



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 063/2005**

**Limited and controlled release of fungal resistant
GM cotton**

Applicant: Hexima Ltd

August 2006

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release of a genetically modified (GM) cotton line with enhanced fungal resistance into the Australian environment, in respect of application DIR 063/2005 from Hexima Ltd (Hexima).

The DIR 063/2005 licence permits the release of one GM cotton line on a limited scale and under controlled conditions.

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

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

The application

Hexima applied for a licence to release a GM cotton line with enhanced resistance to certain disease causing fungi into the environment¹. The release is proposed to take place at two sites in the shire of Pittsworth, Queensland (QLD) and one site in the shire of Narrabri or Moree Plains, New South Wales (NSW) on a maximum total area of one hectare during each of the three summer cotton growing seasons of 2006/07, 2007/08 and 2008/09.

The GM cotton line contains the plant defensin gene, *nad1*, derived from ornamental tobacco (*Nicotiana glauca*). This gene encodes a defensin protein, NAD1, which inhibits the growth of fungi, including three major fungal pathogens that cause fusarium wilt, verticillium wilt and black root rot in cotton. Plant defensins occur naturally in many horticultural and crop plants such as dahlia, tomato, peas and wheat. Their expression can be stimulated by a range of environmental factors, including disease.

The GM cotton line also contains a bacterial gene (*nptII*, conferring resistance to the antibiotic kanamycin) that was used to select successfully modified plants during initial research and development work in the laboratory.

The purpose of the trial is to conduct early stage ('proof of concept') research to assess the extent of enhanced resistance to three fungal diseases and the agronomic performance of the GM cotton line under field conditions; to measure the expression levels of the introduced plant defensin gene and to test for adverse impacts on selected beneficial soil microorganisms. Lint fibres may be tested for quality characteristics. Seed will also be collected for further studies and possible future releases (subject to additional applications and assessment processes). No products from the release will be used for human food or animal feed.

Hexima proposed a number of measures to limit the spread and persistence of the GMO and the introduced genetic material that were considered during the evaluation of the application.

¹ Hexima initially requested approval for the release of three GM lines but subsequently amended the application to one only.

Risk assessment

Background

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

The risks to people and the environment from the proposed limited and controlled release were assessed in comparison to non-GM cotton, in the context of the intended agronomic management practices, and the environmental conditions in the regions proposed for the release.

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

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

Hazard identification

Of the 24 events compiled during the hazard identification process, two were selected for further assessment. The potential adverse outcome to the environment associated with these events was: toxicity for, or growth-inhibition of, invertebrates and/or non-target microorganisms. The remaining 22 events were not assessed further as they were considered not to give rise to an identified risk to human health and safety or the environment (refer to Chapter 2 for more information).

Risk of toxicity to, or growth inhibition of, invertebrates and/or non-target microorganisms

Two events were considered that might cause toxicity for, or growth inhibition in, invertebrates and/or non-target microorganisms as a result of the release of the GM cotton line:

- Direct or indirect ingestion of the introduced protein (NAD1) by invertebrates
- Exposure of non-target microorganisms to the introduced protein (NAD1).

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

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. The level of risk to the health and safety of people or the environment was assessed for two events. The risk estimates for the adverse outcome associated with both events were **negligible**.

The *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation. However, containment and disposal measures have been imposed to restrict the release in locations, size and duration to those requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require the applicant to limit the duration of the release to three summer cotton growing seasons of 2006/07, 2007/08 and 2008/09 on a maximum total area of one hectare at up to three sites; prevent the use of the GMO, or materials from the GMO, in food and animal feed; maintain physical isolation of the release sites; and conduct post-harvest monitoring to ensure all GM plants are destroyed².

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of a GM cotton line with enhanced fungal resistance into the shires of Pittsworth, QLD, Narrabri and Moree Plains, NSW, poses **negligible** risks to the health and safety of people and the environment.

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

² The licence and conditions for DIR 063/2005 are available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>, following the path to DIR 063/2005).

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Abbreviations

AFP	Antifungal peptide
AM	Arbuscular mycorrhiza
AMP	Antimicrobial peptide
ANZFA	Previous name for Food Standards Australia New Zealand
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
ART V 1	A pollen protein from <i>Artemisia vulgaris</i>
ATP	Adenosine triphosphate
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information as declared under section 185 of the Act
CRC	Cooperative Research Centre
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EFB	European Federation of Biotechnology
ELISA	Enzyme-linked Immunosorbent Assay
EMBL	European Molecular Biology Laboratory
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
Glu	Glutamine
Gly	Glycine
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GPS	Global Position System
GTTAC	Gene Technology Technical Advisory Committee
kDa	1000 Dalton, unit of relative molecular mass
kg	Kilogram
LD ₅₀	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
Lys	Lysine
m	Metre
mg	Milligram
mL	Millilitre
M _r	Relative molecular mass
µg	Microgram
µM	Microliter
<i>nad1</i>	Gene encoding a <i>Nicotiana glauca</i> plant defensin
NAD1	Product of the <i>nad1</i> gene
NADH	Nicotinamide adenine dinucleotide
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
<i>nos</i>	Gene encoding nopaline synthase
<i>nptII</i>	Gene encoding neomycin phosphotransferase type II
NPTII	Product of the <i>nptII</i> gene
NSW	New South Wales

OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PAR H 1	A pollen protein from <i>Parthenium hysterophorus</i>
PC2	Physical containment level 2
PCR	Polymerase Chain Reaction
QLD	Queensland
RARMP	Risk Analysis and Risk Management Plan
Ser	Serine
TAD1	A <i>Triticum aestivum</i> defensin gene product
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration
Thr	Threonine
UK	United Kingdom
US FDA	United States Food and Drug Administration
USA	The United States of America
WHO	World Health Organisation

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 063/2005) to Hexima Ltd (Hexima) for dealings involving the intentional release of one genetically modified (GM) cotton line into the Australian environment.

The DIR 063/2005 licence permits the limited and controlled release of a GM cotton line with enhanced resistance to certain fungal pathogens. The release will occur on up to three sites of a maximum total area of one hectare during each of the three summer cotton growing seasons of 2006/07, 2007/08 and 2008/09 in the shires of Pittsworth, QLD, and Narrabri or Moree Plains, NSW.

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

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions³.

The RARMP for DIR 063/2005 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the dealings proposed by the applicant were taken into account by the Regulator in deciding to issue a licence and the licence conditions that have been imposed.

Application

Title:	Field trial of GM cotton expressing natural plant genes for fungal resistance*
Applicant:	Hexima Limited
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	<i>Gossypium hirsutum</i> L.
Modified traits:	Fungal resistance, antibiotic resistance
Identity of the genes responsible for the modified traits:	<i>nad1</i> (<i>Nicotiana glauca</i> defensin) gene, from ornamental tobacco (fungal resistance gene); <i>nptII</i> (neomycin phosphotransferase type II) from the bacterium <i>Escherichia coli</i> (antibiotic resistance selectable marker)
Proposed locations:	Up to 3 sites per season in the shires of Pittsworth (QLD), Narrabri and Moree Plains (NSW)
Proposed release size:	Up to one hectare per season over 3 summer cotton growing seasons
Proposed time of release:	2006 – 2009

* The title of the licence application submitted by the applicant is *Assessment of transgenic cotton expressing natural plant genes for fungal control*.

Hexima applied for a licence to release a GM cotton line with enhanced resistance to certain disease causing fungi into the environment⁴. The release of the GM cotton line is intended to

³ More information on the assessment of licence applications and copies of the *Risk Analysis Framework* are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>> and <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>, respectively.

take place at two sites in the shire of Pittsworth, Queensland (QLD) and one site in the shire of Narrabri or Moree Plains, New South Wales (NSW) on a maximum total area of one hectare during each of the three summer cotton growing seasons of 2006/07, 2007/08 and 2008/09.

The GM cotton line contains the plant defensin gene, *nad1*, derived from *Nicotiana alata*, ornamental tobacco and an antibiotic resistance marker gene (*nptII*). Some details of the gene construct, including the plasmid map and some of the regulatory sequences, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was made available to the prescribed expert groups that were consulted in the preparation of the risk assessment and risk management plan (RARMP). The information was also considered during the preparation of the RARMP.

The purpose of the trial is to conduct early stage ('proof of concept') research to enable the applicant to assess the extent of enhanced resistance to three fungal diseases and the agronomic performance of the GM cotton line under field conditions; to measure the expression levels of the introduced plant defensin gene and to test for adverse impacts on selected beneficial soil microorganisms. Lint fibres may be tested for quality characteristics. Seed will also be collected for further studies and possible future releases (subject to additional assessments and approvals).

Plant defensins occur naturally in many horticultural and crop plants such as dahlia, tomato, peas and wheat. Their expression can be stimulated by a range of environmental factors, including disease. The *nad1* gene encodes a defensin protein, NAD1, which *in-vitro* inhibits the growth of fungi, including three major fungal pathogens of cotton: *Fusarium oxysporum* f.sp. *vasinfectum*, *Verticillium dahliae* and *Thielaviopsis basicola*, which cause fusarium wilt, verticillium wilt and black root rot, respectively.

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

Risk assessment

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

Advice received from a member of the public from consultation on the RARMP and how it was considered is summarised in Appendix D.

In addition, a reference document, *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia*, was used to provide baseline information on non-GM cotton. The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

⁴ Hexima initially requested approval for the release of three lines but subsequently amended the application to one only.

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

A hazard (source of potential harm) may be an event, substance or organism (OGTR 2005). The hazard identification process resulted in the compilation of a list of 24 events that describe sets of circumstances (events) by which the proposed release could potentially give rise to adverse outcomes.

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

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

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

Twenty four events were characterised in the hazard identification process. These 24 events included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, produce unintended changes in the biochemistry, physiology or ecology of the GM cotton plants, or alter characteristics that may impact on spread and persistence of the GMO. In addition, consideration was given to the opportunity for gene flow to other organisms, and unauthorised activities.

Two of the 24 events characterised in the hazard identification process for the proposed release were identified as requiring further assessment. The potential adverse outcome associated with these events was toxicity to, or growth inhibition of, invertebrates and/or non-target microorganisms. This identified risk was assessed in comparison to the parent organism and other GM cotton lines previously approved for commercial release, in the context of the proposed containment measures and the environmental conditions in the regions where the release will occur. The consequence and likelihood assessments used to derive risk estimates from these two events are summarised in Table 1 (the detailed risk assessments are in Chapter 3).

More information on the remaining 22 events that were considered not to give rise to an identified risk is provided in Chapter 2.

If a risk is estimated to be higher than **negligible**, risk treatment measures may be required to protect the health and safety of people or the environment. However, all risks were estimated to be negligible for this release.

Table 1 Summary table for the risk assessment

Event that may give rise to toxicity for, or growth inhibition of, invertebrates and/or non-target microorganisms	Consequence assessment	Likelihood assessment	Risk estimate	Risk evaluation
Event 1 Contact with or ingestion of GM cotton plant materials containing NAD1 protein by invertebrates.	Minor <ul style="list-style-type: none"> The NAD1 protein may be toxic to certain insects. 	Highly unlikely <ul style="list-style-type: none"> Limited exposure of insects to the NAD1 protein expected due to the small size and short duration of the proposed release. Low expression of the protein would further limit the level of exposure. Agronomic practices proposed by the applicant, specifically insecticide use, are expected to have a greater impact on invertebrate survival than the expression of the NAD1 protein in the GM cotton plants. 	Negligible	No specific risk treatment options are required, however, some conditions have been imposed to limit the release in time and space.
Event 2 Contact with NAD1 protein by non-target microorganisms.	Minor <ul style="list-style-type: none"> The NAD1 protein may be toxic to some non-target microorganisms (neutral, beneficial or pathogenic). 	Highly unlikely <ul style="list-style-type: none"> Limited exposure of non-target microorganisms to the NAD1 protein expected due to the small size and short duration of the proposed release. Low expression of the protein would further limit the level of exposure. 	Negligible	No specific risk treatment options are required, however, some conditions have been imposed to limit the release in time and space.

Risk management

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

The risk assessment considered two events that might lead to risk to the health and safety of people or the environment. The risk estimates for the adverse outcome associated with those two events are **negligible**, ie insubstantial with no present need to invoke actions for their mitigation. Therefore, no risk treatment measures for the identified risk have been imposed.

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

Licence conditions to manage this limited and controlled release

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

- surround each release site with a pollen trap of non-GM cotton
- locate the release sites at least 50 m away from natural waterways

- harvest cotton seed from the release separately from any other crop
- prohibit the use of cotton seed and other materials from the release in human food or animal feed
- destroy any plant materials remaining at the sites and clean the sites and any equipment used on the sites
- inspect the sites following harvest and cleaning, any areas used to clean equipment and any irrigation channels associated with the release
- destroy any volunteer plants prior to flowering
- conduct regular inspections of the release sites and other areas following harvest for at least 12 months and until six consecutive months have passed without any volunteer cotton plants.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, June 2001*; *Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been imposed 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 (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ (Food Standards Australia New Zealand), APVMA (Australian Pesticides and Veterinary Medicines Authority), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Health and Medical Research Council (NHMRC) and Australian Quarantine and Inspection Service (AQIS). Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies⁵.

The GM cotton line proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of a fungal growth-inhibiting substance, and therefore it is subject to regulation by the APVMA. Currently, APVMA is assessing a research permit application from Hexima for the proposed release. The Regulator has liaised closely with APVMA to ensure the thorough and coordinated assessment of these parallel applications.

No products from the release will be permitted for use in human food (or animal feed). Therefore, at this stage, no application has been made to FSANZ.

Identification of issues to be addressed for future releases

The risk assessment identified additional information that may be required to assess an application for a large scale release of this GM cotton line or to justify a reduction in the containment conditions, including:

- data on expression levels of the plant defensin NAD1 in the GM cotton line (in whole plants, leaves, pollen, stems, roots, seeds and bolls) and data on root exudation of NAD1

⁵ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>>.

- the mode of action of NAD1
- characterisation of the potential toxicity of NAD1 and the GM cotton line to vertebrates (including people)
- *in-vitro* experiments or field studies addressing the effect of NAD1 on species of invertebrates and non-target microorganisms such as mycorrhiza fungi and *Rhizobium* spp.
- agronomic characteristics indicative of weediness
- any effects resulting from stacking of the *nad1* gene with other introduced traits in commercially released GM cotton plants such as herbicide tolerance and insect resistance.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of a GM cotton line with enhanced resistance to certain fungal pathogens in the shires of Pittsworth, QLD, and Narrabri or Moree Plains, NSW, poses negligible risks to the health and safety of people and the environment posed by or as a result of gene technology.

