monsanto Australia Ltd (Monsanto) for dealings involving the limited and controlled release of genetically modified (GM) cotton lines into the Australian environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Acting Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public⁶.

The application

Monsanto applied for a licence for dealings involving the intentional release of up to 504 cotton (*Gossypium hirsutum* L.) lines⁷ that have been genetically modified for enhanced water use efficiency (WUE) on a limited scale and under controlled conditions. The trial is authorised to take place at up to 20 sites of no more than 2 ha each, on a maximum total area of 40 ha per year between September 2008 and June 2010.

The proposed sites⁸ may be located in the New South Wales local government areas (LGAs) of Balranald, Bourke, Central Darling, Carathool, Coonamble, Gunnedah, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren and Lake Tandou (an unincorporated area); the Queensland LGAs of Paroo, Balonne, Dalby Regional, Goondiwindi Regional, Toowoomba Regional, Somerset Regional, Brisbane City and Lockyer Valley Regional; and the Western Australia LGA of Wyndham-East Kimberley. Glasshouses in the LGAs of Brisbane City and Toowoomba Regional would be producing the seed for planting at field sites.

The cotton lines were genetically modified using one of 56⁹ different gene constructs. All the constructs contain one gene for WUE, except for one construct which contains two different genes. The introduced genes have demonstrated the capacity to produce a water use efficient phenotype in cotton and other plants by regulating expression of endogenous genes, or modulating biochemical pathways in the cotton plants. Most of the introduced genes were derived from the plants *Arabidopsis thaliana* (thale cress), *Zea mays* (corn), *Glycine max*

⁶ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

⁷ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

⁸ The original application indicated that the sites may be located in up to 25 different LGAs. The applicant has requested the addition of Gunnedah (NSW) to the list of LGAs, but due to council amalgamation in Queensland, the number of proposed locations has decreased from 25 to 23.

⁹ The original application was for 63 gene constructs containing 56 different genes. However, Monsanto withdrew 7 constructs leaving 56 constructs containing 50 different genes.

(soybean), *Oryza sativa* (rice), *Gossypium hirsutum* (cotton), *Beta vulgaris* (beetroot), *Cucurbita ficifolia* (figleaf gourd), *Triticum aestivum* (wheat) and the moss, *Physcomitrella patens*. The remainder of the introduced genes were derived from the bacteria *Agrobacterium tumefaciens*, *Bacillus haloduras*, *B. subtilis*, and *Escherichia coli*; or the fungus, *Saccharomyces cerevisiae*.

Additionally, the GM cotton lines contain the antibiotic resistance selectable marker gene, *nptII* or the herbicide tolerance selectable marker gene, *cp4 epsps*. The *nptII* gene, encoding a neomycin phosphotransferase type II enzyme, was originally derived from the common gut bacterium *Escherichia coli* and confers kanamycin or neomycin resistance on the GM plant. 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 and *cp4 epsps* genes were used in the laboratory to select modified plant tissues during the initial development of the plants from which the GM lines are derived.

The purpose of the trial is to conduct proof of concept research involving experiments to evaluate agronomic characteristics including water use efficiency, yield and fibre quality of the GM cotton lines under optimal and water stress conditions. Seed will be collected for further studies, including possible future releases (subject to additional assessments and approvals). The GM cotton will not be used for human food or animal feed

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

Confidential Commercial Information

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

Risk assessment

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

Advice received from the public on the consultation RARMP (4 submissions) and how it was considered, is summarised in Appendix C.

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

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

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

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

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton lines into the environment are considered to be **negligible**. Hence, the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹⁰.

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 seven events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

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

¹⁰ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Licence conditions to manage this limited and controlled release

The Acting Regulator has imposed a number of licence conditions including requirements to:

- conduct the release on up to 20 sites of no more than 2 ha each on a total maximum area of 40 ha per year for two years between September 2008 and June 2010;
- locate the proposed trial sites at least 50 metres (m) away from natural waterways;
- limit pollen flow using one of the following measures:
 - surround the trial site with a 100 m monitoring zone and maintain a 3 kilometre (km) isolation distance between the site and intentionally planted cotton crops, **or**
 - surround the trial site by a 20 m pollen trap of non-GM (conventional) cotton or GM cotton that the Regulator has approved for commercial release.
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering;
- ensure the pollen trap plants are grown in such a way as to ensure flowering at the same time and for the same period of time as the GM cotton;
- locate the glasshouses at least 20 km from the nearest cotton crop;
- implement an insect control program within the glasshouses;
- harvest and gin all cotton plant materials (GM and non-GM) separately from other commercial cotton crops;
- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for future research or release;
- store GM plant materials (required for further study or future release) in certified physical containment level 2 (PC2) facilities or facilities approved in writing by the Regulator;
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil;
- monitor trial sites after harvest for a minimum of 12 months and destroy any cotton volunteers that may grow until no volunteers are detected for a continuous 6 month period;
- restrict personnel with access to the site to authorised personnel only; and
- not permit the use of GM plant material, including cotton seed, cotton seed oil and meal for human food or animal feed, or cotton lint for the production of fabrics and/or other cotton products.

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

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework. Dealings conducted under a licence issued by the Regulator may also be subject

to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)¹¹.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept 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 to evaluate any of the GM cotton lines. FSANZ approval would need to be obtained before they could be used in human food in Australia.

Identification of issues to be addressed for future releases

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

- ♦ characterisation of the genetic material inserted into the plants, including copy number and genotypic stability;
- ♦ additional data on the potential toxicity of plant materials from the GM cotton lines;
- ♦ additional data on the allergenicity of proteins encoded by the introduced genes for water use efficiency; and
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; altered germination; altered flowering time; tolerance to environmental stress; and disease susceptibility.

Suitability of the applicant

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

Conclusions of the consultation RARMP

The risk assessment concludes that this limited and controlled release of the GM cotton lines on up to 20 sites, located in various LGAs in NSW, QLD and WA, totalling no more than 40 ha per year over a two year period between 2008 and 2010 poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

¹¹ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

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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 legislation¹², details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

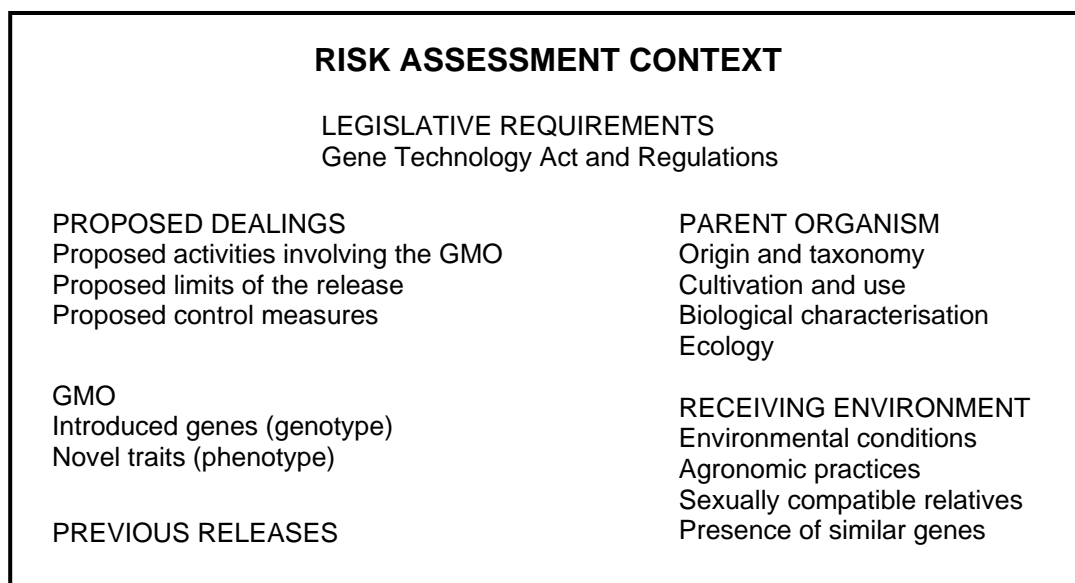


Figure 1 Components of the context considered during the preparation of the risk assessment

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

¹² The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/process-1>> and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

- ♦ the presence of the introduced or similar genes in the environment (Section 6.5)
- ♦ any previous releases of these or other GMOs relevant to this application (Section 7)

Section 2 The legislative requirements

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

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

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

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

Section 3 The proposed dealings

7. Monsanto Australia Ltd (Monsanto) proposes to release up to 504 cotton lines¹³ that have been genetically modified for enhanced water use efficiency (WUE), into the environment under limited and controlled conditions.

8. 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.

3.1 The proposed activities

9. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept research involving experiments with the GM cotton lines to evaluate

¹³ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

agronomic characteristics including WUE, yield and fibre quality under optimal and water stress conditions. Seed would be collected for further studies, including possible future releases (subject to additional assessments and approvals). The GM cotton will not be used for human food or animal feed or in the production of fabric and/or other cotton products.

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

10. The release is proposed to take place at up to 20 sites of no more than 2 hectares (ha) each, on a total maximum area of 40 ha per year over a two year period between 2008 and 2010. The sites may be located¹⁴ in the New South Wales local government areas (LGAs) of Balranald, Bourke, Central Darling, Carathool, Coonamble, Gunnedah, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren and Lake Tandou (an unincorporated area); the Queensland LGAs of Paroo, Balonne, Dalby Regional, Goondiwindi Regional, Toowoomba Regional, Somerset Regional, Brisbane City and Lockyer Valley Regional; and the Western Australia LGA of Wyndham-East Kimberley. Glasshouses in the LGAs of Brisbane City (AQIS quarantine facility in Eagle Farm) and Toowoomba Regional (Monsanto Biotechnology Research Centre) would be producing the seed for planting at field sites.

11. The applicant has proposed that some of the sites may be planted directly over a site used the previous year or directly over a site used under DIR 064/2006.

3.3 Proposed controls to restrict the dissemination or persistence of the GMOs and their genetic material in the environment

12. The applicant has also proposed a number of controls to restrict the dissemination or persistence of the GM cotton lines in the environment including:

- conduct the release on up to 20 sites of no more than 2 ha each on a total maximum area of 40 ha per year for two years between September 2008 and June 2010;
- locate the proposed trial sites at least 50 metres (m) away from natural waterways;
- limit pollen flow using one of the following measures:
 - *maintain a 10 kilometres (km) isolation zone between the site and any other potential outcrossing cropping source, or*
 - *implement a prophylactic insect control program capable of limiting pollen flow by insect vectors and maintain a 5 km isolation zone between the site and any other potential outcrossing cropping source, or*
 - *surround the trial sites by a 20 m pollen trap of non-GM (conventional) cotton or GM cotton that the Regulator has approved for commercial release.*
- ensure the pollen trap plants are grown in such a way as to ensure flowering at the same time and for the same period of time as the GM cotton;
- locate the glasshouses at least 20 km from the nearest cotton crop;
- implement an insect control program within the glasshouses;
- harvest and gin all cotton plant materials (GM and non-GM) separately from other commercial cotton crops;

¹⁴ The original application indicated that the sites may be located in up to 25 different LGAs. The applicant has requested the addition of Gunnedah (NSW) to the list of LGAs, but due to council amalgamation in Queensland, the number of proposed locations has decreased from 25 to 23.

- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for future research or release;
- store GM plant materials (required for further study or future release) in certified physical containment level 2 (PC2) facilities or in facilities approved by the Regulator;
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil;
- monitor trial sites after harvest for a minimum of 12 months and destroy any cotton volunteers that may grow until no volunteers are detected for a continuous 6 month period;
- restrict personnel with access to the site to authorised personnel only; and
- not permit the use of GM plant material, including cotton seed, cotton seed oil and meal for human food or animal feed, or cotton lint for the production of fabrics and/or other cotton products.

13. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter) and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.

Section 4 The parent organism

14. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in New South Wales, and southern and central Queensland. However, the cultivar which has been transformed, Coker 130, is not commercially grown in Australia. More detailed information on cotton can be found in the document, *The Biology of Gossypium hirsutum* L. and *Gossypium barbadense* L. (*cotton*), which was produced to inform the risk assessment process for licence applications involving GM cotton plants (OGTR 2008). This document is available at <<http://www.ogtr.gov.au>>.

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

5.1 Introduction to the GMOs

15. Up to 504 GM cotton lines are proposed for release. Each GM cotton line was created using one of 56¹⁵ different gene constructs. All the constructs contain one gene for WUE, except for one construct which contains two different genes. Some of the gene constructs contain the same gene for enhanced WUE but differ in the regulatory elements controlling expression of that gene. The 56 different gene constructs contain a total of 50 different genes for enhanced WUE. All of the GM cotton lines were generated by *A. tumefaciens*-mediated transformation (see Section 5.4 for further detail).

16. The introduced genes encode proteins that are intended to confer enhanced WUE (drought tolerance) by modulating biochemical pathways, functioning as molecular chaperones, or regulating signal transduction and expression of endogenous genes in the cotton plants. The majority of the introduced genes for WUE encode transcription factors¹⁶.

¹⁵ The original application was for 63 gene constructs containing 56 different genes. However, Monsanto withdrew 7 constructs leaving 56 constructs containing 50 different genes.

¹⁶ A transcription factor is any protein required for recognition, by RNA polymerases, of specific regulatory sequences in genes (eg a promoter) (Lewin 1994).

Some of the introduced genes, or their homologs¹⁷, are also known to confer other agronomic effects in plants, such as tolerance to other abiotic and biotic stresses. However, at this early stage of research, the nature and extent of such stress tolerance is not known. This uncertainty will be taken into account in the risk analysis process.

17. GM cotton lines derived from nine constructs would also contain the herbicide tolerance selectable marker gene *cp4 epsps*. All GM cotton lines derived from the remaining constructs would contain the antibiotic resistance selectable marker gene *nptII*.

18. Most of the introduced genes were derived from the plants *Arabidopsis thaliana* (thale cress), *Zea mays* (corn), *Glycine max* (soybean), *Oryza sativa* (rice), *Gossypium hirsutum* (cotton), *Beta vulgaris* (beetroot), *Cucurbita ficifolia* (figleaf gourd), *Triticum aestivum* (wheat) and the moss, *Physcomitrella patens*. The remainder of the introduced genes were derived from the bacteria *Agrobacterium tumefaciens*, *Bacillus halodurans*, *B. subtilis* and *Escherichia coli*; or the fungus, *Saccharomyces cerevisiae*.

19. The expression of the introduced genes for WUE in the GM cotton lines is controlled by one of eight different promoters derived from the plants *A. thaliana* and *P. x hybrida*; Cauliflower Mosaic Virus (CaMV) and Figwort Mosaic Virus (FMV). The termination region of the messenger RNA (mRNA) for the introduced WUE genes is derived from *Pisum sativum* or *G. barbadense*. Regulatory elements are discussed further in Section 5.3 of this Chapter.

5.2 The introduced genes, encoded proteins and end products

20. The introduced genes for water use efficiency have been classified into groupings according to the gene family (sub-family) in which they have been assigned by their known function or sequence, or both. The groupings reflect the notion that genes belonging to a particular family or subfamily may have very similar, and sometimes the same, function in plants. Further information related to the introduced genes has been declared as CCI and has been removed from this Section. General information about the anticipated effects of the introduced genes is in Section 5.2.4 of this Chapter.

5.2.1 Toxicity/allergenicity of the proteins encoded by the introduced genes for water use efficiency

21. Homologues of all of the encoded proteins occur naturally in a range of organisms, including plants widely consumed by people and animals (see discussion in Section 6.5). On this basis, people and other organisms have a long history of exposure to the introduced proteins involved in water use efficiency.

22. No toxicity/allergenicity tests have been performed on any of the purified encoded proteins as the proposed trial is still at proof of concept stage. Such tests would have to be conducted if approval was sought for the GMOs to be considered for human consumption in Australia (see discussion in Section 7.1.2).

23. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequences of the proteins encoded by each of the introduced genes for water use efficiency were compared to a database of

¹⁷ Homologous genes refers to genes within a single species that diverged by gene duplication or a gene similar in structure and evolutionary origin to a gene in another species.

known allergens. The results of this analysis did not indicate that any of the encoded proteins shared any significant sequence homology with any known allergens (data supplied by applicant).

24. A comprehensive search of the scientific literature also yielded no information to suggest that any of the encoded proteins are toxic or allergenic to people, or toxic to other organisms.

5.2.2 The selectable marker genes and their encoded proteins

25. The GM cotton lines contain the antibiotic resistance selectable marker gene, *nptII* or the herbicide tolerance selectable marker gene, *cp4 epsps*. The *nptII* gene, encoding a neomycin phosphotransferase type II enzyme, was originally derived from the common gut bacterium *Escherichia coli* and confers kanamycin or neomycin resistance on the GM plant. 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 and *cp4 epsps* genes were used in the laboratory to select modified plant tissues during the initial development of the plants from which the GM lines are derived. The applicant does not intend to use these markers during the field trial.

5.2.3 Toxicity/allergenicity of the proteins encoded by the selectable marker genes

The reporter gene CP4 epsps and the encoded protein

26. Nine of the constructs used to generate the water use efficient GM cotton lines would contain the *cp4 epsps* gene, which confers tolerance to glyphosate (N-phosphonomethyl glycine) and was derived from the *Agrobacterium* sp. strain CP4. (Padgett et al. 1996). The *cp4 epsps* gene was used as a selective marker to identify transformed plant tissue during initial development of GM plants in the laboratory.

27. The CP4 EPSPS protein expressed in the GM cotton plants proposed for release is the same as that present in the previously approved, commercially released GM Roundup Ready[®] cotton. Toxicity studies using the purified form of the introduced CP4 EPSPS protein present in Roundup Ready[®] cotton have been conducted. Details of these studies are available in previous RARMPs (see the RARMPs for DIR 020/2002 and DIR 023/2002 for the most detailed discussion). A summary of these studies is given below.

28. Sequence homology does not show any structurally relevant similarity between the CP4 EPSPS protein and any known toxic or pharmacologically active protein relevant to human health. Nor does it show any significant amino acid sequence homology to known allergens in protein databases. Furthermore, Roundup Ready[®] soybean expressing the identical introduced CP4 EPSPS protein has been shown not to be allergenic to humans (Batista et al. 2005). Hence, the *cp4 epsps* gene will not be considered further in this assessment.

The selectable marker gene (nptII) and the encoded protein

29. Forty-seven of the constructs used to generate the water use efficient GM cotton lines also contain the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (*nptII*). This gene, encoding for the enzyme neomycin phosphotransferase, was derived from *Escherichia coli* and confers kanamycin or neomycin resistance on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plant tissue during initial development of GM plants in the laboratory.

30. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, most recently in

DIR 070/2006, regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GMOs as not posing a risk to human or animal health or to the environment. The most recent international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority (EFSA 2007). Hence the *nptII* gene will not be considered further in this assessment.

5.2.4 The end products/effects associated with the introduced genes for water use efficiency

31. Plant molecular responses to drought stress were discussed in a previous RARMP (see Chapter 1, Section 4.2 of the RARMP for DIR 064/2006 for further information and references); a summary of that information is given below.

32. 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.

33. 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. Once sensed, a drought stress signal may be relayed to eventually regulate the transcription of numerous genes involved in the stress response.

34. 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, but other genes encoding proteins involved in chromatin remodelling may also be involved in transcription control. Approximately 7.4% (~2000) of the expressed genes in the experimental model plant *A. thaliana* encode transcription factors (Iida et al. 2005). Certain regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity).

35. 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 and late embryogenesis abundant proteins that 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), which are thought to act primarily as osmoprotectants but may also have secondary functions as gene regulators, 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). 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 (eg water channel proteins and ion transporters). 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).

5.2.5 Toxicity of the end products associated with the introduced genes for water use efficiency

36. Some of the introduced genes for water use efficiency encode particular proteins with specific end products. No evidence was found to suggest that these end products would cause a toxic or allergenic effect at the levels found in either non-GM or GM plants. Further information related to the introduced genes and their end products has been declared as CCI and has been removed from this Section.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the water use efficiency genes

37. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Information on the eight promoters used to regulate expression of the introduced genes for WUE was declared CCI and has been removed from this Section. Part of the purpose of the proposed trial is to determine whether any of these promoters provide adequate expression of the introduced genes in the cotton plants.

38. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the introduced genes for water use efficiency are derived from *P. sativum*, except for one construct which is derived from *G. barbadense*.

39. Two introns have been used in the constructs. The introns are both derived from *A. thaliana* and have been placed with their respective promoter elements. Inclusion of the intron with the promoter has been shown to enhance expression of the gene of interest (Keith & Chua 1986; Callis et al. 1987; Vain et al. 1996).

40. All the constructs contain a 5' untranslated region (5'UTR), or leader sequence. This region extends from the transcription start site to just before the ATG translation initiation codon and although transcribed, these sequences are not translated into protein. The 5' UTRs of eukaryotic mRNAs contain specific motifs that act either as target sites for RNA-binding factors or interact directly with the translation machinery. 5' UTRs play an important role in the regulation of gene expression through the regulation of mRNA transport, translation efficiency, subcellular localization and message stability (Mignone et al. 2005).

41. While some of the regulatory sequences are derived from plant pathogens (CaMV, FMV) the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants. The other regulatory sequences are derived from plants (*A. thaliana*, *P. x hybrida*, *P. sativum*, and *G. barbadense*), that may or may not be associated with allergenic or toxic responses in humans, but are not in themselves allergenic or toxic.

5.3.2 Regulatory sequences for the expression of the nptII and cp4 epsps genes

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

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

44. Although FWV, CaMV and *A. tumefaciens* are plant pathogens, the regulatory sequences comprise only a small part of the total genome, and are not capable of causing disease.

5.4 Method of genetic modification

45. All of the GM cotton lines will be generated by *A. tumefaciens*-mediated transformation.

46. A disarmed binary plasmid vector was used to introduce the gene constructs containing either the *cp4 epsps* or *nptII* gene (see section 5.2.2, above) 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 either glyphosate, or kanamycin and neomycin.

47. The 504 GM cotton lines proposed for release 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.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

48. The applicant states that all 504 GM cotton lines are in early development stage and have not been tested for genotypic stability. In addition, the applicant states that the GM cotton lines form part of a 'proof of concept' field trial.

49. Detailed molecular characterisation using standard molecular biology techniques, such as copy number and insertion sites, would take place once the best performing GM cotton lines have been identified under field conditions.

5.5.2 Characterisation of the phenotype of the GMOs

50. The main purpose of the trial is to conduct proof of concept experiments to evaluate agronomic characteristics including water use efficiency, yield and fibre quality of the GM cotton lines under optimal and water stress conditions. Phenotypic and agronomic data would be collected during the proposed trial.

51. The applicant states that all of the 50 introduced genes have been shown in cotton and/or other plants to confer a WUE phenotype. Literature based evidence supported a role in WUE for most, but not all, of the 50 genes (see Section 5.2, this Chapter). The applicant is not aware of differences in the flowering window of the GM plants compared to the non-GM parent in glasshouse studies. The applicant has not observed any unintended or secondary effects from the genetic modification of the cotton lines.

