Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for **DIR 085/2008**

Limited and controlled release of cotton genetically modified for altered fatty acid composition of the cottonseed oil

Applicant: CSIRO

October 2008

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Executive Summary

Introduction

The Acting Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of one cotton line modified for altered fatty acid composition of the cottonseed oil into the environment in respect of application DIR 085/2008 from the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

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 Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public[1].

The application

CSIRO applied for a licence for dealings involving the intentional release of one GM cotton line on a limited scale and under controlled conditions. The GM cotton line has been modified for altered fatty acid composition of the cottonseed oil. The intent in changing the fatty acid profile of the cottonseed oil, in the GM cotton line, is to increase the stability of the oil for food industry applications and improve the health effects of cottonseed oil. The trial is proposed to take place at one site in the local government area of Narrabri, New South Wales on a maximum total area of 2 hectares between 2008 and 2009.

The GM cotton line contains a genetic construct comprising the partial sequences of three cotton genes involved in fatty acid synthesis. The genetic construct is intended to suppress the expression of the corresponding genes in the GM cotton line. The GM cotton line also contains an antibiotic resistance gene (*nptII*) which provides resistance to kanamycin.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM cotton line to assess a range of agronomic characteristics of the GM cotton line, when grown under natural field conditions, including seed germination rate, fibre yield and quality, seed yield, oil content and fatty acid composition. The GM cotton will not be used for human food or animal feed.

CSIRO proposed a number of controls to restrict the dissemination or persistence of the GM cotton line and the introduced genetic materials in the environment that have been considered during the evaluation of the application.

Risk assessment

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

[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 DIR licence application assessment process and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at Risk analysis framework.

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. Consideration was then given as to 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 line into the environment are estimated 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 limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require CSIRO to **limit** the release to a total area of up to 2 hectares at one site between October 2008 and June 2009. The **control** measures include containment provisions at the trial site, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concludes that this proposed limited and controlled release of one GM cotton line on a maximum total area of 2 hectares over one year in the New South Wales local government area of Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

Table of Contents

Abbreviations

the Act	*Gene Technology Act 2000*
ACRI	Australian Cotton Research Institute
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CaMV	Cauliflower mosaic virus
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv	cultivar
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare(s)
km	kilometre
mRNA	Messenger Ribonucleic Acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nptII	neomycin phosphotransferase type II gene
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
PTGS	post-transcriptional gene silencing
RARMP	Risk Assessment and Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
RNAi	RNA interference
TGA	Therapeutic Goods Administration

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Technical Summary

Introduction

The Acting Gene Technology Regulator (the Regulator) has made a decision to issue a licence (DIR 085/2008) to Commonwealth Scientific and Industrial Research Organisation (CSIRO) for a limited and controlled release of genetically modified (GM) cotton line 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 Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public[2].

The application

CSIRO applied for a licence for dealings involving the intentional release of one line[3] of cotton (*Gossypium hirsutum* cv. Coker 315) that has been genetically modified for altered fatty acid composition of the cottonseed oil. The intent in changing the fatty acid profile of the cottonseed oil, in the GM cotton line, is to increase the stability of the oil for food industry applications and improve the health effects of cottonseed oil. The trial is authorised to take place at one site in the local government area of Narrabri, New South Wales (NSW) on a maximum total area of 2 hectares between 2008 and 2009.

The GM cotton line contains a single copy of the introduced genetic construct comprising the partial sequence of three cotton genes: *palmitoyl-ACP thioesterase* (*ghFatB-1*), *microsomal Δ12-desaturase* (*ghFAD2-1*) and *cyclopropane fatty acid synthase* (*ghCPA-FAS-2*). The introduced sequences were originally isolated from *G. hirsutum* and are intended to suppress the expression of the corresponding genes in the GM cotton line.

In addition, the GM cotton line contains 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 plants during initial development of GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM cotton line to assess a range of agronomic characteristics of the GM cotton line, when grown under natural field conditions, including seed germination rate, fibre yield and quality, seed yield, oil content and fatty acid composition. The GM cotton will not be used for human food or animal feed.

[2] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at DIR licence application assessment process), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at Risk analysis framework.

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

CSIRO proposed a number of controls to restrict the dissemination or persistence of the GM cotton line and its genetic material into the environment. These controls were considered during the evaluation of the application.

Risk assessment

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

A reference document on the parent organism, *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton),* was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR or from the OGTR Website.

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, location and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the dissemination or persistence of the GM cotton plants and their genetic material
- limited capacity of the GM cotton line to spread and persist outside the area proposed for release
- limited ability and opportunity for the GM cotton line 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 antibiotic resistance gene and the protein encoded by it in the environment and lack of known toxicity or evidence of harm from either the gene or the encoded protein
- widespread presence of the end products produced as a result of the activity of the introduced partial gene sequences 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 line into the environment are considered to be **negligible.** Hence, the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the 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 estimated as **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 limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

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

- conduct the release on a total area of up to 2 hectares for one year at one site in the NSW local government area of Narrabri, between October 2008 and June 2009
- surround the release site with a 20 m pollen trap
- locate the trial site at least 50 m away from natural waterways
- harvest and gin seed cotton from the release separately from any other cotton crop
- not permit any materials from the release to be used in human food or animal feed or for the production of fabrics and/or other cotton products
- destroy all plant materials not required for further analysis
- following harvest, clean the site, monitoring zone and equipment used on the site
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- monitor the site for at least 12 months and destroy any cotton plants that may grow until no volunteers are detected for a continuous 6 month period.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs; 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 GMO 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 that avoids duplication and enhances coordinated decision making. 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 Standards 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)[4].

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 line proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM cotton line. FSANZ approval would need to be obtained before it 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 this GM cotton line or to justify a reduction in containment conditions. This would include:

- additional data on the potential toxicity of plant materials from the GM cotton line
- characteristics indicative of weediness including measurement of altered reproductive capacity, germination rates, degree of seed dormancy, tolerance to environmental stresses, and disease susceptibility.

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that CSIRO is suitable to hold a DIR licence under the requirements of section 58 of the Act. The Acting Regulator is satisfied that CSIRO 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 RARMP

The risk assessment concludes that this limited and controlled release of one GM cotton line on a maximum total area of 2 hectares over one year in the New South Wales local government area of Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

[4] 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 Risk analysis framework.

Chapter 1 Risk assessment context

Section 1 Background

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

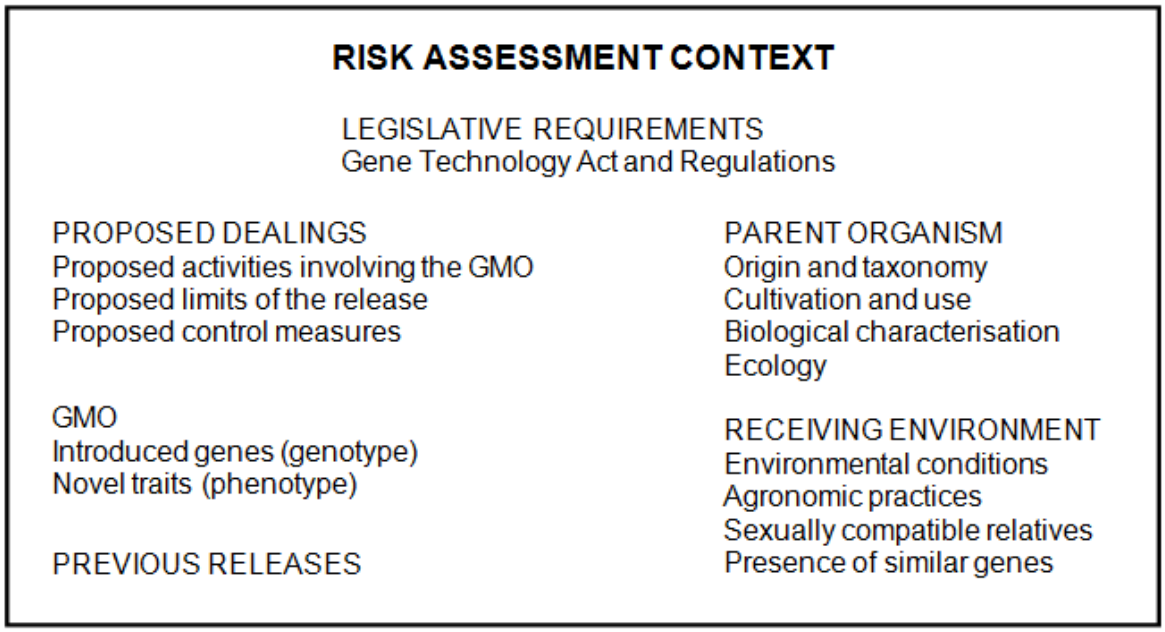

Figure 1 Components of the context considered during the preparation of the risk assessment

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

- the proposed dealings (Section 3.1)
- the limits proposed by the applicant (Section 3.2)
- the controls proposed by the applicant (Section 3.3)
- characteristics of the parent organism (Section 4)
- the nature and effect of the genetic modification (Section 5)
- the environmental conditions in the location where the release would occur (Sections 6.1 and 6.2)
- relevant agricultural practices (Section 6.3)
- the presence of related plants in the environment (Section 6.4)
- the presence of the introduced or similar genes in the environment (Section 6.5)
- any previous releases of this GMO or other GMOs relevant to this application (Section 7).