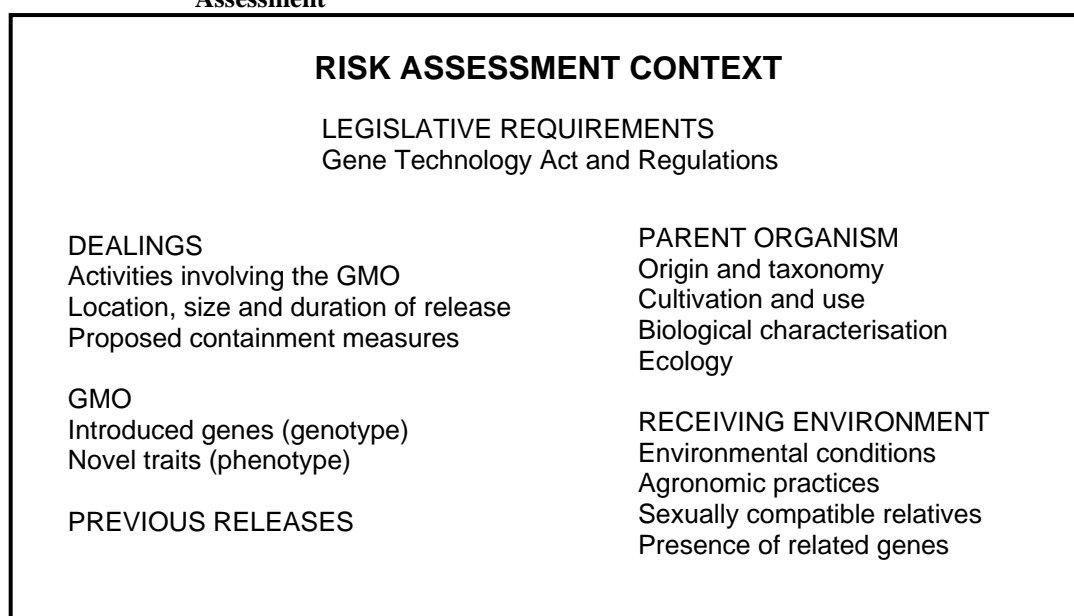
The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the proposed locations, size and duration requested by the applicant.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁶, details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

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



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

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

- the location, size and duration of the trial requested by the applicant
- containment measures for the GMO proposed by the applicant
- characteristics of the parent organism
- the nature and effect of the genetic modification
- the environmental conditions in the locations where the release would occur
- relevant agricultural practices

⁶ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/ir/process.htm>> and in the *Risk Analysis Framework* (OGTR 2005) <<http://www.gov.au/pdf/raffinal2.2.pdf>>.

- presence of related plants in the environment
- presence of the introduced or similar genes in the environment
- any previous releases of these or other GMOs relevant to this application.

4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and the local councils where the release will take place were consulted on matters relevant to the preparation of the RARMP. This advice, and where it was taken into account in the RARMP, is summarised in Appendix B.

5. In accordance with section 52 of the Act, the Regulator notified the public when the consultation version of the RARMP had been prepared and invited written submissions. Advice on the RARMP was also sought from the same experts, agencies and authorities as before. None of the latter raised any issues relating to risks to human health and safety and/or the environment that required further consideration (See Appendix C). Issues raised by a member of the public and how they were addressed in the RARMP are summarised in Appendix D.

Section 2 The application

6. Hexima initially proposed to release three GM cotton lines into the environment under limited and controlled conditions but subsequently reduced the request to one line.

2.1 The proposed location, size and duration

7. The release is approved to take place at two sites in the shire of Pittsworth, Queensland (QLD) and one site in the shire of Narrabri or Moree Plains, New South Wales (NSW) on a maximum total area of one hectare during each of the three summer cotton growing seasons between 2006 and 2009.

2.2 The proposed dealings

8. The purpose of the trial is to conduct early stage ('proof of concept') research to assess the extent of enhanced resistance to three fungal diseases and the agronomic performance of the GM cotton line under field conditions; to measure the expression levels of the introduced gene and to test for adverse impacts on selected beneficial soil microorganisms. Lint fibres may be tested for their quality characteristics. Seed will also be collected for further studies and possible future releases (subject to additional assessments and approvals).

9. The applicant proposed to assess the resistance of the GM cotton line to three agronomically important fungal pathogens causing black root rot (*Thielaviopsis basicola*), fusarium wilt (*Fusarium oxysporum* f.sp. *vasinfectum*) and verticillium wilt (*Verticillium dahliae*)⁷. The proposed sites have a history of high incidence of these diseases. In all cases, plants of the non-GM parent cultivar Coker will be planted, sampled and assessed alongside the GM cotton line to serve as a reference within the trial. At the locations that are infested with *F. oxysporum* f.sp. *vasinfectum* and *V. dahliae*, non-GM industry standards for resistance to fusarium wilt (ie varieties Sicot 189 and/or Delta Emerald) and verticillium wilt (Sicala V2), will also be included in the trial for comparison. Methods for testing disease resistance in some or each of the three growing seasons of the trial include:

⁷ In this RARMP, these pathogens are regarded as 'target' organisms, all other microorganisms as 'non-target'. The latter group may include other pathogenic, neutral and beneficial microorganisms.

- destructive sampling of plants early in the growing season and determining the level of root blackening to assess resistance to black root rot
 - recording the number of surviving plants throughout the growing season and, at the end of the growing season, assessing the level of vascular browning to determine resistance to fusarium wilt
 - assessing the extent of vascular discolouration at the end of the growing season in order to determine resistance to verticillium wilt.
10. Measurement of agronomic characteristics such as emergence, plant height, flowering time, the number of bolls set and the number of seeds produced will be taken during the trial.
11. The applicant intends to measure the expression level of the introduced gene in different plant tissues and at different developmental stages during each of the growing seasons.
12. The introduced gene codes for a protein, NAD1 that inhibits the growth of a range of fungi. In order to test for any effect on beneficial soil organisms, the roots from a number of GM and non-GM cotton plants from the trial sites will be assessed for the presence of arbuscular mycorrhizal colonisation during the first six to eight weeks of growth.
13. The applicant intends to test lint fibres for quality characteristics.
14. The applicant proposed to plant acid-delinted seed, grow the plants to maturity and harvest the seed-containing bolls by hand.
15. No products from the release will be allowed to be used for human food or animal feed.

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

16. The applicant proposed measures to limit the spread and persistence of the GM cotton line into the environment. These were taken into account in the risk assessment context (this chapter) and their suitability evaluated in the following chapters for limiting the release to the locations, size and duration proposed by the applicant.
17. The applicant proposed the following containment measures:
- location of the trial sites 50 m away from natural waterways and 3 km away from nature reserves, national parks or state forests
 - 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 the plants on the trial sites (GM and non-GM cotton plants)
 - cleaning of equipment of any viable plant material, ie seed, as soon as practicable after use
 - destruction of GM plant materials used in laboratory analysis by autoclaving
 - after harvest, destruction of all cotton plant materials remaining on the sites including non-GM control plants and the pollen trap and encouraging of germination of any seed remaining on the sites by cultivation and irrigation
 - transportation of GM cotton seed and plant materials to and from the proposed trial sites in accordance with OGTR transportation guidelines
 - monitoring the trial sites for 12 months post harvest in bimonthly intervals and destruction of any cotton volunteer plants

- storage of GM cotton seeds and other GM plant materials required for further study or future release in certified PC2 facilities.

Section 3 The parent organism

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

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

4.1 Introduction to the GMO

19. The GM cotton line for release contains the plant defensin gene, *nad1*, derived from *Nicotiana glauca*, ornamental tobacco. This gene encodes a plant defensin protein, NAD1, which *in-vitro* inhibits the growth of fungi, including three major fungal pathogens of cotton, *T. basicola*, *F. oxysporum* f.sp. *vasinfectum* and *V. dahliae*. Plant defensins are common in many horticultural and crop plants such as dahlia, tomato, peas and wheat.

20. The GM cotton line with enhanced fungal resistance also contains a selectable marker gene, *nptII*, encoding an antibiotic resistance protein, which was derived from *Escherichia coli*, a common gut bacterium.

21. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genes are also present in the GM cotton line. These are derived from cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*, a common soil bacterium.

4.2 Introduction to plant defensins

22. Plant defensins are found in many parts of plants including seeds, leaves, tubers and bark (Lay & Anderson 2005). Some plant defensins are present at high levels in edible parts of crop plants including potato tubers, corn and capsicum (Colilla et al. 1990; Moreno et al. 1994; Meyer et al. 1996; Balandin et al. 2005). A number of plant defensins are constitutively expressed whereas others are expressed upon external stimuli (eg wounding, contact with an ectomycorrhizal fungus or challenge with a pathogen) (eg Penninckx et al. 1996; Johansson et al. 2004).

4.2.1 Structure of the plant defensins

23. Mature plant defensins are highly basic, 45-54 amino acid residues long peptides involved in plant defence (eg Garcia-Olmedo et al. 1998; Lay et al. 2003a). Eight cysteine residues, two glycine residues in positions 13 and 34 in relation to the plant defensin Rs-AFP1 isolated from radish (*Raphanus sativus*), a serine residue in position 8, an aromatic residue in position 11 and a glutamate residue in position 29 are conserved in plant defensins. These amino acid residues are known or implicated in stabilising the three-dimensional structure of the plant defensins (Broekaert et al. 1995; Lay & Anderson 2005). The three dimensional structure of the plant defensins that have been analysed so far comprises a triple-stranded, antiparallel β -sheet and a single α -helix in parallel with the β -sheet (Broekaert et al. 1995; Lay et al. 2003b). The α -helix is connected by two disulfide bridges to the third β -strand and a third disulfide bridge stabilises the overall fold, resulting in a structural motif known as the cysteine-stabilised $\alpha\beta$ -motif (CS $\alpha\beta$) (Cornet et al. 1995). This structural motif also occurs in a range of other biologically active proteins such as the insect defensins, the antifungal insect

peptide drosomycin and certain scorpion toxins (Bonmatin et al. 1992; Landon et al. 1997; Thomma et al. 2002).

24. Plant defensins can be divided into two groups. Both groups contain an N-terminal signal sequence targeting the protein to the endoplasmic reticulum followed by the core domain. One group contains an additional C-terminal domain that is thought to be involved in vacuolar targeting (Lay & Anderson 2005). Most plant defensins included in the latter group have been isolated from the flowers of solanaceous species (Lay et al. 2003a).

4.2.2 Biological activity of plant defensins

25. Individual members of the plant defensin protein family generally display one or more of a wide range of biological activities: many plant defensins inhibit the growth of a range of fungi (Terras et al. 1992; Almeida et al. 2000; Wisniewski et al. 2003), some inhibit the growth of bacteria (Moreno et al. 1994), some inhibit proteinases (Wijaya et al. 2000; Melo et al. 2002), α -amylases (Bloch & Richardson 1991) or protein synthesis (Mendez et al. 1996) and some are sodium channel blockers (Kushmerick et al. 1998) or calcium channel blockers (Spelbrink et al. 2004). Plant defensins have also been implicated in the increased tolerance to zinc in a zinc hyper-accumulating plant, *Arabidopsis halleri* (Mirouze et al. 2006).

26. This range of biological activities displayed by plant defensins seems to be conferred by the amino acid residues that are **not** highly conserved, especially those in the loop regions between the individual β -strands as well as the β -strands and the α -helix (Lay et al. 2003b). For example, the amino acid sequence of the two plant defensins Rs-AFP1 and Rs-AFP2 isolated from radish (*Raphanus sativus*) differs only in two amino acid residues, yet the antifungal activity of Rs-AFP2 is, depending on the test fungus, 2-30 times higher than that of Rs-AFP1 (Terras et al. 1993). Mutational analysis of the plant defensin Rs-AFP2 demonstrated the importance of two basic amino acid residues located in two structurally adjacent sites for antifungal activity against *Fusarium culmorum* (De Samblanx et al. 1997).

27. Laboratory experiments with GM plants transformed with plant defensin genes have been reported in the literature since 1995. Most experimenters used the model plant tobacco (Terras et al. 1995; Park et al. 2002; Lai et al. 2002); crop plants were also defensin modified, eg rice (Kanzaki et al. 2002), potato (Gao et al. 2000) and canola (Wang et al. 1999). An increase in fungal resistance was observed when the defensin-GM plants were challenged with fungal or bacterial pathogens.

4.2.3 Mechanism of action of plant defensins

28. So far, the exact mechanism of action of plant defensins is unknown (Lay & Anderson 2005). It is currently accepted that fungal growth inhibiting plant defensins interact with membrane components resulting in increased membrane permeability of the fungal cells. In *in-vitro* experiments, the growth inhibitory effect of plant defensins on fungi decreases with increasing ionic strength, especially, when the concentration of calcium ions is increased. This effect depends on the test fungus rather than on the plant defensin and therefore, it is believed that the site of recognition on the fungus is altered rather than the plant defensin itself.

29. The mechanism of growth inhibition of plant defensins on fungi has been investigated in three cases, eg in the case of the growth inhibiting plant defensin Dm-AMP1 from *Dahlia merckii* (Thevissen et al. 2000). The growth of *Neurospora crassa* hyphae and *Saccharomyces cerevisiae* cells is inhibited by Dm-AMP1. Labelled Dm-AMP1 binds strongly to components in the membranes of the two fungal species tested and can be efficiently competed for by preincubation of the fungi either with unlabelled Dm-AMP1 or with highly similar plant defensins. Plant defensins with limited similarity to Dm-AMP1 as

well as structurally unrelated antimicrobial peptides are not able to compete for the binding site. In the course of the experiment, two yeast mutant strains were identified that are resistant to Dm-AMP1. Both strains carry a mutation in the gene encoding an enzyme catalysing the final step in the production of a membrane component, mannose-(inositol-phosphate)₂-ceramide. This finding indicates that Dm-AMP1 interacts with the membrane component either directly or indirectly. Similarly, Rs-AFP2 interacts with sphingolipids in the membranes of *Pichia pastoris* (Thevissen et al. 2004) and growth inhibition of Rs-AFP2, Hs-AFP1 and Dm-AMP1 depends on the presence of certain sphingolipids in the membrane of *Neurospora crassa* (Ferket et al. 2003). In another study, Thevissen et al (2004) showed that, in their purified form, both an insect and a plant defensin – Heliomycin from *Heliothis virescens* and Rs-AFP2, respectively – bound to *P. pastoris* glucosylceramides. Heliomycin, but not Rs-AFP2 bound to soybean glucosylceramides, implicating different motifs within the glucosylceramides in the binding of the two defensins. This conclusion is also supported by the finding that binding of one defensin could not be competed for by the respective other defensin.