52. Fifteen lines of GM cotton, created using four of the same constructs, have undergone preliminary field testing for water use efficiency under licence DIR 064/2006. Observations by the applicant during the preliminary field trials suggest that flowering is largely temperature dependent and that there were no significant differences in flowering time between the GM cotton lines and commercial cotton lines. However, the applicant states the data collected from these trials on water use efficiency is inconclusive in this instance.

53. While there may be changes in the levels of products produced as a result of the activity of the encoded proteins, no new products should be produced by expression of the introduced genes (except for the selectable marker genes). There may, however, be unintended effects due to random insertion of the introduced genes (see Chapter 2, Event 6).

Section 6 The receiving environment

54. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, location and duration of the dealings, any relevant biotic/abiotic properties of the geographic 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 2007).

6.1 Relevant abiotic factors

55. The abiotic factors relevant to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)* document (OGTR 2008). Of particular relevance is the section on water use, which was also discussed in Chapter 1, Section 6.3 of the RARMP for DIR 064/2006.

56. The release is proposed to take place at up to 20 sites totalling no more than 40 hectares (ha) per year over a two year period between 2008 and 2010. The sites may be located in the New South Wales LGAs of Balranald, Bourke, Central Darling, Carathool, Coonamble, Gunnedah, Hay, Lachlan, Moree Plains, Narrabri, Narromine, Walgett, Warren and Lake Tandou (an unincorporated area); the Queensland LGAs of Paroo, Balonne, Dalby Regional, Goondiwindi Regional, Toowoomba Regional, Somerset Regional, Brisbane City and Lockyer Valley Regional; and the Western Australia LGA of Wyndham-East Kimberley. Glasshouses in the LGAs of Brisbane City (AQIS quarantine facility in Eagle Farm) and Toowoomba Regional (Monsanto Biotechnology Research Centre) would be producing the seed for planting at field sites.

57. Selected climatic data representing several geographically distinct commercial cotton growing regions where the majority of the trial is proposed to take place is given in Table 1. These areas have typical climates for summer cotton growing regions in Australia, with warm summers and mostly higher summer than winter rainfall.

58. The applicant has indicated that the proposed sites were selected on the basis of relative isolation from commercial cotton sites, generally low rainfall areas suitable for irrigation and with no history of natural flooding.

Table 1 Climatic data for sites representative of proposed water-efficient GM cotton trial areas

| Representative site (within LGA) | 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 °C /20.3 °C | 19.0 °C /5.6 °C | 38.8 mm | 23.6 mm |
| Hay NSW (Hay) | 32.2 °C /15.9 °C | 16.0 °C /4.2 °C | 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 °C /17.9 °C | 17 °C /3.4 °C | 56.8 mm | 30.3 mm |
| Cunnamulla QLD (Paroo) | 35.3 °C / 21.5 °C | 19.8 °C /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; LGA, local government area.

6.2 Relevant biotic factors

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

- ♦ the glasshouse sites would be located at least 20 km from the nearest commercial cotton crop
- ♦ the majority of field trial sites would be in commercial cotton growing regions of Australia
- ♦ all sites would be located at least 50 m from natural waterways
- ♦ invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products.

6.3 Relevant agricultural practices

60. The size, locations and duration of the proposed limited and controlled release of the GM cotton lines are outlined in Section 3.2 of this Chapter.

61. The applicant intends to trial the GM cotton alongside the non-GM parent 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 watering regimes.

62. The applicant has proposed the use of non-PC2 glasshouse(s) in Brisbane (AQIS Quarantine Facility at the Department of Primary Industries, Eagle Farm) and Toowoomba (Monsanto Biotechnology Research Centre) to produce seed of the GM cotton lines for planting at field sites. The plants would be grown in pots of soil on raised benches. Monsanto intends to implement an insect control program to prevent/limit dissemination of the GMOs. The glasshouses would be located at least 20 km from the nearest commercial cotton crops.

63. The glasshouses are surrounded by mesh fences (2.7m high at Brisbane, 2.5m high at Toowoomba) with barbed wire at the top and have lockable gates. Access to the glasshouses

would be restricted to Monsanto authorised and trained persons and would include staff from the Department of Primary Industries at the Brisbane location.

64. The applicant has proposed several different options to access the site for spraying of insecticides. One proposed method is to use high clearance equipment which would not damage the pollen trap. A second proposed method is low clearance equipment and driving through the pollen trap either on wheel tracks or on a cleared (sprayed) path of approximately 2.5 m width.

65. Following harvest of each site (including glasshouse sites), fuzzy cotton seed will be transported to the Toowoomba Centre (or another PC2 facility) where it will be ginned and acid delinted. This seed will be used for further planting, stored at Toowoomba, incinerated, and/or exported to a Monsanto facility in the USA. After harvest all material other than seed will be destroyed.

66. The applicant proposes to transport the seed from Toowoomba to the field sites or seed and plant material from the field to Toowoomba in accordance with OGTR transportation guidelines. All cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) will be removed and/or destroyed after harvest (except for materials required for future research or release).

6.4 Presence of related plants in the receiving environment

67. The applicant has indicated that it is unlikely that native *Gossypium* species would occur within the proposed release sites or in neighbouring commercial cotton fields. The only sexually compatible species present in Australia that could receive genes from the GM cotton lines (*G. hirsutum*) are other *G. hirsutum* or *G. barbadense* cottons (including commercially grown or naturalised cotton). 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 2008). The presence of remnants of some of these populations has not been confirmed.

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

- insect resistant Bollgard II[®] cotton (also known as MON15985), herbicide tolerant Roundup Ready[®] cotton (also known as MON1445), herbicide tolerant Roundup Ready Flex[®] cotton (also known as MON88913), herbicide tolerant/insect resistant Roundup Ready[®]/Bollgard II[®] cotton (also known as MON1445/MON15985) and herbicide tolerant/insect resistant Roundup Ready Flex[®]/Bollgard II[®] cotton (also known as MON88913/MON15985) (authorised under DIR 12/2002, 23/2002, 59/2005 and 066/2006 licences)
- herbicide tolerant Liberty Link[®] Cotton (previously known as LLCotton25 or Liberty[®] cotton) and herbicide tolerant/insect resistant Liberty Link[®] / Bollgard II[®] MON15985 cotton (authorised under DIR 062/2005)

69. Approvals for commercial releases include releases in the areas proposed in the current application. GM cotton plants are widespread in the agricultural environment comprising approximately 92% of commercially grown cotton crops. In contrast, non-GM *G. hirsutum* and *G. barbadense* comprise approximately 6.7% and 1.4% of commercially grown cotton, respectively (see Section 6.1, chapter 1, RARMP for DIR 074/2007).

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

70. All of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment

71. Most of the introduced genes for WUE were derived from the plants *A. thaliana* (thale cress), *Z. mays* (corn), *G. max* (soybean), *O. sativa* (rice), *G. hirsutum* (cotton), *B. vulgaris* (beetroot), *C. ficifolia* (figleaf gourd), *T. aestivum* (wheat) and the moss, *P. patens*. The remainder of the introduced genes were derived from the bacteria *A. tumefaciens*, *B. halodurans*, *B. subtilis*, and *E. coli*; or the fungus, *S. cerevisiae*.

72. The introduced genes for WUE are highly homologous and most likely orthologous¹⁸, to genes in most, if not all, crop and other plants. The bacteria are widespread species found in soil and/or water, or in the case of *E. coli*, widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). *S. cerevisiae* is commonly known as baker's yeast and has been used since ancient times in baking and brewing. Therefore, it is expected humans routinely encounter, through contact with plants and food, the introduced genes and their gene products, or their homologs.

73. Additionally, the GM cotton lines contain the antibiotic resistance selectable marker gene, *nptII* or the herbicide tolerance selectable marker gene, *cp4 epsps*. 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).

74. 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 7 Australian and international approvals

7.1 Australian approvals of the GM cotton lines

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

75. The Regulator has previously issued a licence to Monsanto for the limited and controlled release of GM cotton lines containing 17¹⁹ of the same gene constructs for enhanced water use efficiency (Licence DIR 064/2006). Fifteen GM cotton lines created using four of the same gene constructs were trialled under DIR 064/2006. There have been no previous releases of the GM cotton lines containing the other genes for WUE in Australia.

7.1.2 Approvals by other Australian government agencies

76. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory

¹⁸ Orthologous refers to genes in different species that derive from a common ancestor.

¹⁹ The original DIR 081/2007 application contained 63 constructs and GMOs containing 21 of the same constructs were approved under DIR064/2006. However, Monsanto withdrew 7 constructs (constructs 1, 2, 6, 9, 14, 15 and 45) on 18 March, 14 April and 16 May 2008, reducing the number of constructs shared by the two applications to 17.

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 Standards Australia New Zealand (FSANZ). This is discussed further in Chapter 3.

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

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

7.2 International approvals

79. Field trials with GM cotton lines containing one of the gene constructs were conducted in the USA in 2005 under the USDA permit number 05-278-02n (information supplied by the applicant). There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Chapter 2 Risk assessment

Section 1 Introduction

80. 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 4) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

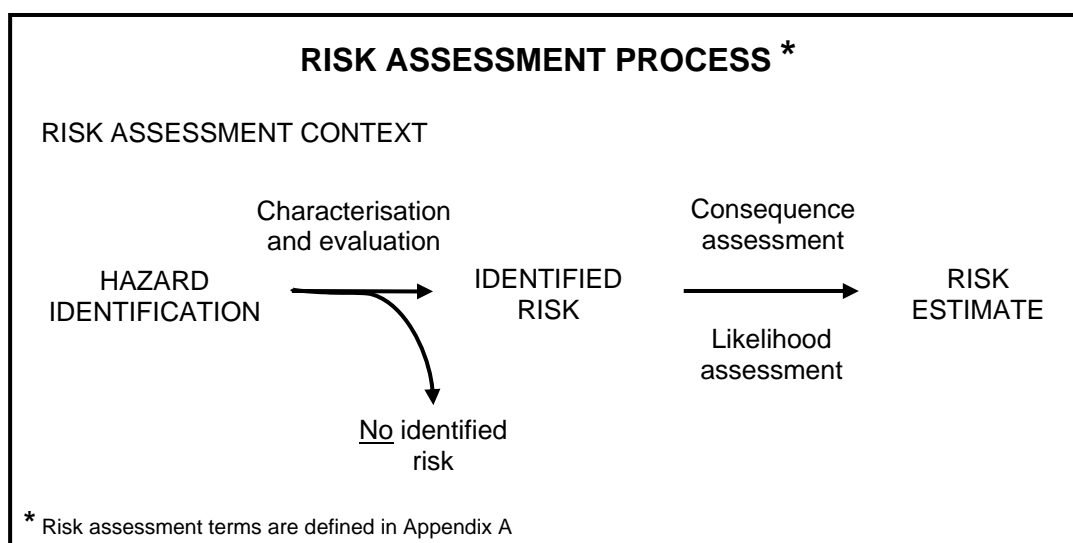


Figure 4 The risk assessment process

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

82. 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 may be an event, a substance or an organism (OGTR 2007).

83. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

84. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

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

Section 2 Hazard characterisation and the identification of risk

86. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

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

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

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

89. The seven events that were characterised are discussed in detail later in this Section. They are summarised in Table 2 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

90. As discussed in Sections 5.2.3, the *cp4 epsps* and *nptII* genes and their products have already been considered in detail in previous RARMPs and by other regulators. They have not been found to pose risks to either people or the environment and will not be considered further.