[5] The legislative requirements and the approach taken in assessing licence applications are outlined in more detail on the OGTR Website and in the Risk Analysis Framework (OGTR 2007).

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 Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions on licence applications. In addition, the *Gene Technology Regulations 2001* (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Acting Regulator has considered information provided in the application and is satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits on the size, location and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMO and 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. No submissions on the consultation RARMP were received from members of the public.

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. The Commonwealth Scientific and Industrial Research Organisation (CSIRO) proposes to release one cotton line[6] that has been genetically modified for altered fatty acid composition of the cottonseed oil into the environment under limited and controlled conditions.

3.1 The proposed activities

8. The applicant has stated that the principal purpose of the proposed release is to conduct experiments to assess a range of agronomic characteristics of the GM cotton line, when grown under natural field conditions, including seed germination rate, fibre yield and quality, seed yield, oil content and fatty acid composition. The trial will also produce cottonseed oil for evaluation of quality by industry. The GM cotton including cotton seed, cottonseed oil and meal will not be used for human food or animal feed.

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

9. The release is proposed to take place at one site in Narrabri, NSW, on a maximum area of 2 hectares. The release is proposed to occur between October 2008 and June 2009.

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

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

10. The trial will occur on a farm approximately 5 km from the Australian Cotton Research Institute (ACRI) near Narrabri, NSW[7]. The ACRI is 25 km from Narrabri (population 7300) and 15 km from Wee Waa (population 2300) and this area is in the centre of the cotton cropping system of the Namoi Valley. Only trained and authorised staff will be permitted access to the proposed location.

11. The applicant proposed a number of controls to restrict the dissemination or persistence of the GM cotton line and its genetic material into the environment including:

- locating the proposed trial site above the normal flood plain and at least 50 m away from natural waterways and other cotton breeding areas
- surrounding the trial sites by a 20 m pollen trap of non-GM cotton and treating all plants in this area in the same way as GM cotton plants
- managing the GM cotton in the same manner as non-GM cotton, including application of insecticides
- cleaning and inspecting all equipment prior to removal from the release site
- harvesting and ginning all cotton plant materials (GM and non-GM) separately from other commercial cotton crops
- cleaning the site and adjacent areas (eg pollen trap) after harvest by removing and/or destroying all cotton plant materials, except for materials required for future research or release
- monitoring the trial site after harvest for a minimum of 12 months and destroying any cotton volunteers
- transporting GM seed and plant materials in accordance with OGTR transportation guidelines
- storing GM plant materials (required for further study or future release) in certified PC2 facilities; and
- not using the GM plant material, including cotton seed, cottonseed oil and meal for human food or animal feed.

12. These controls, and the limits on size, location and duration outlined in Section 3.2, have been taken into account in establishing the risk assessment context, and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.1.1.

Section 4 The parent organism

13. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia but is grown as an agricultural crop in NSW and southern and central QLD. The cultivar Coker 315 is often used as a starting point for research as it can be easily genetically modified in the laboratory. It is not grown commercially in Australia. Further detailed information about the parent organism is contained in a reference document, *The Biology of* Gossypium hirsutum *and* Gossypium barbadense *(cotton)* (OGTR 2008b) that was produced

[7] In the original licence application CSIRO proposed planting the field trial at the ACRI site, this changed during the consultation period. Planting of the trial will take place at a site on a commercial farm approximately 5 km from the ACRI.

to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR or from the OGTR website.

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

5.1 Introduction to the GMO

14. One GM cotton line (known as MonoCott DCS9-34) is proposed for release and contains a single copy of the introduced genetic construct comprising of the partial sequence of three endogenous cotton genes: *palmitoyl-ACP thioesterase* (*ghFatB-1*), *microsomal Δ12-desaturase* (*ghFAD2-1*) and *cyclopropane fatty acid synthase* (*ghCPA-FAS-2*). These sequences were derived from *G. hirsutum* and are intended to suppress the expression of the corresponding endogenous genes (Figure 2; Table 1).

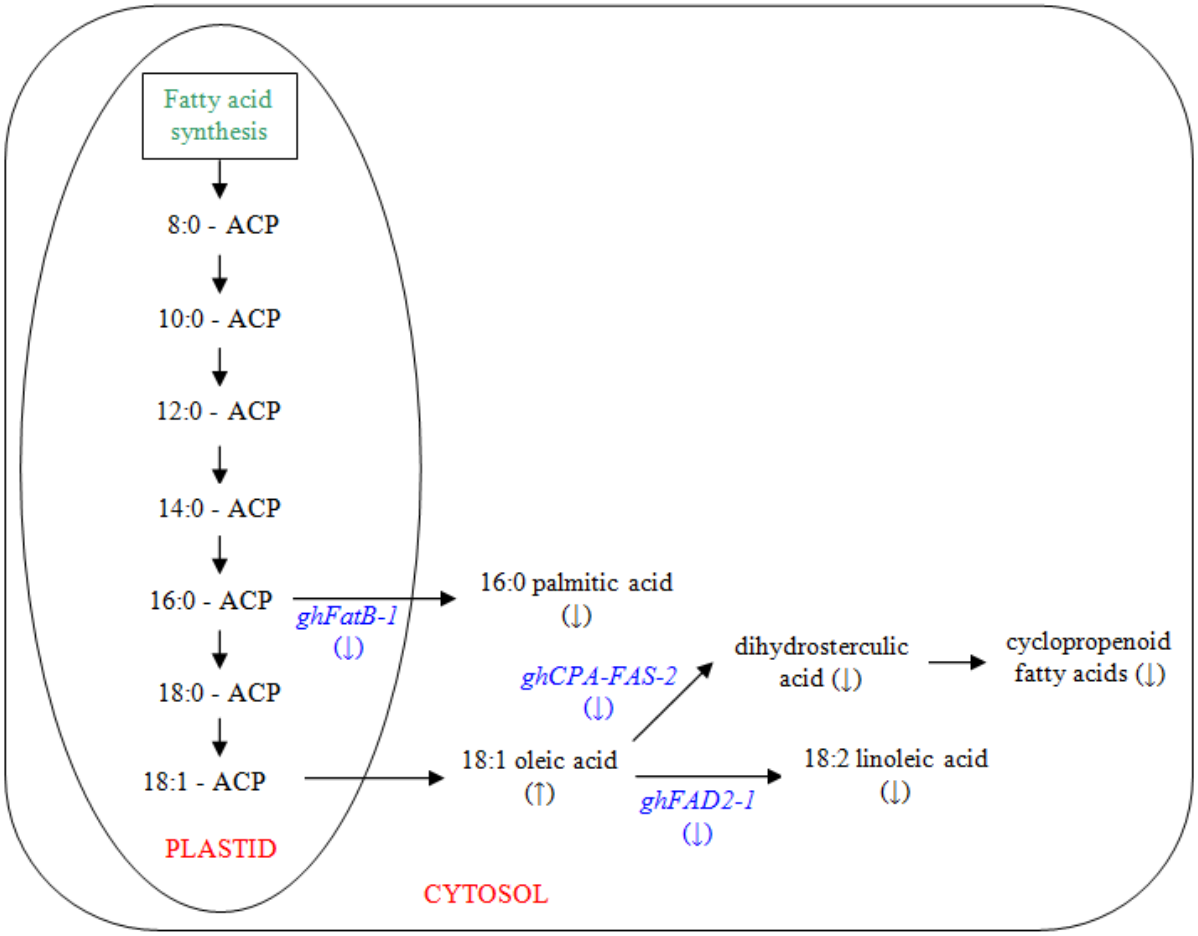

Figure 2 Fatty acid synthesis in the cotton seed

A simplified diagram of the fatty acid synthesis pathway in cotton seeds showing the genes of interest to this proposed release and the end effects of the modification on the fatty acid levels in the seeds. The first number in the set represents the number carbons in the chain and the second number indicates the number of carbon-carbon double bonds in the chain. Gene names i blue indicate the genes targeted in the GM cotton line (MonoCott DCS9-34); ACP: Acyl carrier protein; arrows in parentheses under either gene names or fatty acid names indicate changes either in levels of mRNA or fatty acid relative to the non-GM cotton parental line.