30. Antifungal plant defensins generally exhibit their activity against a limited range of fungi. For example, the defensin gene *Dm-amp1* from *Dahlia merckii* was used to produce GM aubergine plants (Turrini et al. 2004a). Infection of GM aubergine plants with the mycorrhiza fungus *Glomus mosseae* was identical to the non-GM aubergine plants. On the other hand, growth of the phytopathogenic fungus *Verticillium albo-atrum* was reduced in the interaction with GM aubergine plants when compared to the non-GM control (Turrini et al. 2004b).

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

31. Some details of the gene constructs, including the plasmid map and the *nad1* transcription termination sequence, preliminary molecular characterisation of the genetic modifications (Southern blot analysis), western blot analysis and inhibition studies on selected non-target organisms have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was made available to the prescribed expert groups and agencies that were consulted in the preparation and finalising of the risk assessment and risk management plan (RARMP). The information was also considered during the preparation and finalising of the RARMP.

4.3.1 Source organisms for the introduced genes and regulatory sequences

32. Genetic materials from various sources were introduced into cotton (see Table 2).

Table 2 Source organisms of the introduced genes and regulatory sequences

Genetic element	Source organism	Function in the source organism	Hazardous characteristics of the source organism	Comments
35S promoter	Cauliflower mosaic virus	Transcription initiation	Plant pathogen on Brassicaceae	This regulatory sequence does not cause disease symptoms.
<i>nad1</i> gene	<i>Nicotiana glauca</i>	Plant defensin	Poisonous plant	Toxicity mainly inferred by nicotine and other alkaloids.
Terminator	Commercial confidential information	Transcription termination	Commercial confidential information	Commercial confidential information
<i>nos</i> promoter	<i>Agrobacterium tumefaciens</i>	Transcription initiation	Plant pathogen causing crown gall disease	This regulatory sequence does not cause disease symptoms.
<i>nptII</i> gene	<i>Escherichia coli</i>	Antibiotic resistance	Facultative human pathogen	NPTII does not cause ill effects in humans.
<i>nos</i> terminator	<i>Agrobacterium tumefaciens</i>	Transcription termination	Plant pathogen causing crown gall disease	This regulatory sequence does not cause disease symptoms.

33. The genetic elements, their sources and function in the source organisms are discussed below.

4.3.2 The plant defensin gene *nad1* from ornamental tobacco and the encoded protein

34. The gene construct used to genetically modify cotton contains the *nad1* gene. The introduced plant defensin gene, *nad1* (GenBank accession no AF509566), is derived from *N. alata*, a poisonous ornamental plant (Lay et al. 2003a). The toxicity of *N. alata* is in most part conferred by nicotine and other alkaloids.

35. The mature NAD1 is a 5.3 kDa, basic and cysteine-rich protein. It shows clear but limited sequence identity to other members of the plant defensin protein family (also called γ -thionins). It is produced with a N-terminal signal peptide targeting the protein to the endoplasmic reticulum, a central defensin domain and a C-terminal prodomain that may be involved in vacuolar targeting (Lay et al. 2003a).

36. In *N. alata*, the NAD1 protein is developmentally expressed and found predominantly in the vacuoles of certain cells in flowers, especially in developing flowers. Low levels of the transcript were detected in roots but not in leaves (Lay et al. 2003a).

37. The mode of action of NAD1 is currently unknown. Interaction of plant defensins from other plants with membrane components of affected fungi resulting in membrane permeabilisation have been demonstrated (for details see Section 4.2.3).

Toxic or growth inhibitory effects of purified NAD1 protein

38. The applicant supplied data on preliminary tests that indicate that purified NAD1 has no effect on HeLa cells, yeasts (*Saccharomyces cerevisiae*, *Candida albicans* and *Pichia pastoris*) and on both Gram-positive and Gram-negative bacteria.

39. Purified NAD1 was used to assess its effect on various filamentous fungi. At 10 $\mu\text{g/mL}$, the growth of the filamentous fungi *F. oxysporum* f.sp. *dianthi* race 2 was completely inhibited and growth of *Botrytis cinerea* was inhibited by 96% (Lay et al. 2003a). The applicant reported growth inhibition close to 100% of *V. dahliae* and *T. basicola* and 80% of *F. oxysporum* f.sp. *vasinfectum* at a concentration of 2 μM . The applicant found no morphological difference of *F. oxysporum* f.sp. *vasinfectum* or *V. dahliae* treated with NAD1 compared to the untreated controls when viewed with a scanning electron microscope.

Allergenicity of NAD1

40. Comparisons of the NAD1 amino acid sequence to amino acid sequences in the publicly available Farrp Allergen Database (<http://www.allergenonline.com>) was carried out. No matches to known allergens greater than four contiguous identical amino acids were identified, which is below the threshold of six contiguous identical amino acids for allergen epitope screenings suggested by the FAO/WHO (2001) (information supplied by the applicant). It is unlikely for the NAD1 protein to be allergenic as its molecular weight of 5.3 kDa is below the typical molecular weight range (15-70 kDa) of allergens and it also lacks regions of glycosylation, another characteristic of typical allergens. So far, no known plant defensin has been listed as an allergen.

41. An anther-specific cell wall protein from sunflower (*Helianthus annuus*) contains a defensin-like domain (Domon et al. 1990), which shows considerable similarity to the defensin-like domain present in some pollen proteins. These include ART V 1 from mugwort (*Artemisia vulgaris*) and PAR H 1 from feverfew (*Parthenium hysterophorus*) that are known allergens (Domon et al. 1990; Gupta et al. 1996; Himly et al. 2003).

42. The defensin-like portion of those allergens shows 28-34% sequence identity to NAD1 with the identities mainly restricted to structure stabilising amino acid residues (eight cysteine residues, Ser7, Thr9, Gly12, Lys22, Glu27 and Gly32 (numbering relative to NAD1)). In the glycoprotein PAR H 1, the carbohydrate moiety but not the defensin-like domain causes an immune response in humans (Gupta et al. 1996). ART V 1 is a hydroxyproline-rich glycoprotein with the two major variants ranging from approximately 12.9 to 13.5 M_r and from 14.0 to 16.3 M_r. Jahn-Schmid et al (2002) found that a single epitope (amino acid residues 22 – 36) within the defensin-like domain of the molecule was being used by 81% of the patients. The amino acid identity of ART V 1 with the NAD1 protein within the epitope characterised by Jahn-Schmid et al (2002) is 50%.

43. While some proteins containing a defensin-like domain are known allergens, there is no indication of any plant defensin being allergenic for humans.

44. The applicant intends to carry out digestibility assays using simulated gastric fluid if the GM cotton line performs well during the release.

4.3.3 The 35S promoter for *nad1* gene expression

45. Expression of the *nad1* gene is controlled by the 35S promoter from cauliflower mosaic virus (CaMV). CaMV is a plant virus that can infect cruciferous plants. Although CaMV is a plant pathogen, the regulatory sequence does not cause disease symptoms. The 35S promoter leads to constitutive expression of inserted genes in plants.

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

46. The GM cotton line also contains the *nptII* gene, which was isolated from the bacterial Tn5 transposon from *E. coli* (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII) that confers resistance to aminoglycoside antibiotics, eg kanamycin and neomycin. NPTII uses ATP to phosphorylate those antibiotics, thereby inactivating and preventing them from killing the NPTII-producing cells. *NptII* was used as a selectable marker gene during the laboratory stages of cotton plant tissue selection following genetic modification, allowing GM cells to grow in the presence of the antibiotic while inhibiting the growth of non-GM cells. The NPTII protein is in common use as a selectable marker in the production of GM plants (Miki & McHugh 2004).

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

Toxicity of NPTII

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

49. Humans (and, by implication, other animals) continually ingest kanamycin-resistant microorganisms, some containing the NPTII enzyme. The diet, especially raw salad, is the major source: estimated conservatively, each human ingests 1.2×10^6 kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998) with Flavell et

al. (1992) estimating about 10^{12} per person. Kanamycin-resistant bacteria have been isolated from soil, river water and sewage (Smalla et al. 1993).

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

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

Allergenicity of NPTII

52. The NPTII protein is approximately 29 kDa in size, which is within the typical size range of allergenic proteins. However, it does not possess glycosylation sites, is not stable in the mammalian digestive system and is heat labile, decreasing the probability that it is allergenic (US FDA 1998; Fuchs et al. 1993a; FDA 1994; ANZFA 2001e; ANZFA 2001f). Fuchs et al reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both western blot and enzymatic activity (Fuchs et al. 1993b). Protein sequence comparisons using sequences from four separate protein databases (EMBL, GenBank, PIR29 and Swiss-Prot) indicated that NPTII does not have significant sequence identity to any known protein food allergens (Fuchs & Astwood 1996).

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

4.3.5 Regulatory sequences for the expression of the *nptII* gene

54. The bacterial *nptII* gene was modified by the addition of *A. tumefaciens* regulatory sequences (*nos* promoter and termination regions) to allow efficient expression in plant cells.

55. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Although *A. tumefaciens* is a plant pathogen, the regulatory sequences do not cause disease symptoms.

4.4 Method of genetic modification

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

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

58. The gene construct was generated using the disarmed binary vector, pBIN19 (pBIN19 T-DNA: GenBank accession number U12540) (Bevan 1984). A version of the plant defensin gene, *nad1*, the antibiotic selective marker gene, *nptII*, and regulatory sequences for the expression of the genes is present in the gene construct. The applicant states that partial sequencing in and near the inserted *nad1* gene revealed no base changes.

59. *A. tumefaciens* (strain LBA4404) was transformed by electroporation and vector-containing *A. tumefaciens* was used to infect hypocotyl sections of the parent cultivar Coker 315. Infected hypocotyl sections were plated individually onto growth media containing the antibiotic kanamycin. Those that were transformed were resistant to kanamycin and developed embryogenic calli that were selected and embryos germinated. Plants were regenerated and transferred onto soil in contained facilities. Selected plants were tested in fungal bioassays and the best performing plants selected for further characterisation.

60. The genetic modification has not been introduced into any of the Australian elite cotton cultivars; the GMO proposed for release are GM Coker plants.

4.5 Characterisation of the GM cotton line proposed for release

4.5.1 Stability and molecular characterisation

61. The GM cotton line has been propagated by self pollination through four generations in contained facilities in the course of their development. The applicant states that the GM cotton line is genetically stable based on NAD1 expression and that the plant defensin gene and antibiotic marker gene co-segregate.

62. The applicant has used Southern blot analysis and adaptor ligation PCR to estimate the copy number of the inserted genes. The applicant states that using Southern blot analysis, a single band was identified that contained the *nad1* gene. Further analysis by adaptor ligation PCR revealed a single insertion event of two copies of the T-DNA into the cotton genome. The two copies inserted in an inverted repeat with the left borders facing each other. The vector backbone did not insert; only DNA between the borders and the borders themselves inserted. The introduced DNA inserted into a region of the cotton genome that shows high similarity to a nicotinamide adenine dinucleotide (NADH) dehydrogenase gene from *Arabidopsis thaliana* (AC007730).

4.5.2 Characterisation of the phenotype of the GMO

63. The applicant states that under glasshouse conditions agronomic characteristics, eg fertility and seed setting, were the same in the GM cotton line compared to the non-GM parent cultivar Coker. The applicant proposes to compare agronomic data of GM and non-GM parent cotton lines under field conditions.

64. Expression of *nad1* was determined by immunoblot analysis and ELISA using defensin-specific antisera. The applicant states that the plant defensin gene in the GM cotton line is stably expressed. Expression in the GM cotton line was lower than that naturally occurring in *N. alata*. The applicant proposes to collect more data on the expression of NAD1 in various plant tissues and developmental stages under field conditions.

Toxicity and growth inhibitory effects of GM cotton plant materials

Effects on invertebrates

65. In a pilot experiment, leaf discs from one of the GM cotton lines were fed to neonates of the Lepidopteran insect, *Helicoverpa armigera*. The applicant reports no significant difference in the larval mass between the GM cotton line and the non-GM parent cultivar.

Effects on target organisms under glasshouse conditions

66. In collaboration with plant pathologists and industry partners, Australian cotton growers have established a disease ranking of various cotton varieties for fusarium wilt and verticillium wilt, respectively. Commercial cotton varieties with low to moderate disease resistance to either disease have been identified.

67. The GM cotton line was tested by the applicant in bioassays using *F. oxysporum* f.sp. *vasinfectum* infected soil under glasshouse conditions. Fusarium wilt susceptible cotton varieties Siokra 1-4 and Coker and the moderately resistant non-GM industry standard Sicot 189 were used as controls. The two susceptible varieties were the first to show disease symptoms, ie wilting of leaves, and had a higher disease score during the trial and a higher mortality rate than the GM cotton line and the moderately resistant variety.

68. In pilot glasshouse experiments, the level of resistance of the GM cotton line to *T. basicola* was compared to the non-GM parent cultivar. In those experiments, more GM cotton seedlings survived compared to non-GM seedlings. The number of *T. basicola* chlamydospores and the severity of black root rot disease symptoms in tap roots were lower and the root and shoot growth was higher in the GM cotton plants compared to the non-GM control plants. In those experiments, arbuscular mycorrhizal colonisation was not significantly different between GM and non-GM cotton plants.

69. The GM cotton line could not be tested for resistance to *V. dahliae* as the applicant was not able to develop a reliable bioassay under glasshouse conditions, even under conditions where disease pressure was high. In the field, plants would be assessed for verticillium wilt at the end of the growing season.

Section 5 The receiving environment

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

5.1 Relevant abiotic factors

71. The size and duration of the release are outlined in Section 2.1. The release is to occur in the shires of Pittsworth, QLD, and Narrabri or Moree Plains, NSW. These are commercial cotton growing regions and have typical climates for summer cotton growing regions in Australia, with warm summers and higher summer than winter rainfall (see Table 3).

Table 3 Climatic data for Pittsworth, Narrabri and Moree Plains.

	Pittsworth Yandilla St	Moree Post Office	Narrabri Post Office
Average daily max/min temperature (summer)	29.6°C/16.6 °C	34.4 °C /18.7 °C	33.3 °C /18.7 °C
Average daily max/min temperature (winter)	17.5 °C /5.7 °C	19.5 °C /4.3 °C	18.9 °C /4.5 °C
Average monthly rainfall (summer)	89.2 mm	64.1 mm	73.2 mm
Average monthly rainfall (winter)	37.6 mm	39.2 mm	45.7 mm

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

5.2 Relevant agricultural practices

5.2.1 General information

72. The applicant intends to treat the GM cotton in the same way as commercial non-GM or non-insect resistant GM cotton crops, except for the omission of the application of fungicides. The cotton plants in the release sites will therefore receive applications of water, fertilizers, herbicides, insecticides and subject to other agronomic management during the release.

73. With regard to the management of insect pests, the applicant proposed:

- treating seeds with a pre-emergent insecticide
- monitoring the proposed trial sites for insect pests from October to February twice a week and from March to April once a week
- spraying to control all pests as a commercial non-GM or non-insect resistant GM cotton crop with thresholds based on those recommended for non-GM cotton crops.