Table 2. Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for enhanced 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/allergenic to people or toxic to other organisms | 1. Exposure to GM plant material containing proteins encoded by the introduced genes, or their end products. | Allergic reactions in people or toxicity in people or other organisms | No | <ul style="list-style-type: none"> The encoded proteins and their end products are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The limited scale, short duration and other proposed limits and controls, minimise exposure of people and other organisms to proteins encoded by the introduced genes, or their end products. |
| Section 2.2 Spread and persistence (weediness) of the GM cotton lines in the environment | 2. Expression of the introduced genes improving the survival of GM cotton plants. | Weediness; increased allergic reactions in people or toxicity in people and other organisms | No | <ul style="list-style-type: none"> Non-GM cotton and approved GM cotton do not possess weedy characteristics. Many factors other than water availability limit the spread and persistence of cotton in the areas proposed for release including temperature (particularly frost), nutrient levels, roadside management practices, short summer seasons, soil type, competition from other plants and disease. The limits and controls proposed for the release would minimise spread and persistence. |
| | 3. Dispersal of viable GM plant materials through various means, including animals and extreme weather conditions. | Weediness; increased allergic reactions in people or toxicity in people and other organisms | No | <ul style="list-style-type: none"> The proposed limits and controls including transport and storage of seed according to OGTR guidelines, cleaning of equipment and release sites, destruction of plant material, post-harvest monitoring and removal of volunteers, location of release site above flood level and at least 50 m away from natural waterways would all serve to minimise dispersal |
| Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants | 4. Expression of the introduced genes or regulatory sequences in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (including commercially released GM cotton lines) or in other sexually compatible plants | Weediness; increased allergic reactions in people or toxicity in people and other organisms | No | <ul style="list-style-type: none"> Cotton is primarily self-pollinating, 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 GM and non-GM 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. Events 1-3 did not identify any risks to people or the environment associated with expression of the introduced genes. |

| Hazard category | Event that may give rise to an adverse outcome | Potential adverse outcome | Identified risk? | Reason |
|--|---|---|------------------|---|
| Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms | 5. Presence and expression of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer. | Weediness; increased allergic reactions in people or toxicity in people and other organisms | No | <ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via natural mechanisms. Events 1 – 3 did not identify any risks to people or the environment associated with expression of the introduced genes. |
| Section 2.5 Unintended changes in biochemistry, physiology or ecology | 6. Changes to biochemistry, physiology or ecology of the GM cotton lines resulting from altered expression or random insertion of the introduced genes. | Weediness; increased allergic reactions in people or toxicity in people and other organisms | No | <ul style="list-style-type: none"> Adverse effects as a result of unintended changes if any, would be minimised by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process |
| Section 2.6 Unauthorised activities | 7. Use of the GMOs outside the proposed licence conditions. | Potential adverse outcomes mentioned in Sections 2.1 to 2.5 | No | <ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator. |

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

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

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

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

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

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

95. Non-GM cotton tissue, 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 cyclopropanoid fatty acids. The presence of these fatty acids limits the use of whole cotton seed or seed meal in animal feed to a relatively small proportion of the diet and

it must be introduced gradually, to avoid potential toxic effects. For further details see ‘*The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*’ document (OGTR 2008).

96. Although no toxicity studies have been prepared on the GM cotton plant material or encoded proteins, no information was found (Chapter 1, Section 5.2.1) to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms. Some of the introduced genes for water use efficiency encode particular proteins with specific end products. No evidence was found to suggest that these end products would cause a toxic or allergenic effect at the levels found in either non-GM or GM plants (see Chapter 1, Section 5.2.5).

97. It is not expected that any novel products would be produced as a result of the expression of the introduced genes because the introduced genes are likely to be orthologous or homologous to genes in cotton and all other plants (see Chapter 1, Section 6.5). The introduced (or similar) genes and encoded proteins are naturally present in the environment and therefore humans, other vertebrates, invertebrates and microorganisms are already exposed to them.

98. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would all minimise the likelihood of exposure of people and other organisms to GM plant materials. The short duration and small size of the trial will limit the potential for exposure of humans and animals to the GM plant tissues. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff associated with the proposed release. People living nearby are unlikely to be exposed to cotton pollen because it is heavy and not easily dispersed by wind (OGTR 2008). Potential for exposure of the public to plant materials via ingestion, skin contact or inhalation is very limited as no plant material will be used as food, animal feed or plant products and the public will not have access to the site. Public access to glasshouses would be restricted by fences with lockable gates. The applicant intends to destroy plant material remaining on the trial sites after collecting samples for analysis and harvesting seed.

99. **Conclusion:** The potential for allergic reactions in people, or toxicity in people or other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced genes, or their end products as a result of the genetic modification, is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence (weediness) of the GM cotton lines in the environment

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

101. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM cotton plants in particular, is provided in ‘*The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*’ document (OGTR 2008). In summary, this document concludes that non-GM cotton is not a serious weed in Australia. Firstly, cotton does not possess characteristics that are usually associated with weediness 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, large amount of seed dispersed 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.

102. Additional limiting abiotic and biotic factors that determine whether cotton will persist in the environment proposed for release include short summer seasons, soil type, and competition from other plants (Farrell & Roberts 2002). Disease, such as fungal wilt, 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. Reliable availability of water is a limiting factor in many areas of Australia.

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

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

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

105. The impact of the genetic modifications on survival of the GM cotton lines is uncharacterised under field conditions. However, a number of predictions can be made based on knowledge of the gene functions and their predicted effect when expressed in the modified plants. Predictions can also be made based on the observed phenotypes of the GM cotton lines grown under glasshouse conditions and comparing them to the phenotype of the non-GM cotton plants. These predictions are summarised in Chapter 1, Section 5.5.

106. The GM cotton lines contain introduced gene constructs for water use efficiency, which if successful, would confer enhanced tolerance to drought stress. The applicant states the introduced genes have demonstrated the capacity to produce a WUE 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 WUE genes could result in weediness of the GM cotton lines.

107. As discussed previously, 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 stress through different response mechanisms that include biochemical and physiological processes. Additionally, certain regulatory genes can be induced by more than one type of abiotic stress (eg drought, cold and salinity) (see Chapter 1, Section 5.2 for further details). Homologues of the introduced genes encode proteins which have been shown to enhance tolerance to other abiotic and biotic stresses (other than drought stress) such as cold, freezing, pathogen, salinity, nutrient deficiency, and heavy metals.

108. In addition, the introduced genes for WUE could potentially affect fertility, flowering time and seed development (including germination) of the GM cotton lines as compared to commercially grown cotton. For example, homologs of many of the introduced WUE genes

have been implicated in earlier or later flowering, floral induction, flower and inflorescence morphology and pollen development. Homologs of some of the other introduced WUE genes have been shown to be involved in seed germination, embryo development, nutrition in developing seeds, seed development and desiccation tolerance in the developing seed. Thus, it is possible that the encoded proteins could confer tolerances to other abiotic or biotic stresses, or enhance fertility and seed germination, which could lead to increased spread and persistence of the GM cotton plants.

109. However, two issues should be considered. First it should be noted that the anticipated effects of the introduced genes are derived from published literature of the gene (if available) or closely related genes and/or the gene family as a whole. Thus, a much broader range of anticipated effects are considered than would likely result from any single introduced gene. A second consideration is 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, the introduced genes may not confer any phenotypic changes or enhanced stress tolerance, other than drought stress tolerance, to the GM cotton plants.

110. It is possible that altered expression of a regulatory gene involved in plant response to stress could enhance tolerance to several environmental stresses. However, there is no indication from the literature that the introduced genes could alter all of the characteristics which limit the spread and persistence of cotton such as seed dormancy, seed persistence in soil, length of life cycle, large amount of seed dispersed and long-distance dispersal of seeds. In addition, a GM plant that is resistant to multiple stresses may be less fit because of associated metabolic/physiological burdens. For example, a plant with a higher continuous production of an osmoprotectant (Chapter 1, Section 5.2.4) may better tolerate desiccation but show overall stunted growth, produce fewer seeds, and have a decreased ability to tolerate competition from other plants.

111. As discussed above, multiple factors limit the distribution of cotton in the areas proposed for release. Further, the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM cotton lines proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures. These measures include destruction of all plant materials not required for further analysis, irrigation and cultivation of the site and pollen trap areas in the first spring or summer following harvest to promote cotton seed bank reduction and post harvest monitoring of the proposed site with destruction of any volunteers for at least twelve months. These measures would minimise the persistence of the GM cotton lines at the proposed release sites.

112. One of the main purposes of the proposed release is to conduct proof of concept experiments with the GM cotton lines to assess WUE as well as other important agronomic traits. Thus, characteristics that impact the survivability of the GM plants are likely to be closely monitored during of the proposed trial.

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

Event 3: *Dispersal of viable GM plant materials through various means, including animals and extreme weather conditions*

114. If the GM cotton lines were to be dispersed from the release site they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. Toxicity/allergenicity that might arise as a result of exposure to the GM

cotton lines was assessed in Event 1 and was not considered to be an identified risk. The introduced genes improving survival of the GM cotton lines in the environment was assessed in Event 2 and was also found not to be an identified risk.

115. In a natural situation cotton does not reproduce vegetatively (Sheelavantar et al. 1975; OGTR 2008), so dispersal of GM cotton materials other than seed would be highly unlikely to result in the dissemination of the GM cotton lines into the environment. Seed production, dispersal and digestibility characteristics are not expected to be altered in the GM cotton lines compared to non-GM parental cotton lines.

116. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content. They are therefore unlikely to carry bolls any great distance from the cotton fields. The cotton bolls are also unattractive to avian species, so birds are unlikely to transport seeds (OGTR 2008). Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

117. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal (see Chapter 1, Sections 3.2 and 3.3). The proposed release site is located 50 m away from natural waterways on locations with no history of natural flooding. In addition, the applicant proposes using irrigation practices (*Cotton Industry Good Management Practice*) used by cotton growers in Australia, which retains 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. These practices would also reduce the dispersal of seed.

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

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

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

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

121. Baseline information on vertical gene transfer associated with non-GM cotton is provided in '*The Biology of Gossypium hirsutum L. and Gossypium barbadense L. (cotton)*' document (OGTR 2008). In summary, the only sexually compatible species present in Australia that could receive genes from the GM cotton lines (*G. hirsutum*) are other *G. hirsutum* or *G. barbadense* cottons (including commercially grown or naturalised cotton). GM cotton plants are widespread in the agricultural environment comprising approximately 92% of commercially grown cotton crops. In contrast, non-GM *G. hirsutum* and *G. barbadense* comprise approximately 6.7% and 1.4% of commercially grown cotton, respectively (see Section 6.1, chapter 1, RARMP for DIR 074/2007).

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

122. Transfer and expression of the introduced genes for altered WUE in other GM or non-GM *G. hirsutum* or *G. barbadense* plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

123. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM cotton plants by the introduced genes or regulatory sequences. This will be the same if the introduced genes are expressed in other cotton plants. Similarly, if the introduced genes are expressed in *G. barbadense*, or other commercially approved GM cottons, allergenicity and toxicity are not expected to be altered.

124. As discussed in Event 2, the survival of the GM cotton plants proposed for release would be limited by factors such as nutrient availability, temperature and soil type and other environmental factors that normally limit the spread and persistence of cotton plants in Australia. Therefore, similar to the GM cotton plants, expression of the introduced genes in other cotton plants would also result in plants limited by these factors. The expression of the introduced genes in the sexually compatible species *G. barbadense* is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM cotton and *G. barbadense* would be expected to limit the spread and persistence of any hybrids between the GM cotton and *G. barbadense*.