Table 1 The partial gene sequences used to genetically modify cotton

Gene	Accession No (GenBank)	Source	Partial sequence length (no. of nucleotides)	Protein function
ghFatB-1	Not published[8]	*Gossypium hirsutum*	386	Controls palmitic acid content
ghFAD2-1	X97016	*G. hirsutum*	351	Controls conversion of oleic acid to linoleic acid
ghCPA-FAS-2	AY574037	*G. hirsutum*	443	Biosynthesis of cyclic fatty acids

5.2 The introduced construct and associated end products

15. The partial gene sequences were joined in tandem as a single unit which was subsequently joined with its inverted repeat. The single unit and its inverted repeat are separated by an intron derived from the *ghFAD2-1* gene (see Figure 3).

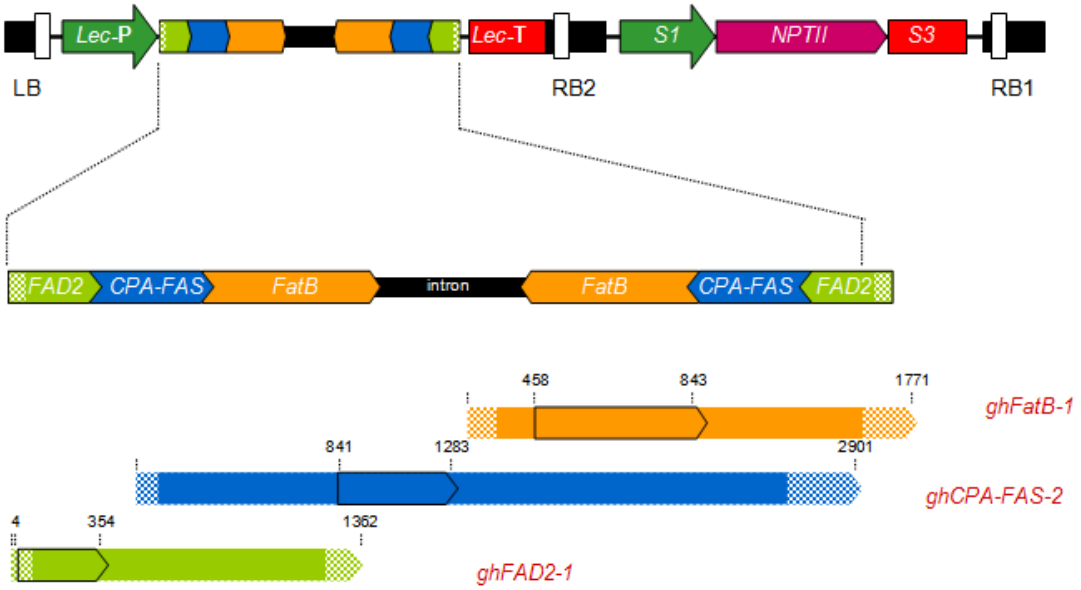

Figure 3. Diagram of the genetic construct used to create the GM cotton line.

LB: T-DNA left border; *LecP*: soybean lec1 promoter; *FAD2*: *ghFAD2-1* or *cotton microsomal Δ12 desaturase* cDNA; *CPA-FAS*:*ghCPA-FAS-2* or *cotton cyclopropane fatty acid synthase*; *FatB*: *ghFatB-1* or *cotton palmitoyl-ACP thioesterase*; *lec-T*: *soybean lectin terminator*; RB2: T-DNA right border 2; *S1*: Subterranean clover stunt virus segment 1 promoter; *S3*: Subterranean clover stunt virus segment 3 terminator; *nptII*: *neomycin phosphotransferase gene*. The sizes of the full length cDNA and the selected fragments of each target gene in making the RNAi construct are also indicated.
Reproduced from the licence application received from CSIRO on 27 March 2008.

16. The construct is designed such that transcription of the introduced partial gene sequences will generate messenger RNA (mRNA) from the tandem unit and its complimentary inverted repeat. The binding of the two mRNA regions produces double stranded RNA (dsRNA). This in turn triggers a conserved biological response to dsRNA, known as RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) and leads to the down-regulation of the targeted gene(s) (Hannon 2002). When the gene silencing system is triggered, the mRNA is degraded together with any RNA that has the same or closely similar sequence. Mechanistically, the main trigger for RNA based gene silencing is small dsRNA segments of 21-25 base pairs, which provide sequence specificity and target degradation by double-stranded ribonucleases (Ahlquist, 2002). Degradation of the mRNA therefore prevents

[8] The *ghFatB-1* gene does not have a GenBank Accession number at this stage. It does have about 80% sequence homology with the published *FatB* gene from cotton, accession number AF034266 (information supplied by applicant).

production of the encoded protein. The use of an intron between the inverted repeats has been shown to enhance RNAi silencing (Smith et al. 2002).

17. Thus, the introduction of the three partial gene sequences is expected to suppress expression of the three endogenous cotton genes involved in fatty acid biosynthesis (see Section 5.1, Table 1) and subsequently alter the fatty acid composition of the cottonseed oil. Analysis of the cottonseed oil from the GM cotton line grown in the glasshouse indicates that the fatty acid composition has been altered (see Section 5.2.4 below).

18. The GM cotton line also contains the *nptII* marker gene which encodes a neomycin phosphotransferase type II enzyme. The *nptII* gene was originally derived from the common gut bacterium *Escherichia coli* and confers kanamycin or neomycin resistance on the GM plant. It was used in the laboratory to select modified plant tissues during the initial development of the plants from which the GM line was derived.

5.2.1 The palmitoyl-ACP thioesterase gene, ghFatB-1 *(low palmitic acid)*

19. The *ghFatB-1* gene belongs to the acyl-acyl carrier protein thioesterase (acyl-ACP thioesterase) family. Acyl-ACP thioesterases act to terminate the elongation of fatty acids by releasing the acyl group of the fatty acid from the acyl carrier protein (ACP; Figure 2) (Yoder et al. 1999). The *ghFatB-1* gene has approximately 80% sequence homology to the published cotton palmitoyl-acyl carrier protein thioesterase (information supplied by applicant). The published cotton palmitoyl-acyl carrier protein thioesterase gene appears to be expressed most abundantly in the cotton embryos (Pirtle et al. 1999).

20. The suppression of the gene expression of the endogenous *ghFatB-1* is intended to reduce the level of palmitic acid in the GM cotton seed by reducing the amount of the sixteen carbon fatty acid that is reduced from the ACP. It would be expected that this ACP bound sixteen carbon fatty acid would then be available for carbon chain elongation down stream (Figure 2).

5.2.2 The microsomal Δ12-desaturase gene, ghFAD2-1 *(high oleic acid)*

21. The microsomal Δ12-desaturase enzyme belongs to the family of enzymes called the fatty acid desaturases which act to convert a single bond between two carbon atoms into a double bond (Los & Murata 1998). In cotton, the enzyme microsomal Δ12-desaturase catalyses the conversion of oleic acid (C18:1) to linoleic acid (C18:2) by introducing a double bond at the Δ12 position. This enzyme is also described as oleoyl-phosphatidylcholine ω6-desaturase or 1-acyl-2-oleoyl-sn-glycero-3-phosphocholine Δ^{12}-desaturase.

22. In *G. hirsutum*, the main seed microsomal Δ12-desaturase is encoded by the *ghFAD2-1* gene. This gene belongs to a small multigenic family in cotton, including *FAD2-1* and *FAD2-2*. The sequences of *ghFAD2-1* and *ghFAD2-2* are significantly divergent; they share approximately 70% nucleotide sequence identity (Liu et al. 2002b). Other members of the *FAD2* gene family may also be present in the cotton genome (Liu et al. 1999).

23. In addition, *G. hirsutum* is an allotetraploid species that has two copies of *FAD2- 1* (one genomic copy of the gene exists in both the A and D genomes). The two copies of *ghFAD2-1* share approximately 98% nucleotide sequence identity. They contribute approximately equally to the conversion of oleic acid to linoleic acid in cottonseed. In contrast, diploid species of Gossypium have only one copy of *FAD2-1* (Liu et al. 1999). The *ghFAD2-1* gene has been silenced previously in *G. hirsutum* and a single copy of the gene silencing construct is able to provide suppression of both genomic copies of the gene to a level considered maximal (Liu et al. 2002b).

24. The two microsomal Δ12-desaturase genes (*ghFAD2-1* and *ghFAD2-2*) have been shown to have different expression patterns in cotton. The major microsomal Δ12-desaturase activity in developing cottonseed embryos is due to the product of the *ghFAD2-1* gene with expression peaking at 30-36 days after anthesis. No RNA transcripts of *ghFAD2-1* are detectable in leaves. In contrast, *ghFAD2-2* shows low level constitutive production of RNA transcripts throughout the plant, including seeds (Liu et al. 2002b).