74. Therefore, the biotic interactions between the GM cotton plants and the proposed release sites are expected to be similar to those occurring already with non-GM or non-insect resistant GM cotton in these environments with the exception of the effect of the introduced plant defensin on certain organisms.

75. The trial sites were selected from fields known to be infested with *F. oxysporum* f.sp. *vasinfectum*, *V. dahliae* and/or *T. basicola*, respectively. According to information supplied by the applicant, one site is infested with *F. oxysporum* f.sp. *vasinfectum* and has been used for cotton variety testing for assessing resistance to fusarium wilt since 1994, another site is known for its infestation with *T. basicola* and some *F. oxysporum* f.sp. *vasinfectum* and the third site will be selected from farms in NSW which have been used for cotton variety testing for assessing resistance to verticillium wilt.

5.2.2 Presence of and current control strategies for black root rot in the receiving environment

76. Black root rot, caused by the fungal pathogen *T. basicola*, was - according to the Australian Cotton CRC (<http://cotton.crc.org.au>) - widespread in all cotton growing areas of NSW, except Menindee in 2001, and was present in all cotton producing areas in QLD in the 2002-2003 growing season. Disease surveys show a steady rise in the number of farms with the disease since it was first detected in 1989. Black root rot cannot be controlled using fungicides, the management of the disease relies on farm management practices that slow down or prevent pathogen infection, eg planting after cold weather has passed, planting varieties that are able to 'catch up' later in the season, pre-irrigation in preference to 'watering

up', planting of non-host crops such as cereals, sunflower, brassicas and onions for more than one season between cotton crops and adapting a 'come clean, go clean' strategy. All cotton varieties and many legumes are hosts for *T. basicola*. Therefore, legumes should be avoided as rotation crops in cotton growing regions infested with *T. basicola* (Allen et al. 2003).

5.2.3 Presence of and current control strategies for fusarium wilt in the receiving environment

77. Fusarium wilt was first detected in Australia in 1993 and the Australian Cotton CRC (<http://cotton.crc.org.au>) predicted that by 2010 the disease would be present on 90% of the farms in NSW. In QLD, no cases have been reported from the Emerald area, but other areas in QLD are affected. The disease is caused by the fungal pathogen *F. oxysporum* f.sp. *vasinfectum* and cannot be controlled by the use of fungicides. The severity of fusarium wilt is strongly influenced by environmental conditions and farm management (plant stress). The control strategies for fusarium wilt recommended by the Australian Cotton CRC include planting moderately resistant and avoiding susceptible cotton varieties, planting of surface-treated seeds, avoiding waterlogging and adapting a 'come clean, go clean' strategy. Cotton and also some weeds, eg bladder ketmia (*Hibiscus trionum*), sesbania pea (*Sesbania cannabina*) and dwarf amaranth (*Amaranthus macrocarpus*), are hosts for *F. oxysporum* f.sp. *vasinfectum* (Allen et al. 2003). Some non-GM cotton varieties are moderately resistant to *F. oxysporum* f.sp. *vasinfectum*.

5.2.4 Presence of and current control strategies for verticillium wilt in the receiving environment

78. Verticillium wilt is caused by the fungal pathogen *V. dahliae*. Its incidence has increased in recent years, mainly due to the use of susceptible varieties (Australian Cotton CRC, <http://cotton.crc.org.au>). Control strategies for verticillium wilt include planting of resistant cotton varieties, planting after cold weather has passed, avoiding waterlogging and adapting a 'come clean, go clean' strategy. *V. dahliae* has a wide host range including the crop plants sunflower, soybean and potato as well as weeds such as saffron thistle (*Carthamus lanatus*) and pigweed (*Portulaca oleracea*) (Allen et al. 2003). Some non-GM cotton varieties are resistant to *V. dahliae*.

5.3 Presence of related plants in the receiving environment

79. In the shires of the release, cotton (*G. hirsutum* and *G. barbadense*) is grown commercially. A number of herbicide tolerant and/or insect resistant GM cotton plants have previously been approved for commercial release (DIR 012/2002, DIR 022/2002, DIR 023/2002 and DIR 059/2005). These approvals include releases in the shires proposed in the current application. As a result of these commercial releases in southern Australia (south of latitude 22 °C), GM cotton plants are widespread in the agricultural environment, comprising about 90% of commercially grown cotton crops.

5.4 Presence of the introduced or similar genes in the environment

80. The NAD1 protein is produced naturally by *N. alata*, an ornamental tobacco. *N. alata* can be grown in most parts of southern Australia, growing best in cool climate. However, *N. alata* is not an important horticultural plant in Australia. Similar defensin proteins have been found in a wide range of plants (see Section 4.2) including a native Australian plant, *Hardenbergia violaceae* (Harrison et al. 1997).

81. The kanamycin resistance protein NPTII is produced by naturally occurring bacteria in soil and in the human gut and a number of GM cotton plants have previously been approved for commercial release that also produce the NPTII protein (for details see Section 4.3.4).

5.5 Presence of mycorrhizal fungi in the environment

82. As for many other plant species, successful cotton growth in most soils depends on the interaction with mycorrhizal fungi (Australian Cotton CRC, <http://cotton.crc.org.au>). The fungal species interacting with cotton roots, eg *Glomus mosseae*, grow intercellularly in the root cortex. They form arbuscules, highly branched, tree-like structures in intimate contact with the plant's plasma membrane within the cortex cells of the plant. The arbuscules are characteristic of this type of endophytic symbiosis called arbuscular mycorrhiza (AM) and are the sites of mineral exchange from the fungus to the plant and of carbohydrate exchange from the plant to the fungus. For the plant, improvement of phosphate uptake is the main advantage in engaging in AM (reviewed in Strack et al. 2003). AM fungi are widespread in the environment.

83. The AM fungal species *Glomus mosseae*, like many other AM fungi, is capable of colonising a variety of plant species. For example, Giovannetti et al. (2004) demonstrated that an isolate of *G. mosseae* is able to colonise cotton (*G. hirsutum*), eggplant (*Solanum melongena*), carrot (*Daucus carota*), lettuce (*Lactuca sativa*) and leek (*Allium porrum*).

84. AM fungi can influence the severity of plant diseases on cotton. Liu (1995) reported mutual inhibition of infection of cotton after simultaneous inoculation with AM fungi and *V. dahliae* as well as reduced disease incidence and disease indices of plants sequentially inoculated with AM fungi and *V. dahliae*. In another report, Zhengjia and Xiangdong (1991) showed reduced severity of fusarium wilt in cotton plants inoculated with *G. mosseae*.

Section 6 Australian and international approvals

6.1 Australian approvals of this GM cotton line

6.1.1 Previous releases approved by GMAC or the Regulator

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

6.1.2 Approvals by other Australian government agencies

86. The *Gene Technology Act 2000* is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication.

87. While the Regulator is responsible for identifying, assessing and managing 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.

88. Food Standard Australia New Zealand (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 proposed for release in food. Accordingly, the applicant has not applied to FSANZ for evaluation of materials from the trial for use in human food. However, FSANZ approval would need to be obtained before such materials could be used for this purpose.

89. In considering applications for registration or permits, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of herbicides, fungicides and insecticides, and their resistance management. The APVMA can impose conditions on the use of agricultural chemical products in registrations and permits. These conditions can include implementation of a

fungal resistance management or insect resistance management plan, and ongoing reporting on compliance and effectiveness.

90. The GM cotton line proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of a fungal growth-inhibiting substance, and therefore it is subject to regulation by the APVMA. Currently, APVMA is assessing a research permit application from Hexima for the proposed release. The Regulator has liaised closely with APVMA to ensure the thorough and coordinated assessment of these parallel applications.

6.2 International approvals

91. There has been no release of this GM cotton line or other defensin modified cotton lines world wide.

92. Other defensin modified crops have been and are currently being trialled in the United States of America (GM avocado, papaya and potato) and in Japan (GM rice) (see Table 4, information supplied by the applicant).

Table 4

Approved overseas trials of defensin modified GMOs

Plant defensin gene source	Recipient organism	Country
<i>Wasabia japonica</i> (wasabi)	Rice	Japan
<i>Arabidopsis thaliana</i>	Avocado	USA
<i>Raphanus sativus</i> (radish)	Papaya	USA
<i>Medicago sativa</i> (alfalfa)	Potato	USA

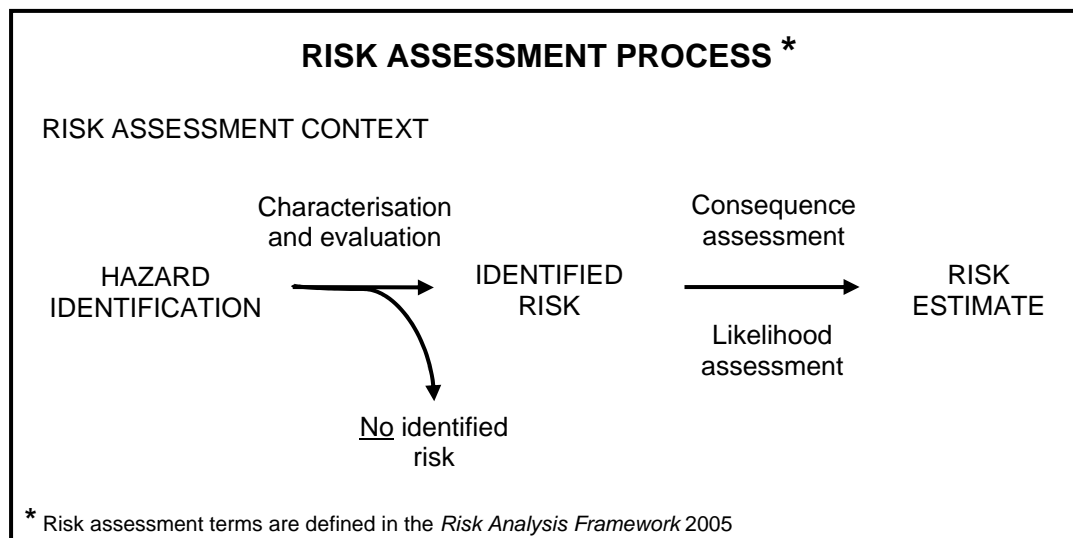
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Chapter 2 Risk assessment

Section 1 Introduction

93. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 2) considers risks from the proposed dealings with the GMO that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

Figure 2 The risk assessment process.



94. 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.

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

96. 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.

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

98. 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

99. The list of events compiled during hazard identification are characterised and evaluated 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.

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

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

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

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

103. There have been no releases of this GMO in Australia or overseas.

104. Events that are discussed in detail later in this Section are summarised below in Table 5. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. Twenty four events were characterised, of which two are considered to lead to an identified risk that required further assessment.

105. The prevalence of the *nptII* gene in the environment and the lack of evidence for toxicity or allergenicity of the NPTII protein are discussed in Chapter 1 Section 4.3.4. In addition, the potential effects of the *nptII* gene and its product were considered in detail in previous DIR applications including some for commercial releases. RARMPs for those DIR applications are available from the OGTR or from the website <<http://www.ogtr.gov.au>>. No risks have been identified from the expression of NPTII in GM-cotton. Therefore, the potential effects of NPTII will not be further assessed for this application, with the exception of events that are specific to the current DIR application.

Table 5 Summary of events that may give rise to an adverse outcome

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic to people	1. Ingestion of GM plant materials or food products containing the introduced protein, NAD1.	Toxicity for people	No	None of the GM plant materials from the proposed release would be used as human food.
	2. Contact with, or inhalation of, GM plant materials containing the introduced protein, NAD1.	Toxicity for people	No	Contact with, or inhalation of GM plant materials would be limited to people working with the GM plants and people living or working nearby. Contact would be further limited by the small size and short duration of the proposed field trial.
Section 2.2 Production of a substance allergenic to people	1. Use of GM plant materials containing the introduced proteins in food.	Allergic reactions in people	No	None of the GM plant materials from the proposed release would be used as human food. No plant defensin has been listed in a publicly available allergen database. The size of the NAD1 protein is small and no glycosylation sites have been identified.
	2. Contact with, or inhalation of, GM plant materials containing the introduced protein, NAD1.	Allergic reactions in people	No	None of the GM plant materials from the proposed release would be used as human food, animal feed or in the production of fabrics and/or other cotton products. Contact with, or inhalation of, GM plant materials would be limited to people working with the GMO or people living nearby. No plant defensin has been listed in a publicly available allergen database. The size of the NAD1 protein is small and no glycosylation sites have been identified. Contact would be further limited by the small size and short duration of the proposed field trial.
Section 2.3 Production of a substance toxic to, or growth inhibiting of, organisms other than people	1. Direct or indirect ingestion of GM plant materials containing NAD1 by vertebrates.	Toxicity for vertebrates	No	None of the GM plant materials from the proposed release would be used as animal feed. Mammals generally do not feed on cotton plants as they find them unpalatable. Exposure of wild vertebrates to the GM cotton lines would be limited by the small size and short duration of the proposed release.
	2. Contact with or ingestion of GM plant materials containing NAD1 by invertebrates.	Toxicity for, or growth inhibition of, invertebrates	Yes	See Chapter 3, event 1
	3. Contact with GM plant materials containing NAD1 by microorganisms.	Toxicity for, or growth inhibition of, non-target microorganisms	Yes	See Chapter 3, event 2