125. A number of insect resistant and/or herbicide tolerant GM cotton lines are currently approved for commercial release in Australia and may be grown in the areas proposed for this release. These GM cotton lines were comprehensively assessed (most recently in the RARMPs for DIR 062/2006 and 066/2006) prior to release and comprised more than 90% of the commercial cotton crop in 2006-07. The commercial GM cotton lines could also be used in the pollen trap proposed by the applicant for the majority of the sites. If so, stacking of the WUE genes with those for insect resistance and/or herbicide tolerance is likely to occur.

126. However, expression of the introduced genes for WUE in the commercial GM cotton lines is not expected to increase their spread and persistence as they would still be limited by many abiotic and biotic factors such as soil nutrition, ability to compete with other plants, seed dormancy, seed persistence in soil, length of life cycle, high seed dispersal and long distance dispersal of seed (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008), and could be controlled by the use of alternative herbicides. Furthermore, if commercial GM cotton is used for the pollen trap, the proposed control measures relating to harvesting, post-harvest procedures and destruction of the experimental GM cotton plants also apply to pollen trap plants. If the highly unlikely 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.

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

128. Cotton is primarily self-pollinating with pollen that is large, sticky and heavy, and not easily dispersed by wind (McGregor 1976; Moffett 1983). The flowers are large and conspicuous and are attractive to insects (Green & Jones 1953), thus it is an opportunistic out-croser when insect pollinators are present (Oosterhuis & Jernstedt 1999). Overseas studies

have shown that insect pollinators can transfer pollen to other nearby cotton plants at rates up to 80% (eg (Oosterhuis & Jernstedt 1999). However, cotton pollen dispersal studies consistently show that outcrossing is localised around the pollen source and decreases significantly with distance ((OGTR 2008), and references therein). The separation distance of 4 metres required in Australia for certified commercial seed production reflects the relatively short distances observed for cotton pollen dispersal in Australian studies (Brubaker et al. 1999).

129. As indicated in Event 2, the genes for WUE could potentially affect fertility and flowering time. Increased fertility and early flowering could lead to increased spread and persistence of the GMOs in the environment. 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 could be increased. However, 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, the effect on fertility and flowering time as a result of the introduced genes may not eventuate in the GM cotton lines. As discussed in Chapter 1, Section 5.5, no differences in the flowering window of the GM plants compared to the non-GM parent was seen in glasshouse studies, or from observations on field trials of a limited number of the same GM cotton lines under licence DIR 064/2006. Nonetheless, the applicant has proposed that the 20 m pollen trap will be grown in such a way as to ensure that the flowering of the pollen trap plants is at the same time and for the same period of time as the GM cotton (see Chapter 1, Section 3.3). Additionally, if there is evidence of asynchronous flowering, the applicant intends to implement a contingency plan which includes the use of insecticides to limit pollen flow by insect vectors.

130. The applicant has proposed that sites near Gatton and Atkinson's Dam (Lockyer Valley Regional LGA) and Chinchilla (Dalby Regional LGA) would not be surrounded by a pollen trap. Instead these sites would be isolated from other cotton plants.

131. The applicant has proposed to plant the GM cotton lines at the same location for successive years and/or at the same location planted under DIR 064/2006. This means that a location (including the pollen trap) may be replanted to WUE GM cotton lines for up to 3 successive growing seasons²⁰. Thus, if a location is replanted, it is possible that seed lost during harvest (including GM cottonseed) of the first planting of a location may germinate in the trial location or pollen trap during subsequent years. The presence of GM cotton plants in the pollen trap could potentially lead to transfer and expression of the introduced genes for WUE to other cotton plants.

132. However, seed loss during harvest is expected to be low and the existence of a soil seed bank is unlikely because dispersed cottonseeds that do not germinate are rapidly weathered, leading to significant decreases in their viability (Halooin 1975; Woodstock et al. 1985). Thus, few volunteer cotton plants are expected and not all volunteers would be the GM cotton proposed for release (ie the volunteers plants could result from cottonseed lost from either the GMOs or pollen trap plants). In addition, the applicant has proposed to irrigate and cultivate the site and pollen trap areas in the first spring or summer following harvest to promote germination of volunteers and then to destroy the volunteers prior to replanting at the site. This practice would serve to further reduce any cotton seed bank due to seed loss at harvest.

²⁰ Under licence DIR 064/2006 the applicant was allowed to replant at the same location in successive years but did not exercise this option. Thus, the locations planted under DIR 064/2006 could potentially be replanted twice under the proposed dealings for DIR 081/2007, for a total of three successive plantings.

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

134. The applicant has proposed a number of measures to limit and restrict the potential for pollen flow and gene transfer to sexually compatible plants (see Chapter 1, Sections 3.2 and 3.3). These include a 20 m pollen trap or isolation distances of up to 10 km between the GM cotton and other cotton plants. 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. This would likely result in the applicant having to adopt a heavy insecticide spraying regime that is necessary 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. The applicant also proposes to perform post harvest monitoring of the site for at least twelve months and to destroy any volunteer plants found. These proposed controls would reduce the already low likelihood of gene flow from the GMOs to other cotton resulting in expression of the introduced genes.

135. Outcrossing from GM cotton at the greenhouse sites is limited by glass walls, an insect control program and an isolation distance of at least 20 km to the nearest commercial cotton crops. The only cotton in close proximity to the glasshouses would be contained within PC2 research facilities which would further limit outcrossing.

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

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

137. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2000). In the context of genetic modification, the major concern has been whether DNA introduced into crops could transfer into bacteria or into the cells of organisms that may eat the crops. Horizontal gene transfer has been considered in previous RARMPs (including in detail in DIR 057/2004), 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 sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. There are no more recent data that alter this conclusion.

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

138. The probability of transferring introduced genes and regulatory sequences contained in the GM cotton plants is no greater than that of transferring any of the native genes and

regulatory sequences. All of the introduced genes and regulatory sequences are isolated from naturally occurring organisms that are already widespread and prevalent in the environment (see Chapter 1, Sections 5.3 and 6.5), therefore these genes and regulatory sequences are already available for transfer via demonstrated natural mechanisms.

139. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied not only 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 (Mercer et al. 1999; Gebhard & Smalla 1998; Nielsen et al. 2000; Nielsen 1998; De Vries et al. 2001). This suggests that the likelihood of natural horizontal gene transfer is remote.

140. The safety of the protein product(s) resulting from the expression of the introduced gene(s), rather than horizontal gene transfer *per se*, is a key consideration in the risk assessment process (Thomson 2000). If the protein products are not associated with any risk to humans, animals or the environment then, even in the unlikely event of horizontal transfer occurring, they should still not pose any such risk. Events 1–4 associated with the proteins encoded by the introduced genes or their end products were not considered to represent an identified risk.

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

2.5 Unintended changes in biochemistry, physiology or ecology

142. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy²¹ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such 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.

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

²¹ Pleiotropy is the effect of one particular gene on the expression of other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

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

144. The GM cotton lines contain introduced genes for water use efficiency. Reduced water availability would induce stress on the plants and plants respond to stress often through interconnected signals and transcriptional controls (see Chapter 1, Section 5.2 for further details). Therefore, there is potential for a number of interrelated biochemical pathways to be affected by the introduced genes. However, it should be noted that the anticipated effects of the introduced genes was derived from published literature of the gene (if available) or closely related genes and/or the gene family as a whole. As such, a much broader range of anticipated effects are considered in the previous events than would likely result from any single introduced gene. Thus, considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, are already discussed for Events 1 - 3 which were not considered to be identified risks.

145. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. However, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). The applicant proposes to identify and eliminate any obvious examples of GM cotton plants showing deleterious or advantageous phenotypes before planting in the field.

146. The applicant proposes the release of up to 504 GM cotton lines and the trial represents the first opportunity for characterisation of many of the GM cotton lines under field conditions, which are difficult to replicate in glasshouse facilities. During this limited and controlled release, the applicant proposes to measure the agronomic performance of the GM cotton lines, and unintended effects, if any, would most likely be detected during the trial. Additionally, the likelihood of any pleiotropic effects causing adverse effects is minimised by the limits and controls outlined in Chapter 1, Sections 3.2 and 3.3. In particular, the proposed release is limited in size, locations and duration and none of the GM plant materials are intended for use in human food or animal feed or in the production of fabrics and/or other cotton products.

147. 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 release of these GMOs could be assessed.

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

2.6 Unauthorised activities

Event 7: Use of GMOs outside the proposed licence conditions (non-compliance)

149. 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.

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

Section 3 Risk estimate process and assessment of significant risk

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

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

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

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

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

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment²².

²² As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Section 4 Uncertainty

155. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. It is recognised that both dimensions of risk (ie consequence and likelihood) are always uncertain to some degree.

156. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability²³. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily treating an identified risk.

157. For DIR 081/2007, which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- ♦ Event 1, associated with the potential for increased allergenicity or toxicity through contact with plant materials containing proteins encoded by the introduced genes or their end products;
- ♦ Event 2, associated with the potential for increased survival of the GMOs;
- ♦ Event 4, associated with vertical gene transfer and expression of the introduced gene in other sexually compatible plants; and
- ♦ Event 6, associated with unintended changes to biochemistry, physiology or ecology of the GM cotton lines.

158. Additional data including information to address this uncertainty would be required to assess possible future applications for a larger scale trial, reduced control measures, or the commercial release of any of these GM cotton lines that may be selected for further development.

159. Section 5 of Chapter 3 discusses additional data that may be required for future releases.

²³ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management

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

Section 1 Background

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

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

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

Section 2 Responsibilities of other Australian regulators

164. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies²⁴.

165. 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.

²⁴ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

166. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from these GM cotton lines 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. FSANZ approval would need to be obtained before they could be used in food.

167. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs, or products known to be mixed with any amount of GM material. As the importation would constitute a dealing under the Act, the importer requires an authorisation under this Act for the import to lawfully proceed. Seed from the WUE GM cotton lines have been imported by Monsanto under an AQIS seed import permit. No other approvals are required.

Section 3 Risk treatment measures for identified risks

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

169. These events were considered in the context of the scale of the proposed release (a maximum total area of 40 ha per year over a two year period between 2008 and 2010 at up to 20 sites in NSW, QLD and WA, the containment measures (Chapter 1, Section 3), and the receiving environment (see Chapter 1, Section 6).

Section 4 General risk management

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

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by Monsanto

171. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls of the release and reference is made to these in the discussion of the events characterised for the release. The following comments are made on the appropriateness of these limits and controls.

172. The applicant has proposed to limit the release to up to 20 sites totalling no more than 40 ha per year over a two year period between 2008 and 2010. The sites may be located in various LGAs in NSW, QLD and WA (Chapter 1, Section 3.2). Proposed sites also include glasshouses in Brisbane and Toowoomba, which would be producing the seed for planting at trial sites.

173. The applicant has proposed to surround the majority of the trial sites (except the glasshouse sites) with a 20 m wide pollen trap of non-GM or GM cotton approved for commercial release to limit gene flow from the GM cotton. As discussed in the *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008) cotton is predominantly self pollinating with the highest level of out-crossing occurring between adjacent rows. Out-crossing data from a large number of GM cotton trials demonstrated an

average rate of 0.0035% measured to the outside row of the 20 m pollen trap (data from 12 sites, over three years, with 56,117 seeds examined, from trials in the cotton growing areas of NSW and QLD). There was no outcrossing recorded at the outside row of the 20m pollen trap at eleven of the twelve sites (Llewellyn et al. 2007). Thus, out-crossing is rare beyond 20 m and a 20 m pollen trap of cotton plants will minimise gene transfer to sexually compatible plants (Event 4).