25. The suppression of the *ghFAD2-1* gene in the GM cotton line is expected to decrease the amount of linoleic acid in the GM cotton by reducing the amount of oleic acid that is converted to linoleic acid in the GM cotton (Figure 2).

5.2.3 The cyclopropane fatty acid synthase gene, ghCPA-FAS-2 *(low cyclic fatty acid)*

26. Cotton seed oil contains small amounts of two cyclopropenoid fatty acids, sterculic and malvalic acid. The cyclopropenoid fatty acids are believed to be synthesised from oleic acid in a two step process with dihydrosterulic acid the intermediator step (Bao et al. 2002). Cyclopropane fatty acid synthase (CPA-FAS) is thought to be responsible for the addition of a methylene group at the Δ9 position of oleic acid to form dihydrosterulic acid (information supplied by the applicant; (Bao et al. 2002). The coding sequence of the *G. hirsutum CPA-FAS-2* gene is 87% identical to the gene sequence of the published *Sterculia foetida* CPA-FAS (GenBank accession number AF470622) [9] (Bao et al. 2002).

27. The amount of oleic acid in the seeds of the GM cotton line will be further increased by the suppression of the *ghCPA-FAS-2* gene which is expected to reduce the amount of cyclopropenoid fatty acids in the produced in the cotton seeds (Figure 2).

5.2.4 The end products/effects associated with the introduced genes for altered fatty acid composition of the cottonseed oil

28. The aim of the genetic modification is to suppress expression of corresponding endogenous genes. Only partial gene sequences have been introduced to achieve this suppression and therefore no new proteins and no novel fatty acids are expected to be produced by the GM cotton line. Suppression of endogenous genes in the GM cotton does not change the types of fatty acids found in cottonseed, but only alters the ratios of palmitic, stearic, oleic, linoleic and cyclopropenoid fatty acids (Table 2).

Table 2. Fatty acid composition in the GM cotton line (MonoCott DCS9-34) and its untransformed parental line Coker 315.

Cotton line	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Total CPFAs (%)
MonoCott DCS9-34	7	1	78	11	0.2
Coker 315	26	2	15	56	1

CPFAs: cyclopropenoid fatty acids. Glasshouse data provided by the applicant.

[9] BLASTN Megablast performed using the National Center for Biotechnology Information's BLAST interface ***Basic Local Alignment Search Tool*** on 22/7/2008 with the following conditions; Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences); Query= gi|50313461|gb|AY574037.1| Gossypium hirsutum cyclopropane fatty acid synthase (CPA-FAS-2) mRNA; nucleotide range 16bp to 2613bp.

5.2.5 Toxicity/allergenicity of the end products associated with the introduced construct for altered fatty acid composition of the cottonseed oil

29. Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids (eg. dihydrosterculic, sterculic and malvalic acids). The applicant has provided glasshouse data which indicates that the total cyclopropenoid fatty acid content of the seeds has been reduced in the GM cotton line compared to the untransformed parent (see Table 2). Thus, the toxicity potential of the cyclopropenoid fatty acids should be reduced in the seeds of the GM cotton line. The applicant has also supplied data, generated from the modified cotton line grown under glasshouse conditions, that suggest the levels of the fatty acids in the root and leaf tissue is not significantly different from the levels in the unmodified control plants.

30. The oleic acid content of the GM cottonseed oil was shown to have increased in comparison with the non-GM cotton (Table 2). Similar levels of oleic acid (75 to 89%) occur naturally in evening primrose, safflower (Chatterjee & Chowdhury 2008) and peanut oil (Liu et al. 2002a). PTGS has also been used to generate similar levels of oleic acid in soybean, canola and mustard (Liu et al. 2002a). Oleic acid is an essential fatty acid not known to be toxic to humans.

31. None of the fatty acids in cottonseed oil are known to cause allergies in humans. Allergic reactions to fats have not been reported in people. It is not expected that the altered fatty acid ratios will affect other properties of GM cotton that may impact on human health and safety when working with or living near GM cotton.

32. While none of the fatty acids in cottonseed oil are known to be toxic or allergenic to humans, consumption of high amounts of some of these fatty acids can have negative health effects. Palmitic acid consumption can raise the level of low-density lipoprotein serum cholesterol which has been associated with an increased risk of cardiovascular disease (Liu et al. 2002a). Consumption of linoleic acid is considered to have the beneficial health effect of lowering the low-density lipoprotein serum cholesterol in the blood. However, linoleic acid is unstable when used as a commercial frying oil and the process of partial hydrogenation used to stabilise the oil can result in oil that has similar negative health effects to the consumption of palmitic acid (Liu et al. 2002a). As the level of palmitic and linoleic acids are reduced in the GM cotton line compared to the non-GM parent line the potential for negative health effects should also be reduced.

33. No toxicity/allergenicity tests have been performed on the cottonseed oil or meal as the proposed trial is still at an early stage. Such tests would have to be conducted if approval was sought for the GMO, or products derived from the GMO, to be considered for human consumption in Australia (see discussion in Section 7.1.2).

5.2.6 The selectable marker gene and the encoded protein

34. The GM cotton line contains the antibiotic resistance selectable marker gene *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.

35. 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 (available on the OGTR Website or by contacting the OGTR), 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). This report concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2007). Hence, the *nptII* gene will not be considered further in this assessment.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the partial gene sequences

36. 3Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Transcription of the introduced partial gene sequences is regulated by a seed specific promoter and terminator derived from the soybean (*Glycine max*) lectin gene (*lec1*; Table 3). In soybean, the lectin protein has been characterised as seed specific (Vodkin & Raikhel 1986) and the *lec1* promoter (*lec-P*) has been shown to be developmentally-regulated in the developing somatic embryo (Buenrostro-Nava et al. 2006). The *lec1* promoter has been tested with the β-*glucuronidase* reporter gene in GM cotton. Strong promoter activity was detected during embryo development, peaking late in embryo development, demonstrating the seed-specificity of the *lec1* promoter in GM cotton (Townsend & Llewellyn 2002). Altered fatty acid composition occurs only in the GM cotton seeds, with leaf membrane lipids being unaffected (data supplied by the applicant).

Table 3. Regulatory sequences for the partial gene sequences.

Genetic Element	Type of regulatory element	Source	Intended purpose	Reference
Lec-P	promoter	soybean (*Glycine max*)	drive seed specific expression of the introduced partial gene sequences	(Buenrostro-Nava et al. 2006)
Lec-T	terminator	soybean (*G. max*)	terminate expression of the introduced partial gene sequences	(Buenrostro-Nava et al. 2006)
intron	*ghFAD2-1* gene intron	*G. hirsutum*	construct spacer	GenBank accession number X97016 (for coding sequence only)

37. The single unit and its inverted repeat are separated by an intron derived from the *ghFAD2-1* gene from *G. hirsutum*. The applicant has indicated that there is no evidence that this intron is involved in the regulation of gene expression and that unpublished data showed that this intron neither enhanced nor suppressed the expression of an introduced reporter gene. However, the use of an intron instead of other DNA as a spacer between the inverted repeats has been shown to increase RNAi silencing efficacy, but this mechanism is not currently well understood (Smith et al. 2002).

38. Gene expression in plants is also regulated by the mRNA termination region or terminator, which includes a site for termination of RNA transcription and a polyadenylation signal. The 3' termination region can also influence stability of the mRNA and expression levels of protein. The termination region for the modified *ghFAD2-1* gene was derived from the same soybean lectin gene used for the promoter region. While these regulatory sequences are derived from plants that are associated with allergenic or toxic responses in humans (cotton, soybean), they are not in themselves allergenic or toxic.

5.3.2 Regulatory sequences for the expression of the selectable marker genes

39. The *nptII* gene is regulated by the SC1 promoter and SC3 termination sequences derived from Subterranean clover stunt virus (SCSV; Table 4). The SC1 promoter has been shown to direct predominantly callus-specific expression and when used to control a selectable marker gene, has proven useful in the selection of GM plants (Schünmann et al. 2003).

Table 4. Regulatory sequences for the selectable marker gene expressed in MonoCott (DCS9-34).

Genetic Element	Type of regulatory element	Source	Intended purpose	Reference
S1	promoter	Subterranean clover stunt virus	drive expression of the *nptII* gene	(Schünmann et al. 2003)
S3	terminator	Subterranean clover stunt virus	terminate expression of the *nptII* gene	

40. While these regulatory sequences are derived from plant pathogens (SCSV), the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants.