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.4 Spread and persistence of the GM cotton in the environment	1. Expression of the <i>nad1</i> gene construct increasing spread and persistence of the GM cotton plants through enhanced fungal resistance.	Weediness	No	Non-GM cotton varieties moderately resistant and resistant to fusarium wilt and verticillium wilt, respectively, have been grown for years without becoming weeds. The GM cotton line proposed for release is expected to show lower levels of disease resistance to these diseases. The modification is not expected to alter susceptibility to the factors that limit the spread and persistence of cotton in the areas proposed for release, which are temperature, soil type and water and nutrient availability. The release would be of small size and short duration and the applicant proposes measures to limit the spread and persistence of the GM cotton line to the proposed release.
	2. Expression of the <i>nad1</i> gene construct increasing spread and persistence of the GM cotton plants through cross tolerance to other environmental stresses.	Weediness	No	Extensive literature search did not reveal any reports showing that NAD1 or any other plant defensin could cause cross tolerance to abiotic environmental stresses. The potential of NAD1 conferring cross tolerance to biotic stresses other than infection by target fungi is addressed in Chapter 3. GM cotton's susceptibility to the main factors limiting the spread and persistence of cotton in the areas proposed for release (temperature, soil type and water and nutrient availability) are not expected to be altered. The release would be of small size and short duration and the applicant proposes measures to limit the spread and persistence of the GM cotton line.
	3. Dispersal of GM seed or other GM plant materials during transport, research, storage or through equipment.	Weediness	No	The applicant proposes to transport and store all GM plant materials including seeds according to OGTR guidelines, to clean equipment on site and to burn all plant material remaining on the sites at the end of each growing season. Additional research would be carried out in certified PC2 facilities and any GM plant waste materials would be autoclaved.
	4. Dispersal of GM plant materials (including seed) via flooding.	Weediness	No	The sites would be located above flood level and at least 50 m away from natural waterways to limit dispersal via flooding. Once established, plants within the pollen trap would act as a physical barrier to GM plant materials being dispersed.
	5. Increased exposure of vertebrates (including people), invertebrates and non-target microorganisms to NAD1 as a result of spread and persistence of GM cotton plants in the environment.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for, or growth inhibition of, invertebrates and/or microorganisms	No	The release would be of small size and short duration and the applicant proposes measures to limit the spread and persistence of the GM cotton line. The potential effects on vertebrates (including people) were discussed in sections 2.1, 2.2 and 2.3.1; the potential effects on invertebrates and microorganisms are addressed in Chapter 3, events 1 and 2.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Gene flow by vertical gene transfer (ie via pollen flow)	1. Gene transfer to native <i>Gossypium</i> species.	Weediness	No	Well established genetic incompatibility prevents vertical gene transfer to native <i>Gossypium</i> species.
	2. Expression of the <i>nad1</i> gene in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (including commercially released GM cotton lines) resulting in increased exposure of vertebrates (including people), invertebrates and microorganisms.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for, or growth inhibition of, invertebrates and/or microorganisms, Weediness, Increased disease burden	No	Outcrossing to other <i>G. hirsutum</i> and <i>G. barbadense</i> including commercial cotton crops would be limited due to the small size, short duration and containment measures proposed by the applicant. Multiple applications of insecticide sprays would further limit outcrossing. Therefore, any exposure of vertebrates (including people) to the NAD1 protein as a result of outcrossing would be limited. The factors limiting the spread and persistence of cotton are temperature, soil type and water and nutrient availability.
	3. Expression of the introduced genes in other <i>G. hirsutum</i> or <i>G. barbadense</i> cotton plants (including commercially released GM cotton lines) resulting in altered dormancy, seed viability, germination rates and/or seedling viability as a result of gene transfer.	Weediness	No	Outcrossing to other <i>G. hirsutum</i> and <i>G. barbadense</i> including commercial cotton crops would be limited due to the small size, short duration and containment measures proposed by the applicant. NAD1 is not expected to alter seed dormancy, seed viability, germination rates and/or seedling viability, or susceptibility to the factors that limit the spread and persistence of cotton in the areas proposed for release (temperature, soil type and water and nutrient availability).
	4. Presence of the introduced regulatory sequences in <i>G. hirsutum</i> or <i>G. barbadense</i> plants as a result of gene transfer.	Unpredictable effects	No	Outcrossing to other <i>G. hirsutum</i> and <i>G. barbadense</i> including commercial cotton crops would be limited due to the small size, short duration and containment measures proposed by the applicant, and by insecticide spraying. The introduced regulatory sequences are not known to behave any differently than endogenous regulatory sequences in plants.
Section 2.6 Gene flow by horizontal gene transfer	1. Presence of the <i>nad1</i> gene, or the introduced regulatory sequences, in other organisms as a result of gene transfer.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for or growth inhibition in invertebrates and/or microorganisms, Weediness, Increased pathogenicity, Increased disease burden	No	The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Gene transfer from plants to bacteria has not been demonstrated under natural conditions.
Section 2.7 Unintended changes in toxicity and/or allergenicity	1. Altered levels of innate toxic or allergenic compounds as a result of random insertion of the gene construct into the cotton genome during genetic modification.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for or growth inhibition in invertebrates and/or microorganisms, Weediness	No	There is no evidence of altered toxicity and/or allergenicity of the GM cotton line proposed for release except for the enhanced resistance to certain fungal pathogens. In addition, the release would be of small size and short duration. Information on toxicity and allergenicity of the GM cotton plants would be a prerequisite for a large scale release.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.8 Unintended changes in biochemistry, physiology or ecology	1. Altered biochemistry, physiology or ecology of the GM cotton line resulting from expression of the introduced genes.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for, or growth inhibition of, invertebrates and/or microorganisms, Weediness	No	The applicant states that there is no evidence of altered biochemistry, physiology or ecology of the GM cotton line compared to the parent cultivar with the exception of enhanced resistance to certain fungal pathogens. One of the aims is to measure agronomic characteristics during the proposed release.
	2. Expression of the introduced genes leading to altered sensitivity to other diseases.	Increased disease burden, Weediness	No	The applicant states that there is no evidence of altered sensitivity of the GM cotton line proposed for release to other diseases. The release would take place on three different sites and this way, the GM cotton line would be exposed to a variety of pathogens. One of the applicant's aims from the proposed release is the evaluation of the agronomic performance of the GM cotton line.
Section 2.9 Unintended effects on existing pests or pathogens	1. Decreased use of fungicide sprays on the GM cotton resulting in the increased prevalence of unaffected pathogenic Oomycetes and/or fungi.	Increased disease burden	No	The release would be of small size and short duration and the applicant proposes to manage the trial sites like normal cotton crop (except for the omission of fungicide applications). Effects would be confined to the areas of the proposed release.
Section 2.10 Secondary impacts	1. Development of resistance in fungal pathogens to plants expressing NAD1.	Loss of fungal resistance trait efficacy, Increased disease burden	No	The release would be of small size and short duration and the applicant proposes to manage the trial sites like normal cotton crop (except for the omission of fungicide applications). Consideration of efficacy is the responsibility of the APVMA.
	2. Adverse impact on populations of organisms that interact with affected organisms.	Toxicity for or allergic reactions in vertebrates (including people), Toxicity for, or growth inhibition of, invertebrates and/or microorganisms, Adverse impact on post-harvest crops	No	The release would be of small size and short duration and the applicant proposes to manage the trial sites like normal cotton crop (except for the omission of fungicide applications). Effects, if any, would be confined to the areas of the proposed release.
Section 2.11 Unauthorised activities	1. Use of GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.10	No	The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic to people

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

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

2.1.1 Ingestion of GM plant materials containing the introduced protein, NAD1

108. The GM cotton line differs from non-GM cotton in the expression of two additional proteins, the NAD1 and NPTII proteins. As explained in paragraph 105, only the NAD1 protein will be considered further. The introduced proteins present in the GM cotton line are the same or similar to those present in many plants or microorganisms. People are exposed to the introduced proteins or similar proteins through normal diet or the environment. There are no reports of toxicity associated with the introduced proteins.

NAD1 protein

109. An extensive search of the literature did not reveal any reports that imply NAD1 or any other plant defensin as a toxin. Plant defensin genes similar to *nad1* are expressed in a wide range of plants. The edible parts of many crop plants, eg maize, capsicum and peas, contain high levels of plant defensins (Kushmerick et al. 1998; Almeida et al. 2000; Cordts et al. 2001) (for details see Chapter 1 Sections 4.2 and 4.3.2).

Additional considerations

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

111. The applicant does not intend to use GM plant materials (including cotton seed oil or linters) from the proposed release in human food or as animal feed, but to destroy those remaining on the trial sites after collecting GM plant materials for analysis and harvesting seed. Ingestion of materials from the GM cotton line containing the NAD1 protein is not expected to occur from the proposed release.

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

2.1.2 Contact with, or inhalation of, GM plant materials containing the introduced protein, NAD1

113. Dermal and inhalation toxicity studies have not been conducted with the NAD1 protein. However, on the basis of the presence of these or similar proteins in many plant species, it is also expected to be of very low acute dermal and inhalation toxicity. In *N. alata*, the NAD1 protein is retained within the plant cells and the same is expected in the GM cotton cells (for more information see paragraph 140). Therefore, dermal and inhalation contact will only

occur when the plant cells have been damaged or broken or via pollen. People working with damaged GM cotton plants may come into contact with the introduced protein during handling and/or processing the GM cotton or its products, including lint, that contain the proteins. People living or working nearby could come into contact with GM cotton pollen. However, cotton pollen is relatively large and heavy, and not easily dispersed by wind (OGTR 2002).

114. Since the proposed release is of small size and short duration (for details see Chapter 1 Section 2.1), the frequency and duration of contact with the introduced proteins is expected to be very limited. In addition, the applicant does not intend to use any cotton plant materials from the proposed release in human food, animal feed or for the production of fabrics and/or other cotton products.

115. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, the introduced proteins will not be further assessed.

2.2 Production of a substance allergenic to people

116. The possibility that exposure of people to the NAD1 protein expressed by the GM cotton plants may result in an allergic reaction is considered. Routes of exposure to the NAD1 protein could include consumption of food containing cotton products or dermal contact with material from cotton plants such as fibres or pollen.

2.2.1 Use of GM plant material containing the introduced proteins in food

117. The NAD1 protein has none of the characteristics of typical allergens (for details see Chapter 1 Section 4.3.2) and no other plant defensin has been reported as an allergen.

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

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

2.2.2 Contact with, or inhalation of GM plant materials containing the introduced proteins

120. As mentioned in event 2.1.2, there would be some contact between a few people and GM cotton materials during the proposed release. There are no reports of NAD1 causing allergic reactions in people, nor does NAD1 have the characteristics associated with known allergens (for details see Chapter 1 Section 4.3). As a result, it is unlikely that contact with GM plant materials, including lint, would cause allergic reactions in people.

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

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

2.3 Production of a substance toxic to, or growth inhibiting for, organisms other than people

123. A range of organisms may be exposed directly or indirectly to NAD1. Through biotic interactions with GM cotton plants organisms may be exposed directly to NAD1 (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure will include organisms that feed on organisms that feed on GM-cotton or degrade it (vertebrates, insects, fungi, Oomycetes and/or bacteria).

2.3.1 Direct or indirect ingestion of GM plant materials containing NAD1 by vertebrates other than people

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

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

126. Contact with the GM plant materials containing the introduced proteins would be limited because of the small scale and short duration of the proposed release, and none of the materials would be used as animal feed.

127. Therefore, **no risk is identified** and the potential for toxicity for vertebrates other than people as a result of direct or indirect indigestion of the NAD1 protein will not be further assessed.

2.3.2 Contact with or ingestion of GM plant materials containing NAD1 by invertebrates

128. Some plant defensins show *in-vitro* inhibitory effects on enzymes that are present in the guts of insects and there is the potential of NAD1 being toxic or growth inhibitory to certain invertebrates. Search of the literature identified a publication that mentioned possible effects of the NAD1 protein on the cotton pests *Helicoverpa armigera* and *H. punctigera*, but no data were presented to support this claim (Lay et al. 2003b). Growth inhibition of an insect species by a plant defensin other than NAD1 has been demonstrated (Chen et al. 2002).

129. Therefore, **a risk is identified** for toxicity or growth inhibiting activity of NAD1 on invertebrates resulting from exposure to the GM cotton line. The level of risk of toxicity or growth inhibiting activity to invertebrates from this event is estimated in Chapter 3 as event 1.

2.3.3 Contact with GM plant materials containing NAD1 by microorganisms

130. *In-vitro*, the purified NAD1 protein is known to inhibit the growth of a range of fungi other than the three pathogens of cotton plants, which are the primary targets of the NAD1 protein (*F. oxysporum* f.sp. *vasinfectum*, *V. dahliae* and *T. basicola*) in the GM cotton line (Lay et al. 2003a).

131. Therefore, **a risk is identified** for toxicity or growth inhibiting activity of NAD1 on non-target microorganisms resulting from exposure to the GM cotton line. The level of risk of

toxicity or growth inhibiting activity to non-target microorganisms from this event is estimated in Chapter 3 as event 2.

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

2.4.1 Expression of the *nad1* gene construct increasing spread and persistence of the GM cotton plants through enhanced fungal resistance

132. The GM cotton line produces the NAD1 protein, which inhibits the growth of certain fungal pathogens, including major pathogens of cotton crops. In an environment in which pathogenic fungi that can be controlled by NAD1 were the main factors limiting the spread and persistence of cotton, expression of NAD1 could result in weediness of the GM cotton plants.

133. In the case of verticillium wilt and fusarium wilt there are already some non-GM cotton varieties resistant or moderately resistant to the disease. These varieties have been grown for many years without becoming weedy. According to the applicant, some commercial non-GM varieties with resistance to fusarium or verticillium wilt are expected to show a higher survival rate than the GM cotton plants in comparisons that would be made during the proposed release. This expectation is in line with the results obtained in glasshouse experiments (for details see Chapter 1 Section 4.5.2).

134. The main factors limiting the spread and persistence of cotton in the areas proposed for release are temperature, soil type and water and nutrient availability (OGTR 2002). Expression of the introduced gene is not expected to alter the susceptibility of the GM cotton to these factors. In addition, the release would be of limited size and short duration and the applicant proposes a number of measures to limit the spread and persistence of the GM cotton line proposed for release (for details see Chapter 1 Section 2.3).

135. Therefore, **no risk is identified** and the potential for the spread and persistence of cotton as a result of the expression of the NAD1 protein will not be further assessed.

2.4.2 Expression of the *nad1* gene construct increasing spread and persistence of the GM cotton plants through cross tolerance to other environmental stresses

136. The GM cotton line produces the NAD1 protein, which enhances resistance to certain fungal pathogens. It could be possible that the NAD1 protein confers cross tolerance to other environmental stresses, which could lead to increased spread and persistence of the GM cotton plants.

Cross tolerance to abiotic environmental stresses

137. If NAD1 conferred cross tolerance to abiotic stresses such as cold, drought or waterlogging, an increase in the spread and persistence of the GM cotton line could occur.

138. Extensive search of the literature did not reveal any reports implicating the *nad1* gene or its product in conferring cross tolerance to abiotic environmental stresses. Two reports were identified, in which the authors found induction of expression of other plant defensins during cold acclimation of winter wheat (*Triticum aestivum* L.) (Koike et al. 2002; Gaudet et al. 2003). Only one of the two studies addressed the possible function of the identified plant defensin, TAD1. TAD1 was demonstrated to have antibacterial activity but lacked antifreeze activity, implying that the plant defensin was not involved in conferring cold tolerance (Koike et al. 2002). Another report showed the involvement of plant defensins for zinc tolerance in the zinc hyper-accumulating plant *Arabidopsis halleri* (Mirouze et al. 2006). However, NAD1 is only distantly related to these plant defensin core domains (with 30% amino acid sequence identity, alignment with ClustalW; <<http://www.ebi.ac.uk/clustalw/#>>) and unlikely to confer tolerance to zinc.