174. It should be noted that the applicant has proposed a cleared (sprayed) 2.5 m path or wheel tracks through the pollen trap created by vehicles accessing the site for insecticide application. Driving through the pollen trap is likely to damage and break some of the pollen trap plants which may reduce the number of flowers in that area. Similarly, clearing a 2.5 m wide path by spraying with herbicide would eliminate a portion of the pollen trap. However, this is unlikely to reduce the effectiveness of the pollen trap because the path makes up a relatively small portion of the total pollen trap area²⁵ and the proposed application of insecticide would minimise insect-mediated pollen flow (Event 4).

175. The applicant has proposed that sites near Gatton and Atkinson's Dam (Lockyer Valley Regional LGA) and Chinchilla (Dalby Regional LGA) would not be surrounded by a pollen trap. Instead, these sites would be isolated from other cotton by at least 10 km or isolated by at least 5 km when a prophylactic insecticidal spray program is implemented. Any application of insecticide would help minimise outcrossing to other cotton plants.

176. In Australia, honeybees are considered to be the most likely insects responsible for any cross-pollination in cotton (Thomson 1966; Mungomery & Glassop 1969). There is little data available on bee foraging ranges in Australia. However, data from overseas indicates that mean foraging distances are at least 0.6 km (Visscher & Seeley 1982; Waddington et al. 1994; Beekman & Ratnieks 2000; Steffan-Dewenter & Kuhn 2003; Beekman et al. 2004), with foraging ranges up to 10 km (Visscher & Seeley 1982; Steffan-Dewenter & Kuhn 2003). Foraging distances varied widely depending on the availability of food, with the longer distances seen in areas in which nectar and pollen sources were scarce. It has also been shown that bees do not find possible forage plants unless they are close by. A study in which flowering buckwheat plants were planted in a forage-poor forest environment indicated that bees foraged on patches within 1 or 2 km of the hive, but failed to find those at 3.2-3.6 km (Seeley 1987).

177. Studies supporting long distance foraging/travel are based on conditions that are unlikely to occur at the proposed release sites. The conditions, such as no other food source are not representative of typical bee foraging and native trees, flowers and other crops grow in the areas proposed for release and therefore the bees are unlikely to be limited to foraging on cotton.

178. Studies have been conducted on insect-mediated gene flow over a heterogenous landscape. A UK study in white clover (*Trifolium repens* L.) indicated that short separation distances (25 m) and interspersing clover plants with barley (non-entomophilous) acted as a barrier to gene flow (Osborne et al. 2001). In Australian canola fields, no gene flow has been seen in canola fields which were more than 3 km from source fields (Rieger et al. 2002). Similarly, a study on cotton in Western Australia indicated that there was no gene flow from a GM cotton crop to the nearest cotton field 1.8 km away (Llewellyn et al. 2007).

²⁵ A 300 m² trial site (10 m x 30 m) surrounded by a 20 m pollen trap would have 3200 m² of pollen trap. A 2.5 m wide path through the pollen trap (2.5 m x 20 m) is 50 m², which represents a 1.5% loss.

179. However, gene flow in cotton has been observed over short distances of bare ground to a cotton field. For instance, Green and Jones (Green & Jones 1953) demonstrated in Oklahoma, USA that out-crossing frequencies over bare ground decreased from 6.0% at 5.0 m, to 4.7% at 10.0 m, and 0.6% at 25.1 m.

180. In comparison, an Australian study has demonstrated out-crossing frequencies of 0.3% (at Kununarra, WA) and 1.02% (at Emerald, QLD) over 50 m of bare ground. In the Emerald study, the out-crossing level dropped to 0.19% at 5 m into the cotton field, suggesting that pollinators did not carry viable pollen far into the field to effect pollination but remained at the edges (Llewellyn et al. 2007).

181. Field trial releases of GM cotton in EU countries such as Spain require a 4 row cotton pollen trap as well as an isolation distance of 200 m from other cotton plants (Directorate General for the Environment & European Commission 2007).

182. There are also isolation distances required when producing certified cotton seed. An isolation distance of at least 600 m is required when producing basic seed of *Gossypium hirsutum* (OECD 2008). In the US, certified seed cotton growers must maintain an isolation of 202 m between cotton fields (AGBIOS 2007).

183. On the basis of the scientific literature on gene flow, international containment measures for GM cotton trials, and the rules for producing basic and certified seed, a 3 km isolation distance from intentionally planted cotton crops is considered adequate to minimise gene flow from the GM cottons plants to other cotton crops (Event 4) and is therefore proposed as a licence condition if a pollen trap is not planted.

184. However, it is possible that feral cotton populations derived from commercial cotton lines may occur on roadsides or in irrigation channels in cotton growing areas. As detailed above, gene flow has been observed between isolated cotton plants over short distances. Thus, the Acting Regulator has imposed a licence condition requiring a 100 m monitoring zone surrounding the GM cotton which must be free of any flowering cotton plants. Implementation of the above measures will minimise gene transfer to sexually compatible plants (Event 4).

185. The application of insecticides (as proposed by the applicant) may reduce bee numbers but the combination of a 100 m monitoring zone and isolating the GM cotton sites 3 km from cotton crops is considered adequate to minimise gene flow without requiring an insecticidal spray program.

186. Outcrossing to native *Gossypium* species has only been demonstrated under conditions involving human intervention and even then the offspring have been sterile. Thus, the likelihood for fertile hybrids occurring, surviving to reproductive maturity and back-crossing to the parental native species is effectively zero (OGTR 2008)(Event 4).

187. The applicant proposed that the pollen trap plants (if any) be grown in such a way as to ensure flowering at the same time and for the same period of time as the GM cotton by planting a mixture of early and late flowering cotton varieties to increase the flowering window of the pollen trap. In addition, if there is evidence of asynchronous flowering, the applicant proposes to implement a specific contingency plan which includes the use of insecticides to limit pollen flow by insect vectors. As indicated in Event 4, some of the introduced genes for WUE may affect flowering time of the GM cotton, thus the measures ensuring synchronicity of flowering are important to minimise the potential for vertical transfer of genetic material from the GMO (Event 4) and dispersal of the GMO in the environment (Events 2 and 3).

188. The applicant has proposed that for some sites (including some sites utilised under DIR 064/200) the GM cotton would be replanted at the same location in successive seasons. A potential outcome resulting from replanting the same location in successive years is that successive year's pollen traps may contain volunteer GM cotton plants, which may provide opportunity for vertical gene flow (see Event 4). However, the applicant has also proposed irrigation and cultivation of the site and pollen trap areas in the first spring or summer following harvest to promote cotton seed bank reduction and minimise the persistence of the GM cotton lines at the proposed release site (Event 2) including the pollen trap. A standard condition of the cotton licences is the inspection of the site for volunteer cotton plants during the period between harvest and replanting and while the replanted GM cotton is growing. Any volunteer cotton found will be destroyed prior to flowering. Inspections of the site after replanting could involve looking for volunteers which emerge between the rows of the pollen trap. The above measures would reduce the number of GM plants in the pollen trap and therefore reduce vertical gene transfer (Event 4).

189. The glasshouses would be located at least 20 km from the nearest commercial cotton crop. An insect control program within the glasshouses and field trials, as well as the physical containment of the glasshouse walls will limit pollen dispersal by insects. The only cotton in close proximity to the glasshouses would be contained within PC2 research facilities which would further limit outcrossing (Event 4) or for the GMO to come into contact with the public (Event 1). Both glasshouses are surrounded by fences (2.5 or 2.7m high) and have lockable gates and access to the glasshouses would be restricted to authorised and trained persons, which would further limit contact with the public (Event 1).

190. All sites would be maintained by personnel who will receive appropriate training in practices relevant to the handling and disposal of GMOs. These measures minimise the potential for vertical transfer of genes or genetic material from the GMO (Event 4), dispersal of the GMO in the environment (Events 2 and 3) or for the GMO to come into contact with the public (Event 1).

191. The GM cotton will be harvested and ginned separately from other cotton crops to prevent mixing and none of the seed or GM plant material will be used in human food, or animal feed or for the production of fabrics and/or other cotton products. These measures will limit the potential exposure of humans and vertebrates to the GMOs (Event 1) and the potential for the GM cotton lines to be dispersed outside the proposed release site (Event 3).

192. The proposed field sites are on level land and at least 50 m from a natural waterway. The applicant has proposed irrigation practices (*Cotton Industry Good Management Practice*) used by cotton growers in Australia to 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 (DIR 059/2005). These practices would reduce the likelihood of plant material being inadvertently moved by landslip or erosion or being washed away from the site (Event 3). Limiting the duration of the release to a two year period would also restrict the opportunity for GM plants to establish outside the site (Event 3).

193. After the GM cotton has been harvested, the applicant proposed to destroy all remaining plant materials not required for further testing. GM plant materials required for further study or future release (eg seed) will be stored in certified PC2 facilities. The site will then be monitored for 12 months and until volunteers have not been observed at the site for at least six months. All volunteers will be destroyed prior to flowering. As discussed in the *The Biology of Gossypium hirsutum and Gossypium barbadense (cotton)* (OGTR 2008), cotton seeds have low dormancy levels and do not generally form a viable seed bank. However, dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture. The

applicant has also proposed irrigation and cultivation of the site and pollen trap areas in the first spring or summer following harvest to promote cotton seed bank reduction and minimise the persistence of the GM cotton lines at the proposed release site (Event 2).

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

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

195. A number of licence conditions have been imposed by the Acting Regulator to limit and control the proposed release, including requirements to:

- conduct the release on up to 20 sites of no more than 2 ha each on a total maximum area of 40 ha per year for two years between September 2008 and June 2010;
- locate the proposed trial sites at least 50 metres (m) away from natural waterways;
- limit pollen flow using one of the following measures:
 - surround the trial site with a 100 m monitoring zone and maintain a 3 km isolation distance between the site and intentionally planted cotton crops, **or**
 - surround the trial site by a 20 m pollen trap of non-GM (conventional) cotton or GM cotton that the Regulator has approved for commercial release.
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering;
- ensure the pollen trap plants are grown in such a way as to ensure flowering at the same time and for the same period of time as the GM cotton;
- locate the glasshouses at least 20 km from the nearest cotton crop;
- implement an insect control program within the glasshouses;
- harvest and gin all cotton plant materials (GM and non-GM) separately from other commercial cotton crops;
- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for future research or release;
- store GM plant materials (required for further study or future release) in certified physical containment level 2 (PC2) facilities;
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil;
- monitor trial sites after harvest for a minimum of 12 months and destroy any cotton volunteers that may grow until no volunteers are detected for a continuous 6 month period;
- restrict personnel with access to the site to authorised personnel only; and

- not permit the use of GM plant material, including cotton seed, cotton seed oil and meal for human food or animal feed, or cotton lint for the production of fabrics and/or other cotton products.

4.1.3 Measures to control other activities associated with the trial

196. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been proposed 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.

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

4.2 Other risk management considerations

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

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

4.2.1 Applicant suitability

199. 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.

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

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

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

4.2.2 Compliance and contingency plans

203. Prior to planting the GM cotton lines, Monsanto is required to submit a plan detailing how it intends to ensure compliance with the licence conditions and document that

compliance. This plan would be required before the planting of the GM cotton lines could occur.

204. Monsanto is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM cotton lines outside of the permitted areas. Additionally, this plan would detail measures to be undertaken to ensure synchronicity of flowering between the GM cotton and pollen trap plants including details of procedures to prevent dissemination of pollen from GM cotton from the site.

205. 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 detection method is required within 30 days of the issue date of the licence.

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

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

4.2.4 Reporting structures

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

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

208. 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.