5.4 Method of genetic modification

41. The GM cotton line was generated by *Agrobacterium tumefaciens*-mediated transformation. This method of transformation was used to create the high oleic acid (HO) GM cotton plants approved for release under DIR 039/2003 and has been discussed in previous RARMPs (the most recent of which is for DIR 070/2006).

42. A disarmed binary plasmid vector was used to introduce the genetic construct containing the three partial gene sequences and the *nptII* gene (see sections 5.1 and 5.2) into cotton cultivar Coker 315 using standard *Agrobacterium* transformation protocols. The cultivar Coker 315 was used as it is readily transformed. Following the transformation process and plant regeneration, screening for successfully modified plants was performed in the presence of kanamycin. Subsequently, cotton plants containing the introduced genetic construct were obtained that are tolerant to kanamycin.

5.5 Characterisation of the GMO

5.5.1 Stability and molecular characterisation

43. Southern blot analysis was used to examine the integration of the introduced genetic construct into the MonoCott (line DCS9-34) cotton genome and to estimate the number of copies of the introduced genetic construct present in this GM cotton line. The selected GM cotton line was found to harbour a single insertion of the genetic construct. Successful integration and the presence of a single copy of the introduced genetic construct has been determined by Southern blot analysis, however the chromosomal location of the insertion has not been determined.

44. The applicant has supplied data, from glasshouse trials, indicating that the introduced genetic construct was stably inherited over several generations. Additionally, the trait of novel fatty acid profile in the cottonseed oil appeared to be completely dominant, with segregation of the introduced trait following a Mendelian single gene segregation pattern in the T2 and T3 seed populations, a clear 3:1 ratio.

5.5.2 Characterisation of the phenotype of the GMO

45. The purpose of the proposed trial is to conduct experiments to assess a range of agronomic characteristics of the GM cotton line under normal cotton growing conditions.

These characteristics include; seed germination rate, fibre yield and quality, seed yield, oil content and fatty acid composition. The trial will also produce cottonseed oil for quality evaluation by industry.

46. Early observations of several other GM cotton lines with altered cottonseed oils, grown under glasshouse conditions, showed normal germination and growth with no obvious differences compared to the parental line (Coker 315). In contrast, the applicant discarded some lines of GM cotton in early generations due to undesirable physiological or agronomic effects (eg smaller bolls and seed sizes).

47. Further observation of plants in glasshouse conditions as well as compositional analysis of the cottonseed oil enabled selection of the cotton line proposed for release, the MonoCott GM cotton line, with an altered fatty acid profile without any deleterious agronomic effects. These observations suggest that the plant morphology and growth characteristics of the GM cotton line are equivalent to non-GM cotton.

48. The fatty acid compositions in the mature cottonseeds from T2, T3 and T4 generations (two, three and four generations after the modified plants were generated) were analysed by gas chromatography. The fatty acid profile in individual cotton seeds appeared to stabilise by the T4 generation, the fatty acid composition appearing constant and stable across individual seeds tested. The fatty acid composition of the leaves and roots appear to be unaffected by the genetic modification (data supplied by applicant).

49. However, there may be unintended effects due to random insertion of the introduced genes (see Chapter 2; Event 6).

Section 6 The receiving environment

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

6.1 Relevant abiotic factors

51. 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 2008b).

52. The release is proposed to take place in NSW local government area of Narrabri. The proposed release site is in the centre of the cotton cropping system of the Namoi Valley, which has typical climate for summer cotton growing regions in Australia, with warm summers and mostly higher summer than winter rainfall. The rainfall and temperature statistics for Narrabri are given in Table 5.

Table 5. Climatic data for Narrabri, NSW

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

Source: Bureau of Meteorology.

53. The proposed release site is on a large farm near the ACRI, the farm has a history of growing cotton. The proposed trial site is not prone to flooding, but even in the event of flooding, cotton fields are laser levelled to control the movement of irrigation water and floods normally rise and fall gently, without disturbing the plants growing in the field.

6.2 Relevant biotic factors

54. 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 proposed field trial site would be in commercial cotton growing regions of Australia
- the proposed site would be located at least 50 m from natural waterways
- the GM cotton will be grown at a site approximately 5 km from the ACRI which conducts cotton breeding trials
- the applicant states that native *Gossypium* species have not been observed in the vicinity; and
- invertebrates, vertebrates and microorganisms would all be exposed to the introduced genetic construct and the altered fatty acid composition of the GM cotton seed in the same manner that exposure would occur to commercial GM and non-GM cotton.

6.3 Relevant agricultural practices

55. The location of the proposed limited and controlled release of the GM cotton line are outlined in Section 3.2 of this Chapter.

56. The applicant intends to trial the GM cotton alongside the non-GM parent to assess a range of agronomic characteristics of the GM cotton line under typical Australian cotton growing conditions. The agronomic traits the applicant intends to assess include; seed germination rate, fibre yield and quality, seed yield, oil content and fatty acid composition. The GM cotton line will be managed in the same manner as non-GM cotton, including application of insecticides.

57. The GM cotton trial site will be located at least 50 m from the nearest natural waterway and other cotton used for breeding. The proposed trial site will be surrounded by a 20 m wide pollen trap of an elite non-GM cotton cultivar. The GM cotton will be ginned separately and all material generated as a result of the ginning process, with the exception of the cotton seeds, will be destroyed by burning.

58. Trials containing both GM and non-GM *G. barbadense* will be grown in the same general cotton growing area as the proposed release. These will take place at the ACRI approximately 5 km from the release site. The applicant has also stated that there will be breeding trials of other *G. hirsutum* cultivars at the ACRI.

59. The plant material remaining at the proposed release site after harvest would include the dry stalks which are left standing, while dry leaves and a small amount of seed would be left on the ground. The applicant estimates that after harvest approximately 2% of the cotton seed could be expected to be present on the ground at the proposed release site. After harvest the applicant has indicated that the proposed site will be cleaned by initially using a mechanical slasher to break the plant material into small fragments followed by root cutting and incorporation of the plant material into the soil for decomposition.

6.4 Presence of related plants in the receiving environment

60. GM cotton plants are widespread in the agricultural environment comprising about 90% of commercially grown cotton crops. In contrast, non-GM *G. hirsutum* and *G. barbadense* varieties comprised less than 7% and 2% of commercially grown cotton, respectively.

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

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

62. The introduced marker gene and partial gene sequences are isolated from naturally occurring organisms that are already present in the environment.

63. The introduced partial gene sequences were derived from three endogenous *G. hirsutum* genes. The introduction of the partial gene sequences suppresses expression of the endogenous genes thus, no new proteins and no novel fatty acids are expected to be produced by GM cotton. Homologues of the three cotton genes can be found in many other plant species including widely grown crop species such as canola, safflower, sunflower, peanut, soybean and maize. Therefore, it is expected humans, animals and microorganisms routinely encounter, through contact with plants and food, the endogenous cotton genes or their homologs.

64. The *nptII* gene is derived from the bacteria *E. coli*; which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM cotton line

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

65. There has been no release of this GM cotton line in Australia. However, the Regulator has previously issued Licence DIR 039/2003 to CSIRO for the limited and controlled release of cotton modified for high oleic acid levels in the cottonseed oil. The GM cotton lines authorised for release under DIR 039/2003 contain one of the partial gene sequences (*ghFAD2-1*) present in the GM cotton line proposed for release in the current application.

66. Approvals for commercial releases of other GM cotton lines include releases in the local government area proposed in the current application. GM cotton (*G. hirsutum*) lines that have 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 066/2006).

- herbicide tolerant Liberty Link® Cotton and herbicide tolerant/insect resistant Liberty Link®/Bollgard II® MON15985 cotton (authorised under DIR 062/2005).

67. GM *G. barbadense* cotton containing two introduced genes for insect resistance (*cry1Ac* and *cry2Ab*) and/or two copies of the herbicide tolerance gene, *cp4 epsps* was approved for limited and controlled release under licence DIR 074/2007. The approval encompassed a number of shires including the Shire of Narrabri, NSW.

7.1.2 Approvals by other Australian government agencies

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

69. 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 line in human food, accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from this GM cotton line could be used in food.

70. Three lines of GM soybean (*G. max*) modified for increased oleic acid have been approved for human consumption in Australia by FSANZ. These soybean lines were also generated using the technique of gene silencing to suppress the expression of the endogenous *gmFad2-1* gene[10].

7.2 International approvals

71. There has been no release of this GM cotton line in any other country. However, there have been releases into the environment of other crop plants modified for high oleic acid. The GM soybean lines approved by FSANZ for human consumption in Australia have also been approved for environmental release in Canada, the United States of America and Japan[11].

72. Notifications of small scale releases of oilseed rape (*Brassica napus*) either modified for increased oil content or altered fatty acid composition in the seeds have been made in Sweden and Germany[12].