Cross tolerance to biotic environmental stresses other than infection by target fungi

139. The potential of NAD1 enhancing resistance to herbivorous insects or microorganisms other than the target fungi is discussed in Sections 2.3.2 and 2.3.3 and will be assessed further in Chapter 3. If NAD1 did enhance insect resistance or resistance to microorganisms other than the target fungi, there would be the possibility of increased spread and persistence of the GM cotton line. Several insect resistant GM cotton lines have been approved for commercial release in southern Australia since 1996 including in the shires proposed for release (DIR 12/2002, DIR 22/2002 and DIR 23/2002). There are no reports of increased weediness in those cotton lines.

140. An increase in spread and persistence of the GM cotton line could only occur in an environment where enhanced resistance to herbivorous insects and microorganisms was limiting the spread and persistence of cotton. The main factors limiting the spread and persistence of cotton in the areas proposed for release are temperature, soil type and water and nutrient availability (OGTR 2002).

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

Cross tolerance to biotic environmental stresses through altered seed dormancy, viability, germination rates and/or seedling viability

142. Expression of the *nad1* gene in the GM cotton plants could lead to altered dormancy, seed viability, germination rate and/or seedling viability resulting in increased spread and persistence of these cotton lines.

143. Extensive search of the literature did not reveal any reports, in which a plant defensin was implicated in altered seed dormancy or viability.

144. One report addressed the possibility of plant defensins, Rs-AFPs from radish (*R. sativus*), to prevent fungal infection of germinating seeds (Terras et al. 1995). Rs-AFPs are plant defensins that lack a C-terminal prodomain and have been shown to be secreted from the plant defensin producing cells into the growth medium (Terras et al. 1995). NAD1 is not expected to be secreted but instead to be contained within the vacuoles of the plant cells as is the case in the source organism, *N. alata* (Lay et al. 2003a), affecting only organisms that come into direct contact with the plant cells, eg insects through feeding or fungi through hyphal growth. This way, seedling viability of cotton plants expressing NAD1 would be increased. In an environment where affected pathogenic or herbivorous organisms were the main factors limiting the spread and persistence of cotton, the GM cotton line could become weedy.

145. The main factors limiting the spread and persistence of cotton in the areas proposed for release are temperature, soil type and water and nutrient availability (OGTR 2002).

146. Therefore, **no risk is identified** and the potential for the spread and persistence of cotton through cross tolerance to environmental stresses other than fungal infection as a result of the expression of the NAD1 protein will not be further assessed.

2.4.3 Dispersal of GM cotton seed or other GM plant materials during transport, research, storage or through equipment

147. In the course of the proposed dealings the applicant proposes to transport seed to and from the release sites, cultivate GM cotton plants, store all GM cotton seed harvested from the crop and collect GM plant materials for research purposes, laboratory research or possible

future release (subject to further applications and assessment processes). Accidental spillage or dispersal of GM plant materials, especially seed, in the course of these dealings could allow the GM cotton plants to spread and persist in the environment.

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

149. The applicant also proposed to clean equipment of any viable plant material by hand at the sites or pollen traps immediately or as soon as possible after use.

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

151. After analysis in certified PC2 facilities, any plant waste materials collected for research purposes would be autoclaved.

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

2.4.4 Dispersal of GM plant materials (including seed) via flooding

153. Severe weather conditions (eg flooding) could lead to the dispersal of GM plant materials, including seed. The three sites where the proposed release will take place are located above flood level (information supplied by the applicant). In the case of a flood, any seed or other GM plant material on the ground is therefore not expected to be dispersed beyond the area of the proposed release (including the pollen trap, which will act as a physical barrier once the plants are established). Cotton does not generally propagate vegetatively, so dispersal of GM cotton plant material other than seed would be highly unlikely to result in the dissemination of the GM cotton line (OGTR 2002).

154. In addition, the applicant proposed sites located at least 50 m away from natural waterways (see Chapter 1 Section 2.3). The standard good management practice for irrigation used by cotton growers in Australia involves retention of irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This practice would also reduce the dispersal of seed (DIR 059/2005).

155. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of dispersal of GM plant materials (including seed) via flooding will not be further assessed.

2.4.5 Increased exposure of vertebrates (including people), invertebrates and non-target microorganisms to NAD1 as a result of spread and persistence of the GM cotton plants in the environment

Increased spread and persistence of the GM cotton line proposed for release

156. The potential of increased spread and persistence of the GM cotton line in the environment was addressed in sections 2.4.1 and 2.4.2 (no risks were identified). However, in the highly unlikely instance of this occurring, spread and persistence of the GM cotton plants in the environment could lead to increased exposure of people to the expressed proteins.

Increased exposure of vertebrates, invertebrates and microorganisms to the NAD1 protein

157. An adverse outcome could occur, if the NAD1 protein were toxic or allergenic for people. The potential for NAD1 causing toxic or allergic reactions in people was assessed in sections 2.1 and 2.2.

158. Organisms other than people may be exposed directly, through feeding on the GM cotton plants or indirectly through eating organisms that have fed on or degrade the GM cotton plants as a result of spread and persistence of the GM cotton in the environment. These organisms include vertebrates, invertebrates and microorganisms. The potential for toxicity of NAD1 to vertebrates other than people was considered in section 2.3.1. The potential for toxicity or growth inhibition of invertebrates and non-target microorganisms will be assessed further in Chapter 3.

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

160. The release would be of limited size and short duration and the applicant proposed a number of measures to limit the spread and persistence of the GM cotton line in the environment (for details see Chapter 1 Section 2.3).

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

2.5 Gene flow by vertical gene transfer

162. Transfer of genetic material to offspring by reproduction, either asexual or sexual (vertical gene transfer) could result in the transfer of the *nad1* gene or its associated regulatory elements to other plants. The only sexually compatible species present in Australia that could receive genes from the GM cotton line are *G. hirsutum* and *G. barbadense* (including both cultivated - GM and non-GM - and naturalised cotton populations).

2.5.1 Gene transfer to native *Gossypium* species

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

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

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

2.5.2 Expression of the *nad1* gene in other *G. hirsutum* or *G. barbadense* plants (including commercially released GM cotton lines) resulting in increased exposure of vertebrates (including people), invertebrates and microorganisms to NAD1 protein

Outcrossing to sexually compatible species

166. The *nad1* gene could be transferred to sexually compatible cotton plants, ie *G. hirsutum* or *G. barbadense* cotton plants (including commercially released GM cotton lines), resulting in increased exposure of vertebrates (including people), invertebrates and microorganisms to NAD1. Although cotton is primarily self-pollinating, low levels of outcrossing can occur. Cotton pollen is relatively heavy and sticky and the low levels of outcrossing are due to insect pollination, decreasing significantly with distance. The separation distance of 4 metres required in Australia for certified commercial seed production reflects the relatively short distances observed for cotton pollen dispersal in Australian studies (OGTR 2002).

167. Although commercial cotton crops will probably be planted adjacent to the trial sites (information provided by the applicant), outcrossing will be limited due to the proposed containment measure of a 20 m pollen trap proposed by the applicant that will surround the GM cotton (see Chapter 1 Section 2.1). Both the GM cotton and the plants of the pollen trap will be destroyed at the end of each growing season along with the GM and non-GM cotton plants on the trial sites (OGTR 2002).

168. In addition, cotton plants within the trial sites and pollen trap plants have no traits conferring insect resistance. As a result, the plants in the proposed trial sites are expected to serve as a refuge for insects susceptible to the insect resistance traits in commercially released GM cotton lines, which currently comprise approximately 90% of plantings. The applicant is expected to adopt the heavy insecticide spraying regime that is necessary for maintaining non-GM cotton. This in turn will further limit the chance of insect-mediated pollen transfer to plants outside the proposed trial sites.

Potential for weediness in plants resulting from outcrossing

169. In the highly unlikely case of outcrossing to a commercially grown non-GM cotton plant, the potential for weediness would be the same as for the GM cotton line itself, which was assessed in section 2.4.2 (no risk was identified). Non-GM cotton varieties with resistance to fusarium or verticillium wilt higher than the GM cotton line proposed for release have been grown for many years without becoming problem weeds, indicating that the spread and persistence of cotton in the areas proposed for release is limited mainly by factors other than fungal disease.

170. If the pollen recipient were a commercially released GM cotton plant, stacking of genes conferring herbicide tolerance and/or insect resistance with *nad1* conferring enhanced fungal (and possibly insect) resistance could occur. This may give any *nad1* carrying plant a fitness advantage in an environment where fungal diseases that can be controlled by NAD1, herbicide tolerance and insect herbivory were the main limiting factors for the spread and persistence of cotton. However, susceptibility to the main factors limiting the spread and persistence of cotton in the areas proposed for release (temperature, soil type and water and nutrient availability (OGTR 2002)) are not expected to be altered. In the rare event of outcrossing to commercially released GM cotton plants, the resulting seed would not be used for subsequent plantings as farmers are required to buy certified GM cotton seed for each growing season. This will further reduce the already limited possibility of spread and persistence of the GM cotton line proposed for release.

Increase in the spread and persistence of cotton plants with nad1

171. Toxicity and/or growth inhibition could be conferred by NAD1 resulting in a decrease of affected pathogenic, neutral or beneficial soil organisms as well as potentially affected insects and therefore, an increase in the spread and persistence in the environment could result. The potential of NAD1 conferring toxicity and/or allergenicity to vertebrates (including people) would be similar to the GM cotton line proposed for release. It was assessed in section 2.1, 2.2, and 2.3.1. The potential of toxicity for, and/or growth inhibition of, invertebrates and non-target microorganisms will be assessed further in Chapter 3.

172. A decrease in the abundance of affected pathogenic, neutral or beneficial soil organisms could allow unaffected and normally suppressed pathogenic soil organisms to proliferate resulting in an increased disease burden. However, any effects will be confined to the proposed release and mitigated by the application of fungicide sprays in the follow-on crop. In addition, the chain of events that would lead to this outcome depends on the most unlikely event, ie outcrossing to commercially grown cotton crops and planting of their progeny in the subsequent season or their dispersal to an environment supporting cotton growth, which is highly unlikely.

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

174. Therefore, **no risk is identified** and the potential for increased exposure of vertebrates (including people), invertebrates and microorganisms to NAD1 resulting from expression of the *nad1* gene in other *G. hirsutum* and *G. barbadense* cotton plants will not be further assessed.

2.5.3 Expression of the nad1 gene in other G. hirsutum or G. barbadense cotton plants (including commercially released GM cotton lines) leading to altered dormancy, seed viability, germination rates and/or seedling viability as a result of gene transfer

175. Transfer of the *nad1* gene to other *G. hirsutum* or *G. barbadense* cotton plants could lead to altered dormancy, seed viability, germination rate and/or seedling viability resulting in increased spread and persistence of these cotton lines.

176. The potential adverse outcomes of these cotton lines would be highly similar to the GM cotton line proposed for release, which was assessed in section 2.4.2 (no risk was identified).

177. Seedling viability of cotton plants expressing NAD1 would be increased in an environment where affected pathogenic or herbivorous organisms were the main factors limiting the spread and persistence of cotton. However, the main factors limiting the spread and persistence of cotton in the areas proposed for release are temperature, soil type and water and nutrient availability (OGTR 2002).

178. Therefore, **no risk is identified** and the potential of expression of *nad1* in other *G. hirsutum* or *G. barbadense* cotton plants leading to altered dormancy, seed viability, germination rates and/or seedling viability will not be further assessed.

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

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

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

2.6 Gene flow by horizontal gene transfer

2.6.1 Presence of the *nad1* gene, *nptII* gene or the introduced regulatory sequences in other organisms as a result of gene transfer

181. Transfer of the *nad1* and *nptII* genes, or the introduced regulatory sequences, from the GM cotton plants to sexually incompatible plants, animals or microorganisms (horizontal gene transfer) could occur only rarely without human intervention.

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

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

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

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

2.7 Unintended changes in toxicity and/or allergenicity

2.7.1 Altered levels of innate toxic or allergenic compounds as a result of random insertion of the gene construct into the cotton genome during genetic modification

186. Cotton tissue (from either GM or non-GM plants), particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional compounds including gossypol and cyclopropenoid fatty acids (eg dihydrosterculic, sterculic and malvalic acids). Further discussion regarding the toxicity and allergenicity of non-GM cotton is provided in *The Biology and Ecology of Cotton (Gossypium hirsutum L.) in Australia* (OGTR 2002). There is potential for the GM cotton plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the random insertion of the gene construct during the genetic modification.

187. Thus far, exposure to plant materials (including pollen) from the GM cotton line has been limited to a few workers maintaining these plants in the glasshouse. The applicant has

reported no adverse outcomes from exposure to the GM cotton plant materials, suggesting that expression of the introduced genes has not altered the toxicity or allergenicity endogenous to non-GM cotton. Compositional analysis comparing the non-GM and GM cotton line is not available at this proof of concept stage of research. This data would be required for possible future applications involving commercial release of the GM cotton line. However, this information is not required for assessing the risks of this proposed release because the trial is limited in size, duration and location and none of the GM cotton plant materials are intended for use in human food, animal feed or in the production of fabrics and/or other cotton products.

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

2.8 Unintended changes in biochemistry, physiology or ecology

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

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

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

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

2.8.1 Altered biochemistry, physiology or ecology of the GM cotton line resulting from expression of the introduced genes

192. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single genes inserted into a plant by genetic modification can also result in pleiotropy. It is therefore necessary to evaluate GM plants for unintended pleiotropic effects, such as changes in agronomic characteristics, which may be a consequence of the gene insertion.

193. The GM cotton line was selected from a number of initial individual transformation events. It was selected on the basis that the plants did not show any altered agronomic properties beyond expression of the introduced proteins (information supplied by the applicant). Based on the applicant's observations, there was no difference in fertility, flowering, seed setting and germination rates between the parental and GM cotton line grown under glasshouse conditions. The applicant proposed to take agronomic measurements such as plant height, seed production and viability during the proposed release.

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

2.8.2 Expression of the introduced genes leading to altered sensitivity to diseases other than black root rot, fusarium wilt and verticillium wilt

195. Expression of the introduced genes could lead to altered sensitivity to diseases other than those targeted by the applicant resulting either in an increased disease burden (if susceptibility to non-target pathogens were enhanced) or in increased weediness (if resistance to non-target pathogens were enhanced and if these were the main factors limiting the spread and persistence of cotton in the areas proposed for release). The latter was discussed in events 2.4.1 and 2.4.2 (no risks were identified).

196. The applicant proposed to trial the GM cotton line at three different locations, this way exposing it to a variety of environments with various plant pathogens.

197. Increased susceptibility to pathogens that in cotton plants other than the GM cotton line can be controlled through expression of endogenous genes could be a result of either pleiotropic effects (discussed in Event 2.8.1) or interaction of the *nad1* gene or its product with intermediates or products of biochemical pathways in cotton, including possible endogenous plant defensins.