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

- ♦ expected and actual dates of planting
- ♦ expected and actual dates of harvest
- ♦ expected and actual dates of destruction and cleaning after final harvest.

4.2.5 Monitoring for Compliance

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

211. 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.

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

Section 5 Issues to be addressed for future releases

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

- ♦ characterisation of the genetic material inserted into the plants, including copy number and genotypic stability;
- ♦ additional data on the potential toxicity of plant materials from the GM cotton lines;
- ♦ additional data on the allergenicity of proteins encoded by the introduced genes for water use efficiency; and
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; altered germination; altered flowering time; tolerance to environmental stresses; and disease susceptibility.

Section 6 Conclusions of the RARMP

214. The risk assessment concludes that this limited and controlled release of the GM cotton lines on up to 20 sites, located in various LGAs in NSW, QLD and WA, totalling no more than 40 ha per year over a two year period between 2008 and 2010 poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

References

- AGBIOS (2007). ACS-GHØØ1-3 (LLCotton25).
<http://www.agbios.com/dbase.php?action=Submit&evidx=234>.
- Arts, J., Mommers, C., de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical review in Toxicology* **36**: 219-251
- Axelos, M., Bardet, C., Liboz, T., Le Van, T.A., Curie, C., Lescure, B. (1989). The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1 alpha: molecular cloning, characterization and expression. *Molecular and General Genetics* **219**: 106-112
- Barry, G., Kishore, G., Padgett, S., Taylor, M.L., Kolacz, K., Weldon, M., Re, D., Eichholtz, D., Fincher, K., Hallas, L.E. (1992). Inhibitors of amino acid biosynthesis: Strategies for imparting glyphosate tolerance to crop plants. In: BK Singh, HE Flores, JC Shannon, eds. *Biosynthesis and molecular regulation of amino acids in plants*, Volume 7. American Society of Plant Physiologists Rockville, USA. pp 139-145.
- Batista, R., Nunes, B., Carmo, M., Cardoso, C., Sao Jose, H., Bugalho de Almeida, A., Manique, A., Bento, L., Pinto Ricardo, C., Oliveira, M.M. (2005). Lack of detectable allergenicity of transgenic maize and soya samples. *Journal of Allergy and Clinical Immunology* **116**: 403-410
- Beekman, M., Ratnieks, F.L.W. (2000). Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology* **14**: 490-496
- Beekman, M., Sumpter, D.J.T., Seraphides, N., Ratnieks, F.L.W. (2004). Comparing foraging behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers. *Functional Ecology* **18**: 829-835
- Blattner, F.R., Plunkett, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462
- Bradford, K.J., van Deynze, A., Gutterson, N., Parrot, W., Strauss, S.H. (2005). Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* **23**[4], 439-444
- Brubaker, C.L., Brown, A.H.D., Stewart, J.M., Kilby, M.J., Grace, J.P. (1999). Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* **108**: 199-213
- Callis, J., Fromm, M., Walbot, V. (1987). Introns increase gene expression in cultured maize cells. *Genes and Development* **1**: 1183-1200
- Datta, N., LaFayette, P.R., Kroner, P.A., Nagao, R.T., Key, J.L. (1993). Isolation and characterization of three families of auxin down-regulated cDNA clones. *Plant Molecular Biology* **21**: 859-869

- De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215
- Directorate General for the Environment, European Commission (2007). Various field trials for GM *G. hirsutum* in Spain. http://gmoinfo.jrc.it/gmp_browse.aspx.
- Eastick, R., Hearnden, M. (2006). Potential for Weediness of *Bt* Cotton (*Gossypium hirsutum*) in Northern Australia. *Weed Science* **54**: 1142-1151
- EFSA (2007). Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *nptII* antibiotic resistance marker gene in genetically modified plants. Adopted on 22-23 March 2007. European Food Safety Authority, available online at [http://www.efsa.europa.eu/EFSA/Statement/gmo_statement_nptII .pdf](http://www.efsa.europa.eu/EFSA/Statement/gmo_statement_nptII.pdf)
- Farrell, T. and Roberts, G. (2002). Survey of cotton volunteers north of latitude 22° south. Australian Cotton CRC and CSIRO Plant Industry Narrabri.
- Felsot, A.S. (2000a). Herbicide tolerant genes. Part 1: squaring up Roundup Ready crops. *Agrichemical & Environmental News* **173**: 9-15
- Felsot, A.S. (2000b). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7
- Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554
- Glover, J. (2002). Gene flow study: Implications for the release of genetically modified crops in Australia. Bureau of Rural Sciences, Australian Government Department of Agriculture, Fisheries and Forestry Canberra.
- Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R. (2008). Allergenicity assessment of genetically modified crops - what makes sense? *Nature Biotechnology* **26**: 73-81
- Green, J.M., Jones, M.D. (1953). Isolation of cotton for seed increase. *Agronomy Journal* **45**: 366-368
- Halloin, J.M. (1975). Solute loss from deteriorated cotton seed: relationships between deterioration, seed moisture and solute loss. *Crop Science* **15**: 11-15
- Haslberger, A.G. (2003). Codex guidelines for GM foods include the analysis of unintended effects. *Nature Biotechnology* **21**: 739-741
- Iida, K., Seki, M., Sakurai, T., Satou, M., Akiyama, K., Toyoda, T., Konagaya, A., Shinozaki, K. (2005). RARTF: Database and tools for complete sets of Arabidopsis transcription factors. *DNA Research* **12**: 247-256
- Kahl, G. (2001). *The dictionary of gene technology: genomics, transcriptomics, proteomics*. Wiley-VCH Weinheim, Germany. pp 1-941.

- Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. *Environ Biosafety Res* Preprint: -Available online at: <http://www.ebr-journal.org/>
- Keith, B., Chua, N.-H. (1986). Monocot and dicot pre-mRNAs are processed with different efficiencies in transgenic tobacco. *EMBO J* **5**: 2419-2425
- Kurland, C.G., Canback, B., Berg, O.G. (2003). Horizontal gene transfer: a critical view. *Proceedings of the National Academy of Science of the United States of America* **100**: 9658-9662
- Lewin, B. (1994). Building the transcription complex: promoters, factors, and the RNA polymerases. Chapter 29. In: *Genes V*. Oxford University Press New York. pp 847-877.
- Llewellyn, D.J., Tyson, C., Constable, G.A., Duggan, B., Beale, S., Steel, P. (2007). Containment of regulated genetically modified cotton in the field. *Agriculture, Ecosystems & Environment* **121**: 419-429
- McGregor, S.E. (1976). *Insect pollination of cultivated crop plants*. Agricultural Research Service, United States Department of Agriculture Washington, D.C. pp 1-approx. 400 (see note above).
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10
- Mignone, F., Grillo, G., Licciulli, F., Iacono, M., Liuni, S., Kersey, P.J., Duarte, J., Saccone, C., Pesole, G. (2005). UTRdb and UTRsite: a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. *Nucleic Acids Research* **33**: D141-D146
- Miki, B., McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* **107**: 193-232
- Moffett, J.O. (1983). Pollination of entomophilous hybrid seed parents - hybrid cotton. Chapter 8. In: CE Jones, RJ Little, eds. *Handbook of experimental pollination biology*. Van Nostrand Reinhold New York. pp 508-514.
- Mungomery, V.E., Glassop, A.J. (1969). Natural cross-pollination of cotton in central Queensland. *Queensland Journal of Agricultural and Animal Sciences* **26**: 69-74
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *Acta pathologica, microbiologica, et immunologica Scandinavica* **106**: 77-84
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242
- Odell, J.T., Nagy, F., Chua, N.H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* **313**: 810-812
- OECD (2008). OECD Seed Schemes 2008. Organisation for Economic Co-operation and Development

- OGTR (2007). Risk Analysis Framework. Report No. Version 2.2, Document produced by the Australian Government Office of the Gene Technology Regulator, available online from <http://www.ogtr.gov.au/>
- OGTR (2008). The Biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton). Document prepared by the Office of the Gene Technology Regulator, Canberra, Australia, available online at <http://www.ogtr.gov.au/>
- Oh, S.J., Song, S.I., Kim, Y.S., Jang, H.J., Kim, S.Y., Kim, M., Kim, Y.K., Nahm, B.H., Kim, J.K. (2005). Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiology* **138**: 341-351
- Oosterhuis, D.M., Jernstedt, J. (1999). Morphology and anatomy of the cotton plant. Chapter 2.1. In: CW Smith, JT Cothren, eds. *Cotton: Origin, History, Technology and Production*. John Wiley & Sons New York. pp 175-206.
- Osborne, J. L., Williams, I. H., Marshall, A. H., Michaelson-Yeates, T. P. T. (2001). Pollination and gene flow in white clover, growing in a patchy habitat. Benedek, P. and Richards, K. W. eds, ISHS, International Society for Horticultural Science Leven, Belgium. pp. 35-40.
- Padgett, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996). New weed control opportunities: development of soybeans with a Roundup Ready gene. Chapter 4. In: SO Duke, ed. *Herbicide-resistant crops: agricultural, environmental, economic, regulatory and technical aspects*. CRC Press Boca Raton. pp 53-84.
- Richins, R.D., Scholthof, H.B., Shepherd, R.J. (1987). Sequence of figwort mosaic virus DNA (caulimovirus group). *Nucleic Acids Research* **15**: 8451-8466
- Rieger, M.A., Lamond, M., Preston, C., Powles, S.B., Roush, R. (2002). Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* **296**: 2386-2388
- Seeley, T.D. (1987). The effectiveness of information collection about food sources by honey bee colonies. *Animal Behaviour* **35**: 1572-1574
- Sheelavantar, M.N., Prabhakar, A.S., Patil, S.V. (1975). Propagation of hybrid cotton through cuttings. *Indian Journal of Agricultural Sciences* **45**: 91-92
- Steffan-Dewenter, I., Kuhn, A. (2003). Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society of London: Biological Sciences* **270**: 569-575, available online at <http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1691282&blobtype=pdf>
- Thomson, J.A. (2000). Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science* **66**: 188-193
- Thomson, N.J. (1966). Cotton variety trials in the Ord valley, North Western Australia: 4. Natural crossing of cotton. *Empire Cotton Growing Review* **43**: 18-21

Vain, P., Finer, K.R., Engler, D.E., Pratt, R.C., Finer, J.J. (1996). Intron-mediated enhancement of gene expression in maize (*Zea mays* L.) and bluegrass (*Poa pratensis*). *Plant Cell Reports* **15**: 489-494

Visscher, P.K., Seeley, T.D. (1982). Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest. *Ecology* **63**: 1790-1801

Waddington, K.D., Visscher, P.K., Herbert, T.J., Richter, M.R. (1994). Comparisons of forager distributions from matched honey bee colonies in suburban environments. *Behavioral Ecology and Sociobiology* **35**: 423-429

Waines, J.G., Hedge, S.G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science* **43**: 451-463

Woodstock, L.W., Furman, K., Leffler, H.R. (1985). Relationship between weathering deterioration and germination, respiratory metabolism and mineral leaching from cotton seeds. *Crop Science* **25**: 459-466

Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

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

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

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

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

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

Risk

the chance of something happening that will have an undesired impact

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

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

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

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

Risk analysis

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

Risk analysis framework

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

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

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

Risk Context

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

Risk estimate

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

Risk evaluation

the process of determining risks that require treatment

Risk management

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

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

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

States

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

Uncertainty

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

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities²⁶ on the consultation RARMP for DIR 081/2007