[10] Application A387 - Food derived from high oleic acid soybean lines G94-1, G94-19 and G168, approved in 2000 by FSANZ,accessed 28 July 2008.
[11] agbios.com accessed on 28 July 2008.
[12] European Commission accessed 28th July 2008.

Chapter 2 Risk assessment

Section 1 Introduction

73. 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 GMO that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

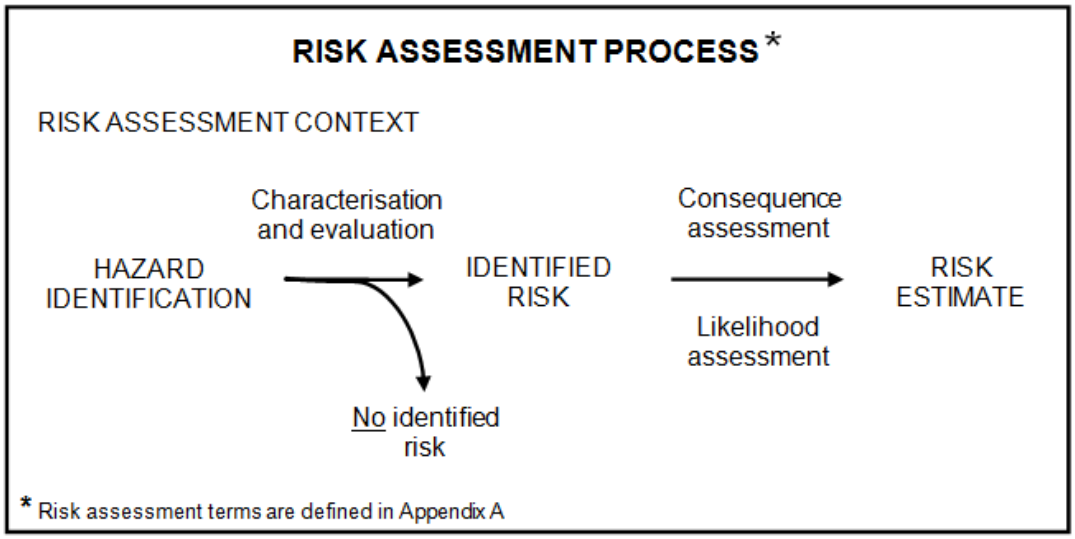

Figure 4 The risk assessment process

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

75. 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).

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

77. 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.

78. 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

79. 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.

80. 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.

81. 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 GMO
- the proposed limits
- the proposed controls
- characteristics of the non-GM parent
- routes of exposure to the GMO, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMO[13]
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic factors at the site(s) of release
- agronomic management practices for the GMO.

82. The seven events that were characterised are discussed in detail later in this Section. They are summarised in Table 6 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.

[13] As discussed in Section 5.2.6, the *nptII* gene and its product has 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 6 Summary of events that may give rise to an adverse outcome through the expression of the introduced partial gene sequences for altered fatty acid composition in cottonseed oil.

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 partial gene sequences, or their end products.	Allergic reactions in people or toxicity in people or other organisms	No	• The introduced partial gene sequences suppress the expression of endogenous cotton genes. With the exception of the *nptII* gene, no new proteins or novel fatty acids have been introduced. • The genetic modification is seed specific and only the ratio of the fatty acids in cottonseed oil has been altered, thus allergenicity and toxicity of the GM cotton is unlikely to be greater than that of non-GM cotton. • The proposed release is limited and controlled; further reducing exposure of people and other organisms to products of the introduced partial gene sequences.
Section 2.2 Spread and persistence (weediness) of the GM cotton line in the environment	2. Expression of the introduced partial gene sequences improving the survival of GM cotton plants.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	• Commercial cotton plants do not possess weedy characteristics. • The applicant has provided information that indicates the germination rate of the GMO is not significantly different to the parental cultivar. • The limits and controls proposed for the release would minimise persistence.
	3. Dispersal of reproductive (sexual or asexual) GM plant material through various means, including animals and extreme weather conditions.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	• Cotton seeds have limited dispersal characteristics, which are not expected to be changed in the GMO. • GM cotton seeds are unlikely to be transported by mammals and birds because naturally occurring toxins make seeds unpalatable. • The proposed limits and controls would minimise dispersal.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced partial gene sequences or regulatory sequences in commercial, GM and non-GM cotton plants (*G. hirsutum* or *G. barbadense*) or in other sexually compatible plants.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	• Cotton is predominately self-pollinating and outcrossing is limited. • The applicant proposed a number of controls which would minimise pollen mediated gene flow.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced partial gene sequences, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	• Complete genes from which the introduced partial gene sequences were derived, 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.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM cotton line resulting from altered expression or random insertion of the introduced partial gene sequences.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	• Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. • One purpose of the trial is to identify any unintended phenotypic effects through the measurement of agronomic performance under field conditions.
Section 2.6 Unauthorised activities	7. Use of the GMO outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMO 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

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

84. 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).

85. A range of organisms may be exposed directly or indirectly to the introduced partial gene sequences (and end products) for altered fatty acid composition of the cottonseed oil. Workers cultivating the cotton would be exposed to all plant parts. Organisms may be exposed directly 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 the introduced partial gene sequences, or their end products.

86. Expression of the introduced partial gene sequences for altered fatty acid composition could potentially result in the production of novel toxic or allergenic compounds in the GM cotton line, 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.

87. The aim of the genetic modification is to suppress expression of corresponding endogenous cotton genes and only partial gene sequences have been introduced, thus, no new proteins and no novel fatty acids are expected to be produced by the GM cotton. The altered fatty acid composition of GM cotton does not change the types of fatty acids found in cottonseed, but only alters the ratios of palmitic, stearic, oleic, linoleic and cyclopropenoid fatty acids in the GM cottonseed oil.

88. Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids (Chapter 1, Section 5.5.2). As the total cyclopropenoid fatty acid content has been reduced in the GM cotton line (Table 2, Chapter 1), the toxicity potential for the GM cotton

seeds should be reduced compared to the parent cotton line. The fatty acid composition of the GM cotton leaves and roots appear to be unaffected by the genetic modification (from data supplied by applicant). Therefore, the other plant tissues are not expected to be any more toxic than non-GM cotton or commercial GM cotton.

89. The genetic modification has increased the oleic acid content in the GM cottonseed oil (to approximately 78% of the seed oil in glasshouse grown plants). Oleic acid is a component of all plant and animal cells and is not known to be toxic or allergenic to humans (as discussed in RARMP for DIR 039/2003). Similar levels of oleic acid occur naturally in other oils used for human consumption, for example canola oil contains up to 62% oleic acid (OGTR 2008a). After harvest of the cottonseed plant litter will be incorporated into the soil for decomposition and there may be a small amount of cottonseed also incorporated into the soil. However, the composition of the fatty acids is only altered in the cottonseed oil and not the leaf or root tissues and the increased levels of oleic acid in the cottonseeds are not dissimilar to levels found naturally in other crop species. Therefore, exposure to the GM plant material is not expected to adversely affect the health of humans or other organisms that may come into contact with or consume the leaf or root tissues.

90. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Seed from the trial may be saved for further research and oil may be extracted for quality evaluation by industry. Public exposure to GM plant materials via ingestion, skin contact or inhalation would be minimal as no plant material will be used as food, animal feed and public access to the trial site is restricted. Human exposure to the GM plant materials would be limited to trained and authorised staff associated with the field trial.

91. ***Conclusion***: The potential for allergic reaction in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing the introduced partial gene sequences is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence of the GM cotton line in the environment

92. 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 2008b). In summary, cotton lacks most characteristics that are common to many weeds, such as the ability to produce a persisting seed bank, rapid growth to flowering, continuous seed production, very high seed output, high seed dispersal and long-distance seed dispersal.

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

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

Event 2: Expression of the introduced partial gene sequences improving the survival of the GM cotton plants

95. If the GM cotton line was to establish or persist in the environment it could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials, the introduced partial gene sequences or end products has been considered in Event 1 and was not considered an identified risk.

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

97. Although the impact of the genetic modification on survival of the GM cotton line is uncharacterised under field conditions, the genetic modification is seed specific and the applicant has supplied data indicating that effects of the modification are limited to altered fatty acid composition in the GM cottonseed oil. No new proteins and no novel fatty acids are expected to be produced by the GM cotton line.