198. While the release involves proof of concept research with a preliminarily characterised GM cotton line, to date there is neither indication of endogenous plant defensins in cotton nor an indication of the GM cotton line showing altered susceptibility to diseases other than black root rot, fusarium wilt and verticillium wilt.

199. Therefore, **no risk is identified** and the possibility of expression of NAD1 leading to altered sensitivity to diseases other than black root rot, fusarium wilt and verticillium wilt will not be further assessed.

2.9 Unintended effects on existing pests or pathogens

2.9.1 Decreased use of fungicide sprays on the GM cotton resulting in the increased prevalence of unaffected pathogenic Oomycetes and/or fungi

200. The applicant does not intend to apply fungicide sprays during the trial as this could compromise the assessment of the efficacy of the genetic modification (enhanced fungal resistance). Omission of fungicide sprays in large areas for prolonged periods of time could result in the increased prevalence of human, animal and/or plant pathogenic Oomycetes and/or fungi.

201. However, the release will be of limited size and short duration. Any increase of pathogenic Oomycetes and/or fungi will be confined to the areas proposed for release and mitigated by the application of fungicide sprays in the follow-on crop.

202. Therefore, **no risk is identified** and the possibility of expression of NAD1 leading to increased prevalence of pathogenic microorganisms other than the target fungi will not be further assessed.

2.10 Secondary impacts

2.10.1 Development of resistance in fungal pathogens to plants expressing NAD1

203. APVMA has a complementary regulatory role in respect of this application due to its responsibility for agricultural chemical use in Australia, including insecticides, fungicides and herbicides, under the *Agricultural and Veterinary Chemicals Code Act 1994*.

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

205. Widespread and long-term use of the GM cotton line with enhanced fungal resistance expressing the *nad1* gene could result in fungal species becoming resistant to NAD1, a fungal growth-inhibiting protein, decreasing the efficacy of this GM trait. Although efficacy is not covered by the Act, this issue has been considered. The proposed release will be of small size and short duration and therefore, it is not expected to lead to the development of resistance.

206. Therefore, **no risk is identified** relating to the health and safety of people or the environment and the potential for decreased efficacy of the GM trait will not be further assessed.

2.10.2 Adverse impact on populations of organisms that interact with affected organisms

207. Widespread and long-term use of the GM cotton line with enhanced fungal resistance expressing the *nad1* gene could adversely affect some organisms that interact with impacted organisms in the environment, including the food web. This could result in reduced biodiversity. However, as the trial will be of small size and short duration, there will be limited opportunity for the GM cotton line expressing the NAD1 protein to affect the wider environment including the food web.

208. Therefore, **no risk is identified** and the potential for an adverse impact on populations of organisms that interact with affected organisms, as a result of the expression of the *nad1* gene in the GM cotton line, will not be further assessed.

2.11 Unauthorised activities

2.11.1 Use of GMOs outside the proposed licence conditions (non-compliance)

209. 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 GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

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

Section 3 Risk estimate process for identified risks

211. Two events from the hazard identification process (Events 2.3.2 and 2.3.3 in Table 5) are considered to lead to an identified risk for the adverse outcome of toxicity for, or growth inhibition of, non-target organisms. Those two events from this release of a GMO into the environment are assessed in further detail in Chapter 3 to obtain estimates of the level of risk.

212. Chapter 3 gives detailed consideration to the consequences and likelihood of these two events. The risk is assessed against the baselines established by reference to characteristics of the parent organism and aspects of the receiving environment (including the agronomic practices proposed by the applicant).

213. Information contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, the proposed dealings and potential impacts on the health and safety of people and the environment), current scientific knowledge and submissions received during consultation with expert groups and authorities were also considered.

214. The consequence assessment considers the seriousness of the harm that could potentially result from each event, while the likelihood assessment considers the chance of the event resulting in harm. Consequence and likelihood assessments are combined to give an overall risk estimate using the Risk Estimate Matrix (Figure 3). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty that arise from a lack of knowledge.

Figure 3 The OGTR Risk Estimate Matrix (OGTR 2005)

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

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

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

216. After an estimate is obtained for each identified risk, risks higher than negligible are evaluated to determine if risk treatment measures are required to mitigate potential harm (see Chapter 4).

Chapter 3 Risk estimates for toxicity for, or growth inhibition of, non-target organisms

217. This chapter estimates the risks associated with two events that could lead to the adverse outcome of toxicity for, or growth inhibition of, non-target organisms (insects and microorganisms) arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

Section 1 Background

218. Toxicity is the cascade of reactions resulting from exposure to a dose of a chemical that is sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992).

219. Criteria for measuring toxicity of a substance may include decreased feeding, weight loss, decreased weight gain, altered development, reduced reproductive capacity and death.

220. The GM cotton line expresses two proteins as a result of the genetic modification. Events that may give rise to toxicity for non-target organisms as a result of the expression of these two proteins during the proposed release were considered in Chapter 2. Expression of the antibiotic resistance protein, NPTII, is not expected to be a novel source of harm to non-target organisms as it and similar proteins are naturally widespread in the environment as it is expressed by ubiquitous bacterial species. There are indications that the NAD1 protein is not toxic to vertebrates and is expected to pose a risk only to invertebrates and non-target microorganisms. Therefore, this chapter will be limited to assessing the risk of toxicity for, or growth inhibition of, invertebrates and microorganisms as a result of expression of the NAD1 protein by the GM cotton line. The toxicity of NAD1 to fungal pathogens of cotton, in particular the target species *Fusarium oxysporum* f.sp. *vasinfectum*, *V. dahliae* and *T. basicola*, is not considered to be an adverse outcome.

Section 2 Consequence and likelihood assessments

221. Consideration is given to events 1 (Section 2.3.2) and 2 (Section 2.3.3) identified in Chapter 2 (Hazard identification) that may give rise to toxicity for, or growth inhibition of, non-target organisms. For each event the level of risk is estimated from assessments of the seriousness of harm (**consequence** – ranging from marginal to major) and the chance of harm (**likelihood** – ranging from highly unlikely to highly likely).

222. The Regulator can only consider risks posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMO is considered relative to the baselines of toxicity for, or growth inhibition of, invertebrates and non-target microorganisms of the non-GM parent and the environment, in which the GM cotton plants are proposed for release. Therefore, other sources of the introduced genes or similar genes in the environment (such as natural occurrence in other plants) and the agronomic management practices proposed by the applicant, eg use of insecticide sprays on the GM cotton plants to control insect pests and fungal pathogens are relevant to the risk estimate.

2.1 Toxicity of non-GM cotton

223. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton line being considered in this risk assessment. Cotton is a well established field crop with a long history of use. A comprehensive review of the biology of non-GM

cotton, is provided in the document *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia* (OGTR 2002).

224. Gossypol, a phenolic compound produced by cotton, is known to be toxic to insects (Percival et al. 1999). However, a study by Wilson et al (1981) (cited in Percival et al. 1999) showed that high levels of gossypol, even in combination with morphological characteristics that discourage insect infestation, such as okra or lacinate leaf forms, were not sufficient to provide protection against the pink bollworm (*Pectinophora gossypiella*), a major pest of cotton in the USA.

225. As indicated by surveys carried out under previous cotton DIR licences (eg DIRs 017/2002 and 025/2002), large numbers of invertebrates can be found in unsprayed cotton crops. These include herbivorous, predatory and beneficial invertebrates. GM cotton crops without insect resistance and non-GM cotton crops require multiple insecticide applications to maintain growth and productivity.

226. Cotton seed contain a number of antifungal components, some of which are active against *Botrytis cinerea*, *Alternaria brassicicola*, *Chalara elegans* and/or *Fusarium oxysporum* (Chung et al. 1997). However, most non-GM cotton seedlings and adult plants are susceptible to many fungal pathogens, such as the target fungi of the NAD1 protein expressed by the GM cotton line.

2.2 Event 1: Contact with or ingestion of GM plant materials containing NAD1 protein by invertebrates as a result of the proposed release

227. Plant defensins display one or more of a range of biological activities, including inhibition of the activity of enzymes (for details see Chapter 1 Section 4.2.2). Therefore, it was hypothesised that the biological function of some plant defensins may be the defence against herbivorous insects through inhibition of digestive enzymes. Extensive search of the literature identified one report of a plant defensin, VrCRP, from mungbean (*Vigna radiata*) with growth-inhibiting activity on insect larvae (Chen et al. 2002). In that report, VrCRP was expressed in *E. coli*, purified and artificial seeds were made containing a known amount of the plant defensin. The growth of bruchid (*Callosobruchus chinensis*) larvae was inhibited in this *in-vitro* experiment.

228. Toxicity for insects may also result in indirect adverse impacts:

- effects on populations of specialist parasitoids and predators that feed on insects affected by the NAD1 protein and
- effects on populations of other organisms that interact with insects affected by the NAD1 protein.

229. The risk of toxicity for, or growth inhibition of, invertebrates from direct or indirect ingestion of the NAD1 protein would depend upon the level of toxicity of the NAD1 protein (consequence assessment) and the level of exposure to the protein during this release (likelihood assessment). The risk is assessed against the baseline of the toxicity of the non-GM parent organism for insects, toxicity due to the presence of genes similar to the introduced gene, *nad1*, in the environment in native and crop plants and the toxicity of the pesticide regime proposed by the applicant.

2.2.1 Consequence assessment

230. Lay et al (2003b) reported a potential growth-inhibiting effect of the NAD1 protein to the cotton pests, *Helicoverpa armigera* and *Helicoverpa punctigera*. However, the authors did not support their claim with data. Preliminary experiments on *Helicoverpa armigera* larvae

reported by the applicant did not show growth-inhibiting activity of the GM cotton plants compared to non-GM plants. Although many plant defensins are present in the environment, it is currently unknown whether any of those possess toxic or growth-inhibiting activity for insects (for details see Chapter 1 Section 4.2).

231. Therefore, the consequences of expression of the *nad1* gene in GM cotton plants in relation to possible toxicity for, or growth inhibition of, invertebrates are assessed as **minor**.

2.2.2 Likelihood assessment

232. Invertebrates may be exposed directly, through feeding on the GM cotton plants, or indirectly through feeding on organisms that have fed on or degraded GM plant materials. However, the NAD1 protein is produced at low levels (for details see Chapter 1 Section 4.5.2). Relative exposure would be greatest for herbivorous species feeding on the GM cotton plants.

233. Pollinator species and various insects that feed on pollen, and species (eg predators or parasitoids) feeding on insect larvae, may also be exposed to low levels of the NAD1 protein. Sap feeders, such as aphids, could also be exposed to NAD1, as the size of the protein may determine whether it is present in phloem sap or not: Snowdrop lectin toxin was present in the honeydew of aphids feeding on GM tobacco plants expressing this protein, indicating that it is present in the phloem sap (Shi et al. 1994). The snowdrop lectin toxin is 12,500 M_r and the NAD1 protein is 5,300 M_r in size. It is therefore possible that the NAD1 protein could enter the phloem of the GM cotton plants.

234. However, agronomic practices for the proposed release, specifically insecticide use, are expected to have a greater impact on invertebrate survival than the expression of the NAD1 protein in the GM cotton plants (for details on proposed pest management see Chapter 1 Section 5.2.1).

235. In addition, the proposed release will be of limited size and short duration (for details see Chapter 1 Section 2.3).

236. Therefore, the **likelihood** of toxicity for invertebrates resulting from event 1 is assessed as **highly unlikely**.

2.2.3 Uncertainty

237. The toxicity of the NAD1 protein for invertebrates has not been studied in detail. Data on toxicity for a range of invertebrate indicator species would be required for risk assessments of applications for large scale releases of this GM cotton line. Such information is not required to assess this application for proof of concept research, particularly as the applicant intends to use insecticide sprays on the cotton, which are expected to have a greater impact on invertebrate populations than the GM cotton line.

238. More detailed expression data would be required for the GM cotton line for risk assessments of applications for large scale releases of this GM cotton line, to enable the likely level of exposure to the NAD1 protein to be determined.

239. The effect of stacking of genes conferring insect and/or herbicide resistance in commercially released GM cotton plants with *nad1* is not known. However, stacking is not expected to occur during this limited and controlled release.

240. Such information may be required before a large scale release of the NAD1-expressing GM cotton could be assessed.

2.3 Event 2: Contact of microorganisms with GM plant materials containing NAD1 protein as a result of the proposed release

241. Plant defensins display one or more of a range of biological activities, including fungal growth-inhibiting activity. Purified NAD1 is known to be growth-inhibiting to the fungi, *F. oxysporum* f.sp. *vasinfectum*, *F. oxysporum* f.sp. *dianthi* race 2, *B. cinerea*, *V. dahliae* and *T. basicola*, but has no effect on tested yeasts and bacteria (for details see Chapter 1 Sections 4.2.2 and 4.3.2). An adverse impact on other microorganisms, especially beneficial microorganisms (eg arbuscular mycorrhiza (AM) fungi), could result from exposure of those organisms to NAD1 expressed by the GM cotton line during the proposed release.

242. Toxicity for, or growth inhibition of, microorganisms may also result in indirect adverse impacts:

- effects on populations of organisms that feed on microorganisms affected by the NAD1 protein and
- effects on populations of other organisms that interact with microorganisms affected by the NAD1 protein.

243. The risk of toxicity for, or growth inhibition of, microorganisms from contact with the NAD1 protein would depend upon the level of toxicity of the NAD1 protein (consequence assessment) and the level of exposure to the proteins during this release (likelihood assessment). The risk is assessed against the baseline of the toxicity of the non-GM parent organism for microorganisms, toxicity due to the presence of genes similar to the introduced gene, *nad1*, in the environment in native and crop plants and the toxicity of the pesticide regime proposed by the applicant.

2.3.1 Consequence assessment

Effects of GM cotton expressing NAD1 on arbuscular mycorrhiza fungi

244. Mycorrhizal colonisation is important for the growth of cotton plants and the applicant reported no obvious difference in agronomic characteristics in the GM cotton plants when grown in a glasshouse. A negative effect of the NAD1 protein on AM fungi should have been reflected in stunted growth of the GM cotton plants compared to the non-GM parent. Additionally, a preliminary glasshouse bioassay was carried out by the applicant, with no statistical difference in the number of AM on roots of GM and non-GM cotton plants.

245. The *nad1* gene is derived from *N. alata*, an ornamental plant. The NAD1 protein is produced mainly in developing flowers. Various *Nicotiana* and other solanaceous species including *N. tabacum*, *N. longifolia*, *N. rustica*, *N. glauca*, *N. sanderae* and tomato (*Lycopersicon esculentum*) are known to form symbioses with the AM fungus *Glomus intraradices* (eg Shaul et al. 1999; Maier et al. 2000).