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

| Summary of issues raised | Comment |
|--|---|
| The OGTR should require Monsanto to undertake further screening of these GM cotton lines in a controlled environment, to substantially reduce the number of GM cotton lines proposed for field trial release. | The proposed dealings for application DIR081 were for a limited and controlled release into the environment. The field trials will assess a number of agronomic traits including yield and water use efficiency and this will reduce the number of lines in future releases. The limits and controls proposed by the applicant as well as licence conditions imposed by the Acting Regulator were discussed in the RARMP and found to be adequate to limit the proposed release to the size, locations and duration proposed by the applicant (see Chapter 3, Section 4.1.1) |
| The GM cotton lines do not have GM insect resistance traits. The voracious cotton-eating insect population in the Wyndham-East Kimberley area means the applicant would be unlikely to harvest any cotton from plants with no insect resistant traits. | The applicant could use insecticides and has indicated that fertilizers, insecticides and other <i>Cotton Industry Good Management Practices</i> would be applied, similar to those used for commercially grown non-GM cotton (see Chapter 1, Section 6.3 of the RARMP). |
| Expand the licence conditions for the GM cotton lines to include requirements to obtain and report the additional information outlined by the OGTR in the RARMP (Chapter 3, Section 5) as part of the field trials. | The RARMP did not identify risks that required specific risk treatment measures and therefore the research suggested was not considered necessary for the management of risks to human health and safety or the environment posed by the proposed limited and controlled release. The proposed licence conditions include a requirement for the applicant to report any additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence. Issues to be addressed by the applicant for future releases have been identified in the RARMP, including further characterisation of the introduced genetic material in the plants (see Chapter 3, Section 5 and licence conditions) |
| The large number and variety of GM cotton lines proposed for release coupled with the limited information provided due to CCI restrictions prevents any detailed analysis. Lack of knowledge about the characterisation of the | CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application. Uncertainty regarding characterisation of the genes or gene products was acknowledged in the RARMP and taken into account when assessing possible risks that may |

²⁶ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment, Heritage & the Arts and the Local council(s) where the release may occur.

| Summary of issues raised | Comment |
|---|---|
| introduced genes and associated encoded proteins, even in a limited and controlled release is of concern. | be associated with the proposed trial (see Chapter 2, Section 4). Risks to human health and the environment resulting from the proposed limited and controlled were considered to be negligible. |

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 081/2007

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

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

Issues raised: A: administration; Ap: application; C: containment measures; DR: data requirements; EN: Environmental risks, HGT: Horizontal Gene Transfer; M: marketing; SA: sustainable agriculture; UE: Unintended effects

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

Type: A: Agricultural/industry organisation; IG: interest group; I: individual

| Sub. No: | Type | Position | Issue | Summary of issues raised | Comment |
|----------|------|----------|-------|--|---|
| 1 | I | x | none | Objects to the release of GM cotton into the Australian environment and believes that segregation is not possible. | Noted. Marketing issues are outside the scope of assessments conducted under the Act. |
| 2 | A | y | none | <p>Welcomes the research and development programme for an enhanced WUE trait in Australia as the cotton industry progresses its goal of greatly improving water use on a number of fronts, including cultivar improvement and crop management. This trait, in addition to the current commercial traits, will result in decreased inputs of water and pesticides and a significantly improved industry environmental outcome.</p> <p>The technology providers have a good record of stewardship of the introduced GM cotton traits and the limited and controlled conditions proposed under DIR081/2007 are consistent with our expectation of the process for development of the WUE trait.</p> | Noted. |

| Sub. No: | Type | Position | Issue | Summary of issues raised | Comment | | |
|----------|------|----------|---|--|--|--|--|
| 3 | I | x | none | Opposes the granting of a licence for DIR 081/2007. | Noted. | | |
| | | | Administrative concerns | | | | |
| | | | A, CCI | <p>Concerned that the application and submissions opposing the granting of the licence will not be properly considered by competent staff.</p> <p>Considers that the application will be 'rubber stamped' by the Regulatory Authority.</p> <p>Considers that CCI permits avoidance of scrutiny</p> | <p>Noted. The Regulator is required to invite submissions from the general public on the RARMP (Section 52 of the Act) and similarly, must have regard to any submissions received under section 52, prior to issuing a licence (see Section 56 of the Act).</p> <p>Each application is considered on a case by case basis. The Gene Technology Regulator makes a decision whether to issue a licence after having regard to matters outlined the Gene Technology Act (2000), including a thorough assessment of the risks to human health and safety and the environment.</p> <p>Submissions from the public were considered and are addressed in Appendix C of the finalised RARMP.</p> <p>Under section 185 of the Act, the Regulator has declared certain information in application DIR081/2007 as CCI. In doing so the Regulator was satisfied that the public interest in disclosure did not outweigh the prejudice that the disclosure would cause the applicant (Section 185(2)).</p> <p>CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.</p> | | |
| | | | Concern regarding the application. | | | | |
| | | | Ap | Application DIR 081/2007 is below acceptable standards. | Noted. | | |
| | | | Ap | Concerned that untried and untested GMOs that were rejected or not trialled overseas are being trialled in Australia. | <p>Sections 13.2 and 13.3 of the application require the applicant to disclose previous applications (both successful and unsuccessful) for these GM cotton lines in Australia and overseas. The applicant has indicated details of permits for a number of trials involving water use efficiency traits in the USA (Section 13.2)(see also Chapter 1, Section 7.2 of RARMP).</p> <p>GM cotton and corn with enhanced tolerance to drought, cold, salt and environmental stress have been tested in many states of the USA (http://www.isb.vt.edu/CFDOCS/fieldtests3.cfm)</p> | | |

| Sub. No: | Type | Position | Issue | Summary of issues raised | Comment |
|----------|------|----------|--|--|--|
| | | | Ap | Concerned the response to questions about gene sources are unacceptable and will allow approval of uncharacterised or non-existent GMOs containing genes from poorly identified sources or perhaps any plant, bacteria, fungi or yeast source. | The source of the genes and regulatory elements is given in Chapter 1, Section 5 of the RARMP. However, some details including the names of the introduced genes, their encoded proteins and functions have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The licence only allows dealings with GM cotton lines containing specified genes and constructed using specified gene constructs as detailed in Appendix B of the licence. |
| | | | Ap | Considers that information provided on homology of the introduced genes to genes with known toxicity or allergenicity; or to exogenous cotton genes is misleading. | Noted. At the request of the Regulator, Monsanto has supplied the results of the database searches and the analysis indicated that the encoded proteins did not share any significant sequence homology with any known allergens. There was no literature to suggest that the proteins encoded by the introduced genes were toxic or allergenic (Chapter 1, Section 5.2.1) The introduced genes are derived from plants, bacteria and yeast. In the RARMP for DIR081, it was considered that the introduced genes are highly homologous and most likely orthologous, to genes in most, if not all, crop and other plants and this would include cotton (Chapter 1, Section 6.5; Chapter 2, Event 1) |
| | | | Ap | Considers that lack of characterisation of the GM cotton lines is not appropriate | Noted. The current licence is for a limited and controlled release and licence conditions have been imposed to limit the release to the size, locations and duration specified in the licence. Information suggested was not required for the assessment of this application. This is a 'proof of concept' trial and issues to be addressed by the applicant for future releases have been identified in the RARMP, including further characterisation of the introduced genetic material in the plants (Chapter 3, Section 5) |
| | | | Concerns about Horizontal Gene Transfer (HGT) | | |
| | | | HGT | Considers that the issues raised on HGT in earlier submissions to DIR 077/2007, DIR 080/2007 and DIR 083/2007 apply equally to this application and should be considered prior to a decision being made on this application. | Previous submissions were considered in other RARMPs, see Appendix C in the RARMPs for DIRs 077/2007, 80/2007, and 83/2007. The risk of harm occurring as a result of HGT from the GM cotton to microorganisms in the soil has also been considered in the RARMP for DIR081/2007. In the context of this limited and controlled release, the potential for an adverse outcome as a result of horizontal gene transfer was not considered an identified risk. |

| Sub. No: | Type | Position | Issue | Summary of issues raised | Comment |
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| | | | HGT | <p>Considers that HGT is highly likely because:</p> <ul style="list-style-type: none"> Some of the inserted DNA has homology to DNA of microorganisms, particularly viruses. Microorganisms, particularly prokaryotes, unicellular eukaryotes and fungi will be in close proximity to the GMOs and thus available for HGT. The introduced genes are less stable than endogenous cotton genes, therefore more susceptible to HGT The dry conditions of the trial site will create selection pressure enhancing HGT. <p>Considers that if HGT occurs there will be adverse outcomes because:</p> <ul style="list-style-type: none"> The introduced genes are associated with pathogenicity or ecologically opportunistic genes. HGT will make the microorganisms more virulent and pathogenic and have broader niches in the environment. | <p>The risk arising from such transfer from GMOs to microorganisms (including fungi) was assessed using the available evidence and was considered to be negligible (Event 5). Reasons include:</p> <ul style="list-style-type: none"> Transfer of plant DNA to microorganisms is extremely rare; although examples have been identified the genome sequencing of bacteria shows a lack of plant genes There is no evidence that the introduced genes are unstable or more likely to transfer to other organisms. However, further evidence would be required if a larger scale release application was made. There are a large number of events that must successfully occur before an adverse outcome could arise as a result of HGT, should it occur. Many of these events were considered unlikely. If HGT did occur, the gene would have to be expressed and function in the recipient organism. However, plant gene promoters and introns, for example, may prevent expression of the transferred gene in the recipient organism The introduced genes are from common plants, a common moss, common bacteria or a very common yeast. Therefore, these genes have been in the environment and available for transfer for a significant amount of time. Nonetheless, microorganisms have preferentially evolved other mechanisms to deal with drought and other adverse conditions. For example fungi develop spores which allow them to survive dry conditions. The small size and short duration of the proposed trial greatly reduce the chance of any adverse effect as a result of HGT (if HGT did occur). |
| | | | HGT, C | <p>Concerned that there are no management measures imposed by the licence to control HGT if it occurred.</p> | <p>Noted. The risk of harm occurring as a result of HGT from the GM cotton to microorganisms in the soil has been considered in the RARMP (Chapter 2, Event 5). In the context of this limited and controlled release, the potential for an adverse outcome as a result of horizontal gene transfer was not considered an identified risk, and as such there was no present need to impose management measures for their mitigation.</p> |
| | | | HGT | <p>Concerned that the OGTR has not required Northern and Western blot evidence to exclude expression in micro organisms.</p> | <p>Noted. The risk of harm occurring as a result of HGT from the GM cotton to microorganisms in the soil has been considered in the RARMP (Chapter 2, Event 5). In the context of this limited and controlled release, the potential for an adverse outcome as a result of horizontal gene transfer was not considered an identified risk. The information suggested was not required for this release.</p> |

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| | | | HGT | The Gene Technology Regulator can only conclude "no identifiable risk" from HGT if the Keese (2008) review (from the OGTR) is disregarded. | Based on the weight of the evidence in the available scientific literature and given the context of this limited and controlled release, the potential for an adverse outcome as a result of horizontal gene transfer was not considered an identified risk (Chapter 2, Event 5). |
| 4 | I | x | C | We are not in favour of any licence being issued to Monsanto Australia for any GM trial in WA. It is nonsense to think that any plant trial can be 'controlled' so there is no contamination. | Noted. The risk assessment and risk management plan for DIR081/2007 concluded that the proposed release of GM cotton lines poses negligible risk to the health and safety of people and the environment. |
| | | | SA | Australia must face up to the fact that in conditions of changing climate, the growing of water-demanding crops such as cotton must cease. | Noted. Sustainability of any agricultural crop is outside the scope of assessments conducted under the Act. |
| | | | M | It is important that WA maintains its GM free trade advantage. | Noted. Marketing issues are outside the scope of assessments conducted under the Act. |