98. It is possible that the altered seed oil composition may affect germination and survival of the GM cotton seed/seedlings. Fatty acids are used preferentially by the germinating cotton seed, with linoleic acid and the saturated fatty acids (including palmitic and stearic fatty acids) depleted relatively faster than oleic acid (White 1958). Cotton genetically modified for high stearic fatty acid content in the cottonseed oil had reduced germination and seedling survival compared to GM cotton with high oleic acid content in the cottonseed oil or the non-GM parent (Liu et al. 2002b). The applicant has indicated that under glasshouse conditions, the seed germination rate of the GM cotton line proposed for release appears equivalent to the non-GM parental cotton cultivar. The effect of the altered fatty acid composition of the GM cottonseed oil on seed dormancy or tolerance to abiotic stresses is not known. However, if there are any developmental advantages conferred to the GM cotton line by the altered fatty acid composition their persistence at the release site would be limited by the controls proposed by the applicant.

99. The altered fatty acid composition in the cottonseed oil may also affect the susceptibility of the GM cotton line to insect attack. However, the major lepidopteran insect pests of cotton feed mainly on the leaves and developing flowers and bolls and not on the seeds enclosed within the bolls. The applicant has provided data, generated under glasshouse conditions, which indicate the fatty acid composition is altered in the seeds of the GM cotton line but not the leaf or root tissues. The effect of the altered fatty acid composition of the cottonseed oil on the susceptibility of the GM cotton line to insect attack is unknown. If susceptibility to insect attack was altered, this would be identified during the agronomic evaluation of the growth of the GM cotton line under field conditions.

100. A large number of abiotic and biotic factors determine whether cotton will persist in the environment. These include short summer seasons, soil type and competition from other plants (Farrell & Roberts 2002). Diseases, such as fungal wilts, are also expected to play a role in limiting spread and persistence of cultivated cotton plants. The relative impact of each of these factors is dependent on whether the cotton plants are in the northern or southern areas of Australia. For example, frost is a major limiting factor in southern areas of Australia. Whereas, the distribution of pathogenic organisms is variable and susceptibility is determined by the cultivar. The reliable availability of water is a limiting factor everywhere in Australia.

Expression of the introduced partial gene sequences are not expected to alter these characteristics.

101. Therefore, the genetic modification is not expected to increase the survival of the GM cotton plants in the environment or change their invasive potential.

102. The proposed limits and controls of the trial (Chapter 1, Section 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM cotton line proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures to restrict dissemination or persistence of the GM cotton plants, including monitoring for and destroying any volunteer cotton plants.

103. ***Conclusion:*** The potential for increased survival of the GM cotton line as a result of expression of the introduced partial gene sequences is **not an identified risk** and will not be assessed further.

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

104. If the GM cotton line was to be dispersed from the release site the exposure of humans and other organisms to the GM plant material could be increased and/or the GM plant material could establish and persist in the environment. The effects of exposure to the GM cotton line have been assessed in Event 1 and were not an identified risk. The potential for the introduced partial gene sequences to increase survival of the GM cotton line in the environment was assessed in Event 2 and was also found not to be an identified risk. Therefore, the dispersal of reproductive GM plant material is not expected to adversely affect humans or other animals; or to increase the weediness of the GM cotton line compared to non-GM cotton.

105. It is possible that a reduction in the natural toxin levels in the cotton seed could result in increased consumption of the seeds by animals and subsequently lead to increased dispersal of cotton seed. Cotton tissue, particularly seeds, can be toxic if ingested in large quantities because of the presence of anti-nutritional and toxic compounds including gossypol and cyclopropenoid fatty acids (OGTR 2008b). If either or both of these compounds were reduced, the GM cotton may be less toxic or more palatable to animals.

106. The total cyclopropenoid fatty acid content in the GM cotton line has been reduced through suppression of cotton genes involved in the fatty acid biosynthesis pathway, (see Table 2, Chapter 1). In contrast, gossypol is a terpenoid aldehyde, the end-product of the sesquiterpene pathway (Townsend & Llewellyn 2007), and as such is unlikely to be altered by the genetic modification. Additionally, the genetic modification is limited to the cotton seed, while the lipids in vegetative tissue are unaffected. Therefore, the toxicity and palatability of the vegetative tissue of the GM cotton is not likely to be altered and the likelihood of consumption and dispersal of the seeds by animals is not expected to be altered by the modification.

107. Cotton seeds are not normally dispersed by natural mechanisms, although it is conceivable that flooding could disperse them. The applicant has indicated that the trial site is not prone to flooding, is above the normal flood plain and is not within 50 m of any natural waterways. Additionally, the cotton fields are laser levelled to control the movement of irrigation water into storage and recirculation dams. The applicant has proposed post-harvest monitoring of the trial site and all adjacent irrigation channels for GMOs as well as destruction of any GMOs found. These practices would also reduce the dispersal of cottonseed.

108. All GM plant material will be transported in accordance with the OGTR transport guidelines which will minimise dispersal of GM plant material.

109. ***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

110. 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).

111. Baseline information on vertical gene transfer associated with non-GM cotton plants is provided in *The Biology of* Gossypium hirsutum *and* Gossypium barbadense *(cotton)* (OGTR 2008b). In summary, cotton is predominantly self-pollinating and outcrossing is rare, although crosspollination can occur at low levels over short distances.

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

113. The only sexually compatible species present in Australia that could receive genes from the GM cotton are *G. hirsutum* (including both cultivated GM and non-GM, and naturalised cotton) and *G. barbadense*.

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

114. Transfer and expression of the introduced partial gene sequences in other cotton or sexually compatible plants could alter the allergenicity and/or toxic potential, or increase the weediness potential of the resulting plants.

115. 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 partial gene sequences or regulatory sequences. This will be the same if the introduced genes are expressed in other GM or non-GM commercial or volunteer cotton plants.

116. While some of the regulatory sequences are derived from plant pathogens (SCSV), the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants. Those regulatory sequences derived from plants that are associated with allergenic or toxic responses in humans (cotton, soybean) are not in themselves allergenic or toxic.

117. A number of insect resistant and/or herbicide tolerant GM cotton lines are currently approved for commercial release in Australia and may be grown in the areas proposed for this release. These GM cotton lines were comprehensively assessed (most recently in the RARMPs for DIR 062/2005 and 066/2006) prior to release and comprised more than 90% of the commercial cotton crop in 2006-07. The commercial GM cotton lines could also be used in the pollen trap proposed by the applicant. If so, stacking of the introduced partial gene sequences with those for insect resistance and/or herbicide tolerance is likely to occur.

118. However, the spread and persistence of cotton in the area proposed for release is limited by multiple factors including water availability, temperature (particularly frost), nutrient levels, roadside management practices, short summer seasons, soil type, competition from other plants and disease (Farrell & Roberts 2002; OGTR 2002; OGTR 2008b) and will be further limited by the proposal of the applicant to destroy all plant material from the buffer rows. If outcrossing to commercial GM cotton crops 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. Additionally, as discussed in Event 2, the expression of the introduced partial gene sequences is not expected to increase the survival of the GM cotton plants in the environment or change their invasive potential. Therefore, if outcrossing to a commercially released GM cotton plant were to occur, the potential for weediness would be expected to be no greater than that of the GM cotton parent lines. Similarly, if outcrossing to a commercially grown non-GM cotton plant were to occur, the potential for weediness would be the same as for the GM cotton line itself.

119. 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*.

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

121. Cotton is primarily self-pollinating, but it may outcross when insect pollinators are present. Outcrossing decreases significantly with distance and it is estimated only 1 to 2% of seeds in adjacent rows are a result of crosspollination (OGTR 2008b). The applicant has proposed to surround it with a 20 m pollen trap of an elite non-GM cotton cultivar. The applicant has also proposed to destroy seed from the pollen trap, which may include seed resulting from outcrossing by the GM cotton line.

122. In addition, the GM cotton line proposed for release and the non-GM parent cotton plants within the trial site 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 site. The above practices (isolation, pollen trap, destruction of pollen trap seed, insecticide spraying regime) would serve to limit vertical gene transfer to sexually compatible species.

123. In addition to the proposed measures to limit the spread and persistence of the GM cotton line, the applicant proposes a release of limited size and short duration and also proposes to conduct post harvest monitoring of the trial sites for at least 12 months and destroy all volunteers.

124. ***Conclusion:*** The potential for the expression of the introduced genes and regulatory sequences in GM and non-GM 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

125. All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the disruption of endogenous gene expression. The novel trait may result in negative, neutral or positive effects (Keese 2008).

126. The likelihood of an adverse effect and any potential adverse outcome are important considerations of any risk assessment of HGT from GM plants. In order for harm to arise from HGT several conditions must be met. These include the recipient organism encountering genetic material from a GM plant, the occurrence of HGT involving the genetic material introduced as a result of gene technology, a resulting novel phenotype being expressed in the recipient organism, the persistence of the recipient organism and the transfer of the novel genetic material to its offspring, and a selective advantage allowing the genetic material to be spread and be maintained in the recipient organism's population and species (Keese 2008).