246. Some of those and closely related species, eg *N. tabacum*, *N. excelsior*, *N. paniculata* and tomato, have been shown to express plant defensins indicating that there are AM fungal strains, which are unaffected by similar plant defensins (Gu et al. 1992; Milligan & Gasser 1995; Yamada et al. 1997; Komori et al. 1997).

247. A protein similar to NAD1, the plant defensin Dm-AMP1 from *Dahlia merckii*, was introduced into aubergine plants and has been found to inhibit the growth of a plant pathogen, *Verticillium albo-atrum*, but not to affect AM colonisation by *G. mosseae* negatively (Turrini et al. 2004b).

248. The applicant proposed to assess non-GM and GM cotton plants for the presence of arbuscular mycorrhizal colonisation during the first six to eight weeks of growth and to compare those data (for details see Chapter 1 Section 2).

Effects of GM cotton expressing NAD1 on other soil microorganisms

249. The effects of NAD1 on other microorganisms (especially neutral and beneficial microorganisms) are largely unknown. However, the applicant reported no adverse effects of purified NAD1 on selected yeasts and bacteria (see Chapter 1 Section 4.3.2).

250. Similar genes are found and expressed in a wide range of plants including an Australian native plant, *Hardenbergia violaceae*, and many crop plants, eg wheat and peas (Harrison et al. 1997; Koike et al. 2002; Lai et al. 2002; Gaudet et al. 2003) both of which can form symbioses with rhizobacteria (eg Iniguez et al. 2004; Skorupska et al. 2006). In the case of *A. thaliana*, transcripts of the plant defensin gene *pdf1.2* was accumulated upon challenge with the non-pathogenic rhizobacterium *Bacillus amyloliquefaciens* strain EXTN-1 (Ahn et al. 2002).

251. Taken together, reports in the literature indicate that many beneficial microorganisms are not affected by plant defensin expression whereas plant defensins can have growth inhibiting effects on various plant pathogenic microorganisms.

252. The consequences of expression of the *nad1* gene in GM cotton plants with regard to toxicity for, or growth inhibition of, microorganisms are assessed as **minor**.

2.3.2 Likelihood assessment

253. Microorganisms may be affected if they fed on or degraded mainly species that are affected by the NAD1 protein produced by the GM cotton line and if the numbers of individuals of those affected species declined considerably. Other microorganisms may be exposed directly, through pathogenic or symbiotic interaction with the GM cotton plants, or indirectly through feeding on organisms that have fed on or degrade GM plant materials. However, the NAD1 protein is produced at low levels (for details see Chapter 1 Section 4.5.2). Relative exposure will be greatest for species directly interacting with the GM cotton plants and through possible root exudation. So far, no data are available on the exudation or the amount of exudation of NAD1 by the GM cotton plants.

254. The proposed release will be of limited size and short duration.

255. The **likelihood** of toxicity for non-target microorganisms resulting from event 2 is assessed as **highly unlikely**.

2.3.3 Uncertainty

256. The toxicity of the NAD1 protein for non-target microorganisms has not been studied in detail. Data on toxicity for a range of non-target microorganisms and on the root exudation of NAD1 would be required for risk assessments of applications for large scale releases of this GM cotton line.

Section 3 Risk estimates

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

258. The risk estimates for toxicity for, or growth inhibition of, invertebrates and microorganisms as a result of the proposed release of the GM cotton line have been made relative to the baseline of the toxicity of cotton for invertebrates and non-target microorganisms and in the context of the applicant's proposed containment measures and agricultural practices for the release, including spraying with pesticides to control insect pests in the crop.

259. The consequences of direct or indirect ingestion by invertebrates of the NAD1 protein in the GM cotton line have been assessed as **minor**, and the likelihood of this resulting in toxicity to invertebrates as **highly unlikely**. Therefore, the risk estimate for event 1 is **negligible**.

260. The consequences of exposure of non-target microorganisms to the NAD1 protein have been assessed as **minor**, and the likelihood of this resulting in toxicity to non-target microorganisms as **highly unlikely**. Therefore, the risk estimate for event 2 is **negligible**.

Table 6 Summary of risk assessment

Event that may give rise to toxicity for, or growth inhibition of, invertebrates and/or non-target microorganisms	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 1 Contact with or ingestion of GM plant materials containing NAD1 by invertebrates.	Minor <ul style="list-style-type: none"> The NAD1 protein may be toxic to certain insects. 	Highly unlikely <ul style="list-style-type: none"> Limited exposure of insects to the NAD1 protein expected due to the small size and short duration of the proposed release. Low expression of the protein would further limit the level of exposure. Agronomic practices proposed for the release, specifically insecticide use, are expected to have a greater impact on invertebrate survival than the expression of the NAD1 protein in the GM cotton plants. 	Negligible	No
Event 2 Contact with NAD1 protein by non-target microorganisms.	Minor <ul style="list-style-type: none"> The NAD1 protein may be toxic to some microorganisms (neutral, beneficial or pathogenic). 	Highly unlikely <ul style="list-style-type: none"> Limited exposure of non-target microorganisms to the NAD1 protein is expected due to the small size and short duration of the proposed release. Low expression of the protein would further limit the level of exposure. 	Negligible	No

Chapter 4 Risk management

261. This Chapter evaluates the risk identified in Chapter 3 to determine whether or not specific treatments are required to mitigate harm to human health and safety or the environment that may arise from the release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures were used to inform the decision-making process and determine licence conditions that have been imposed by the Regulator under section 62 of the Act.

Section 1 Background

262. 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.

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

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

Section 2 Other Australian regulators

265. Australia's gene technology regulatory system operates as part of an integrated legislative framework (OGTR 2005). Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, NICNAS, NHMRC and AQIS. Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies⁸.

266. 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.

267. FSANZ is responsible for human food safety assessment, including GM food. The proposed release involves early stage ('proof of concept') research and the applicant does not intend any material from the GM cotton line to be used for human food. Accordingly, the applicant has not applied to FSANZ for evaluation of materials from the trial for use in human food. However, FSANZ approval would need to be obtained before such materials could be used for this purpose.

268. The GM cotton line proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its

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

production of a fungal growth-inhibiting substance, and therefore it is subject to regulation by the APVMA. Currently, APVMA is assessing a research permit application from Hexima for the proposed release.

269. The APVMA and the OGTR have worked closely to ensure the thorough, coordinated assessment of these parallel proposals, and that the decisions by both Regulators coincide.

Section 3 Risk treatment measures for identified risks

270. The detailed risk assessment of events 1 and 2 contained in Chapter 3 concluded that the risk estimates from the proposed release are **negligible**. These events were considered in the context of the release on three sites on a maximum total area of one hectare during each of the three growing seasons between September 2006 and May 2009, containment measures and agricultural practices proposed by the applicant and the receiving environment. The release will be carried out in the shires of Pittsworth (QLD), Narrabri and Moree Plains (NSW).

271. The *Risk Analysis Framework* (OGTR 2005), which guides the risk assessment and risk management process, defines **negligible** risks as insubstantial with no present need to invoke actions for their mitigation. Containment measures have been imposed to limit the release to the locations, size and duration proposed by the applicant.

Section 4 General risk management

272. The applicant proposed measures to limit the spread and persistence of the GM cotton line in the environment. These were used in establishing the risk assessment context (Chapter 1) and their suitability for limiting the release to the locations, size and duration proposed by the applicant was evaluated in Chapters 2 and 3. The Regulator also imposed licence conditions, a summary of which is given below.

4.1 Summary of licence conditions associated with managing the limited and controlled release

4.1.1 Measures to limit and control the release

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

- surround each release site with a pollen trap
- locate the release sites at least 50 m away from natural waterways
- harvest the cotton seed from the release separately from any other crop
- not permit cotton seed or other materials from the release to be used in human food or animal feed
- destroy all plant materials remaining at the trial sites after harvest
- clean the sites and any equipment used on the sites
- inspect the sites following harvest, any areas used to clean equipment and any irrigation channels associated with the release
- destroy any volunteers prior to flowering
- conduct regular inspections of the release sites and other areas following harvest for at least 12 months and until the last six consecutive months have passed without any volunteer cotton plants.

4.1.2 Measures to control other activities associated with the release

274. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

4.2 Other risk management considerations

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

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

4.2.1 Applicant suitability

276. 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.

277. Before making the decision to issue a licence for this application (DIR 063/2005), the Regulator determined that Hexima Limited (Hexima) is suitable to hold a licence.

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

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

4.2.2 Compliance and contingency plans

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

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

282. Hexima 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 instrument would be required within 30 days of the issue date of the licence.

4.2.3 Reporting structures

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

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

284. 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.

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

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

Section 5 Monitoring and Compliance

286. A range of monitoring and compliance activities are undertaken on behalf of the Regulator (OGTR 2005) to check compliance with licence conditions. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

287. 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.

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

Section 6 Issues to be addressed for future releases

289. The risk assessment identified additional information that may be required to assess an application for a large scale release of this GM cotton line or to justify a reduction in the containment conditions, including:

- data on expression levels of the plant defensin NAD1 in the GM cotton line (in whole plants, leaves, pollen, stems, roots, seeds and bolls) and data on root exudation of NAD1
- the mode of action of NAD1

- characterisation of the potential toxicity of NAD1 and the GM cotton line to vertebrates (including people)
- *in-vitro* experiments or field studies addressing the effect of NAD1 on non-target organisms such as invertebrates, mycorrhiza and *Rhizobium* spp.
- agronomic characteristics indicative of weediness
- any effects resulting from stacking of the *nad1* gene with other introduced traits in commercially released GM cotton plants such as herbicide tolerance and insect resistance.

Section 7 Conclusions of the RARMP

290. The risk assessment concludes that this limited and controlled release of a GM cotton line into the shires of Pittsworth (QLD), Narrabri and Moree Plains (NSW) poses a **negligible** risk to the health and safety of people and the environment.

291. The risk management plan concludes that this **negligible** risk does not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the proposed locations, size and duration requested by the applicant.

Section 8 DIR 063/2005 Licence

292. The licence DIR 063/2005 is available on the OGTR website (<http://www.gov.au/gmorec/ir.htm#table>, following the path to DIR 063/2005).

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Appendix A Definitions of risk analysis terms used by the Regulator

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

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

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

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

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

Risk

the chance of something happening that will have an undesired impact

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

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

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

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

Risk analysis

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

Risk analysis framework

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

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

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

Risk Context

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

Risk estimate

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

Risk evaluation

the process of determining risks that require treatment

Risk management

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

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

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

States

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

Uncertainty

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

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities⁹ on application DIR 063/2005

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

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

- Risk of toxicity to all organisms other than the target fungi (Chapter 1 Section 4.3.4, Chapter 2 Events 2.1.1, 2.1.2, 2.3.1, 2.3.2, 2.3.3 and Chapter 3)
- Risk resulting from any gene flow or dissemination of the GM pollen and seed beyond the intended areas (Chapter 2 Events 2.1.1 including 2.4.5)
- Risk of weediness (Chapter 2 Events 2.4.1 and 2.4.2)
- Risk of development of resistance to the NAD1 protein in target organisms (Chapter 2 Event 2.10.1)
- Risk of toxicity as a result of any changes to the levels of endogenous toxic compounds and pleiotropic effects (Chapter 2 Events 2.7.1 and 2.8.1).
- The potential usefulness of the fungal resistance trait in commercial GM cottons and other crops was noted. Stacking of the fungal resistance gene with other traits, or its introduction into different species, would require separate applications and assessments.

Issues raised relating to the Risk Management Plan (considered in Chapter 4 and the Licence):

- Adequate containment measures
- Methods to minimise seed bank
- Adequate labelling and cleaning of transport equipment
- Adequate containment and labelling during transport
- Adequate disposal of GM plant materials, or storage and use in contained facilities.

⁹ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and Local councils where the release may occur.

Appendix C Summary of submissions received from prescribed experts, agencies and authorities on the consultation RARMP

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

Appendix D Summary of public submissions received on the consultation RARMP

The Regulator received one submission from the public on the consultation RARMP from an individual, which does not relate to the proposed release.

All issues relating to risks to human health and safety and the environment were considered following the case-by-case assessment as required by the legislation and in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulators decision to issue the licence.

Issues raised: **EN:** environmental risks; **G:** Gene transfer; **H:** Health concerns; **M:** Market and trade concerns; **OSA:** Outside the scope of the assessment; **RARMP:** Risk assessment and risk management plan; **W:** Weediness.

Other abbreviations: **APVMA:** Australian Pesticides and Veterinary Medicines Authority; **FSANZ:** Food Standards Australia and New Zealand; **GM:** genetically modified; **GMO:** genetically modified organism.

Summary of issues raised	Issue	Consideration of issue
Expresses disapproval of genetically engineered (GE) produce. Cited a report (The case for a GM-free sustainable world, published by scientists from seven countries).		Noted.
Their conclusions are:		
- GM crops failed to deliver the promised benefits	M	OSA. The regulatory system focuses upon assessing risks to people and the environment that may be posed by GMOs. Benefit (or lack thereof) cannot be considered by the Regulator.
- Glyphosate resistant weeds and Bt resistant insects can evolve	EN	OSA. The GM cotton line approved for release contains a gene found in many horticultural and crop plants that confers tolerance to certain fungal diseases. It does not contain introduced herbicide tolerance or insect resistance genes. APVMA evaluates, registers and regulates agricultural and veterinary chemical products including fungicides, herbicides and insecticides.
- Transgenic contamination of non-GM crops (eg corn)	EN, G	Licence conditions have been imposed to contain the release to the size, location and duration requested by the applicant.
- GM crops are not proven safe and - Dangerous gene products are incorporated into the food crops (eg Bt)	H	Chapters 1, 2 and 4 and Licence The release is a small scale proof of concept study and no GM plant material is to be used as human food or animal feed. Assessment of the safety of GM food is the responsibility of Food Standards Australia New Zealand (FSANZ) and FSANZ approval would be required if the applicant decides to develop the GMO further.
- GM foods are increasingly used to produce pharmaceuticals and drugs	H, EN	The GMO approved for release does not contain a gene for a pharmaceutical or drug.
- Crops engineered with suicide genes for male sterility spread both male sterility and herbicide	EN, G, W	The GMO approved for release does not contain a male sterility gene.

Summary of issues raised	Issue	Consideration of issue
tolerance traits via pollen		
- Broad-spectrum herbicides are found to be highly toxic to humans and other species of animals	H, EN	The GMO approved for release does not contain herbicide tolerance traits.
- Genetic tampering with foods may be inadvertently creating super-viruses and bacteria	H, EN	The genetics of food crops differ significantly from animal viruses and also from bacteria. Only some short regulatory sequences from a plant virus or bacteria are present in the GMO approved for release. The sequences are not capable of replication or causing disease. As noted above, no product from this release is to be used as human or animal food.