127. Features that have an effect on the frequency of HGT include the nature of the donor and recipient organisms, their genetic and ecological relationship, and the type and function of the genetic material that is transferred. However, the frequency of gene transfer by HGT for all organisms (including viruses and bacteria) is orders of magnitude lower than gene transfer by sexual or asexual reproduction.

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

Event 5: Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

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

HGT from GM cotton plants to bacteria

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

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

these genes are often abundant in the environment and more readily transferable by conjugation and transduction from other bacteria (Keese 2008).

HGT from GM cotton plants to animals

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

HGT from GM cotton plants to viruses

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

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

HGT from GM cotton plants to other eukaryotes

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

Nature of introduced genetic material

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

137. The probability of transferring the introduced partial gene sequences contained in the GM cotton plants is no greater than that of transferring any of the native genes. Non-GM cotton contains the complete sequence from which the three partial sequences were derived

(Chapter 1, Section 5.1 and 5.2). Therefore, these genes are already available for transfer via demonstrated natural mechanisms. In addition, homologues of the three cotton genes involved in fatty acid biosynthesis occur in other plant species including many crop plants (Chapter 1, Section 6.5), and thus are widespread in the environment.

138. A key consideration in the risk assessment process should be the safety of the protein product(s) resulting from the expression of the introduced partial gene sequences rather than horizontal gene transfer *per se* (Thomson 2001). The partial gene sequences are designed to suppress genes involved in fatty acid biosynthesis, and no novel proteins or new products are expected to be produced. If the introduced partial gene sequences or their end products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Events 1-4 associated with the expression of the introduced genes or end products did not represent an identified risk.

139. ***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

140. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy[14] (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.

141. 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).

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

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

143. As described in Chapter 1, Section 5.2, the function of the introduced partial gene sequences is to suppress the expression of three endogenous cotton genes involved in fatty

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

acid biosynthesis through a process called post-transcriptional gene silencing (PTGS). The process is well defined and suppression should be limited to genes with high sequence similarity to the introduced partial gene sequences. Data from the applicant indicates that the modification is limited to the cottonseed. Altered fatty acid composition has not been detected in other cotton plant tissues, suggesting that similar genes involved in fatty acid biosynthesis in these other cotton tissues have not been altered. While there is no evidence in the scientific literature suggesting that the introduced partial gene sequences may affect another pathway, the possibility cannot be completely ruled out. However, the likelihood of any pleiotropic effects causing adverse effects is minimised by the limits and controls proposed by the applicant.

144. As discussed in Event 2, it is possible that the altered seed oil composition may affect germination and survival of the GM cotton seed/seedlings. For example, cotton genetically modified for high stearic fatty acid content in the cottonseed oil had reduced germination and seedling survival compared to GM cotton with high oleic acid content in the cottonseed oil or the non-GM parent (Liu et al. 2002b). However, the applicant has provided data which show that under glasshouse conditions the seed germination rate of the GM cotton line proposed for release appears equivalent to the non-GM cotton.

145. Observations made under glasshouse conditions and over several generations revealed no apparent differences in appearance or growth between the GM cotton line proposed for release and the non-GM parent. Additionally, data collected under glasshouse conditions showed that the altered fatty acid profile was inherited stably over several generations. The trait appears to be dominant and segregated following a Mendelian single gene segregation pattern in a clear 3:1 ratio (see Chapter 1, Section 5.2). However, there is uncertainty with regard to how the GM cotton plants might behave when grown under field conditions.

146. 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) and the agronomic evaluation of the growth of the GM cotton line under field conditions, proposed by the applicant, would identify any adverse characteristics.

147. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2 and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects.

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

2.6 Unauthorised activities

Event 7: Use of the GMO 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 line 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 the GMO. 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 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.

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:

- the genetic modification is seed specific and only alters the ratio of fatty acids in the cottonseed oil. No new proteins or novel fatty acids have been introduced
- limits on the size, location and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the dissemination or persistence of the GM cotton plants and their genetic material
- limited capacity of the GM cotton line to spread and persist outside the areas proposed for release
- limited ability and opportunity for the GM cotton line 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 gene sequences from which the partial gene sequences were derived 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 line into the environment are considered to be **negligible.** Hence, the Acting Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

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. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

156. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[15]. For field trials some knowledge gaps are inevitable because they involve the conduct of research. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMO and their genetic material in the environment, rather than necessarily to treat an identified risk.

157. For DIR 085/2008 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- Event 1, regarding potential increases in toxicity through contact with plant material containing an altered fatty acid composition
- Event 2, associated with a potential for increased survival of the GMO.

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

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

[15] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at Risk analysis framework 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 that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[16].

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.

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

[16] 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 Risk analysis framework.

GM cotton lines to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of the GM cotton line for use in human food. FSANZ approval would need to be obtained before it could be used in food.

167. 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 and the environment from the proposed trial of GM cotton. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

169. These events were considered in the context of the scale of the proposed release (a maximum total area of 2 hectares over one growing season from October 2008 to June 2009) on one site in the local government area of Narrabri, NSW, the containment measures (Chapter 1, Section 3.3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

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

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by CSIRO

171. Chapter 1 Sections 3.2 and 3.3 provide details of the limits and controls proposed by CSIRO in their application, and discussed in the events characterised for the release in Chapter 2. Many of these proposed control measures are considered standard GM cotton licence conditions and have been imposed by the Regulator in previous DIR licences. The appropriateness of these controls has been assessed in detail in previous GM cotton RARMPs, most recently in DIR 083/2007 and will be discussed briefly here.

172. The proposed release would be limited to one site in the local government area of Narrabri, NSW. The trial, including planting, harvesting and post-harvest monitoring, will be carried out by qualified staff from CSIRO based at ACRI, who have had extensive experience with GM cotton field trials, most recently under DIRs 056/2004, 067/2006 and 074/2007. All personnel have appropriate training in practices relevant to the handling and disposal of GMOs. Additionally, the duration of the proposed release will be limited to one growing season. These measures will limit the potential exposure of humans and vertebrates to the GMO (Event 1) and the potential for the GM cotton line to persist or to establish outside the proposed release site (Event 2).

173. The applicant proposes to surround the trial site with a 20 m wide pollen trap of non-GM cotton plants to limit gene flow from the GM cotton. As discussed in *The Biology of* Gossypium hirsutum *and* Gossypium barbadense *(cotton)* (OGTR 2008b), cotton is predominantly self pollinating with the highest level of outcrossing occurring between adjacent rows. Outcrossing is rare beyond 20 m (Llewellyn et al. 2007), and a 20 m pollen

trap of non-GM cotton plants will minimise gene transfer to sexually compatible plants (Event 4).

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

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

176. 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*. 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 Regulator to limit and control the proposed release

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

- conduct the release on a total area of up to 2 hectares for one year at one site in the NSW local government area of Narrabri, between October 2008 and June 2009
- surround the release site with a 20 m pollen trap
- locate the trial site at least 50 m away from natural waterways
- harvest and gin seed cotton from the release separately from any other cotton crop
- not permit any materials from the release to be used in human food or animal feed or for the production of fabrics and/or other cotton products
- destroy all plant materials not required for further analysis
- following harvest, clean the site, monitoring zone and equipment used on the site
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- monitor the site for at least 12 months and destroy any cotton plants that may grow until no volunteers are detected for a continuous 6 month period.

4.1.3 Measures to control other activities associated with the trial

178. 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 GMO for the purposes of, or in the course of, the authorised dealings.

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

4.2 Other risk management considerations

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

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

4.2.1 Applicant suitability

181. 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.

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

183. 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.

184. CSIRO 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

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

186. CSIRO 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 line outside of the permitted areas.

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

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

188. 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

189. 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.

190. 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.

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

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

4.2.5 Monitoring for Compliance

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

193. 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.

194. 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

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

- additional data on the potential toxicity of plant materials from the GM cotton line

- characteristics indicative of weediness including measurement of altered reproductive capacity, germination rates, degree of seed dormancy, tolerance to environmental stresses, and disease susceptibility.

Section 6 Conclusions of the RARMP

196. The risk assessment concludes that this proposed limited and controlled release of one GM cotton line on a maximum total area of 2 hectares over one year in the NSW local government area of Narrabri poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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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 authorities[17] on the consultation RARMP for DIR 085/2008

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
Considers that this controlled release does not pose an unacceptable risk to public health or occupational health and safety. Indicates that if a future application was received for this GMO the OGTR would need to consider, in greater detail, the potential for altered toxicity or allergenicity and the potential for altered metabolism to occur.	Noted. Chapter 3, Section 5 of the RARMP outlines issues that the applicant may need to address if a future licence application was made to release this GMO on a larger scale or if reduced containment measures were proposed.

[17] 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.