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

Risk Assessment and Risk Management Plan for **DIR 058/2005**

Limited and controlled release of insect resistant (VIP) GM cotton

Applicant: Deltapine Australia Pty Ltd

October 2005

Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release (DIR) of genetically modified (GM) insect resistant cotton lines into the environment, in respect of application DIR 058/2005 from Deltapine Australia Pty Ltd (Deltapine).

The DIR 058/2005 licence permits Deltapine to conduct a limited and controlled release of GM insect resistant (COT102, COT202 and COT203) cotton lines. The small scale trial may take place on a maximum of two sites in the Shires of Narrabri, New South Wales and Emerald, Queensland during the 2005–06 summer growing season. Each site must not exceed half a hectare in size.

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 prepared by the Regulator in consultation with a wide range of expert groups and authorities and the public.

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

The application

Deltapine has applied for a licence to grow three GM cotton lines on one or two sites of half a hectare each for one summer season (2005–06). The proposed release sites are in the Shires of Narrabri, NSW, and Emerald, QLD.

The GM cotton lines contain an insect resistance gene (*vip3A*) isolated from *Bacillus thuringiensis*, a common soil bacterium. The gene expresses an insect resistance protein (VIP3A) that is toxic to caterpillars of some lepidopterans (butterflies and moths), including cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*).

One of the GM cotton lines also contains a bacterial gene (*aph4*, conferring resistance to the antibiotic hygromycin B) that was used to select successfully modified plants during initial research and development work in the laboratory.

The purpose of the proposed release is to produce pure seed from the GM cotton lines. This seed may be used in future releases, which would require separate assessments and approval processes. The applicant also requested permission to sell lint from the cotton plants grown during this release. Lint (cellulose fibres removed from the surface of the cotton seed during ginning) does not contain protein or genetic material after it has been processed.

Risk assessment

Background

This is the first time that the revised *Risk Analysis Framework* has been fully applied to the preparation of a RARMP. Copies of the *Risk Analysis Framework* can be obtained from the OGTR by free call 1800 181 030, or at <http://www.ogtr.gov.au/pdf/public/raffinal2.1.pdf>.

The restructured RARMP aims to explain the risk analysis process in greater detail and introduces new terminology to differentiate the steps involved.

The risk assessment 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 GMOs into the environment (**hazard identification** refer to Chapter 2 for more information).

Thirty one hazards were identified for this proposed release. These 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.

Eight events were selected for further assessment. The potential adverse outcomes associated with these events were: allergic reactions in people, toxicity for non-target invertebrates and weediness. The remaining 23 events compiled during the hazard identification were not assessed further as they were considered not to give rise to an identified risk.

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). A brief summary of the conclusions of the risk assessment is provided below.

The GM cotton lines proposed for release have been approved previously for limited and controlled releases under DIR licences 017/2002, 025/2002, 034/2003 and 036/2003. release (PR-151) occurred under the former voluntary system overseen by the Genetic Manipulation Advisory Committee.

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

Risks of allergic reactions in people

Three events were considered that might cause allergic reactions in people as a result of the release of the GM cotton lines.

- Contact with GM plant materials containing the insect resistance or hygromycin resistance proteins
- Exposure of people to the insect resistance or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment
- Exposure of people to the insect resistance or hygromycin resistance proteins as a result of gene transfer to other cultivated or naturalised cotton plants (GM or non-GM).

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**.

Risks of toxicity to non-target invertebrates

Three events were considered that might cause toxicity in non-target invertebrates, in particular some non-target lepidopteran insects, as a result of the release of the GM cotton lines.

- Direct or indirect ingestion of the insect resistance proteins by non-target invertebrates as a result of this release
- Exposure of non-target invertebrates to the insect resistance protein as a result of spread and persistence of the GM cotton in the environment.
- Exposure of non-target invertebrates to the insect resistance protein as a result of gene transfer to other cultivated or naturalised cotton plants (GM or non-GM).

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**.

Risks of weediness

Two events were considered that might cause weediness as a result of the release of the GM cotton lines.

- Expression of the insect resistance gene increasing spread and persistence of the GM cotton plants through reduced insect attack on the plants.
- Expression of the insect resistance gene in other cultivated or naturalised cotton plants (GM or non-GM).

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 level of risk to the health and safety of people or the environment was assessed for eight events. The risk estimates for the adverse outcomes associated with all eight events were **negligible**. The *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation. However, as the small size and short duration of the release were important parts of the context for assessing the risks, containment measures have been imposed to limit the size and duration of the release to that requested by the applicant.

The licence conditions, detailed in Chapter 7 of the RARMP, require the applicant to limit the area and duration of the release to 1 hectare for a single season; prevent use of the GMOs, or materials from the GMOs, in human food or animal feed; maintain physical isolation of the release sites; and conduct post-harvest monitoring to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of insect resistant GM cotton lines into the Shires of Narrabri, NSW and Emerald, QLD poses **negligible** risks to the health and safety of people and the environment as a result of gene technology. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. Licence conditions have been imposed to contain the release to the proposed size, duration and regions requested by the applicant.

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine Inspection Service
bp	Basepair of nucleic acid
Bt	*Bacillus thuringiensis*
Btk	*Bacillus thuringiensis* variety *kurstaki*
CCI	Confidential Commercial Information as declared under section 185 of the Act
cry	Gene encoding a Cry protein
Cry protein	Crystal insecticidal protein isolated from *Bacillus thuringiensis*
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	Gram
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
IgE	Immunoglobulin E
km	Kilometre
LD_{50}	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
m	Metre
mRNA	Messenger ribonucleic acid
M_r	Relative molecular mass
µg	Microgram
ng	Nanogram
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nos	Gene encoding nopaline synthase
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
vip3A	Gene encoding the Vegetative Insecticidal Protein from *Bacillus thuringiensis*
VIP3A	Vegetative Insecticidal Protein from *Bacillus thuringiensis*
WHO	World Health Organisation

Technical summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release of genetically modified (GM) insect resistant cotton lines into the environment, in respect of application DIR 058/2005 from Deltapine Australia Pty Ltd (Deltapine).

The DIR 058/2005 licence permits Deltapine to conduct a limited and controlled release of GM insect resistant (COT102, COT202 and COT203) cotton lines. The small scale trial may take place on a maximum of two sites in the Shires of Narrabri, New South Wales and Emerald, Queensland during the 2005–06 summer growing season. Each site must not exceed half a hectare in size.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 (the Regulations) and corresponding state and territory law govern the process undertaken by the Regulator before a decision is made on whether or not to issue a licence.

While the gene technology legislation provides the scope and boundaries for assessment under the Act and Regulations, it is not explicit in directing how the evaluator should undertake a risk analysis. The Regulator's *Risk Analysis Framework* explains the approach used to evaluate applications for licences to deal with GMOs. The *Risk Analysis Framework* has been revised based on the recently published Australia and New Zealand Standard 4360:2004 on Risk Management (AS/NZS 4360:2004), which is recognised internationally as reflecting best practice in this field.

This is the first time that the revised *Risk Analysis Framework* has been fully applied to the preparation of a RARMP. Copies of the *Risk Analysis Framework* can be obtained from the Office of the Gene Technology Regulator (OGTR) (Freecall 1800 181 030, or at <http://www.ogtr.gov.au/pdf/public/raffinal2.1.pdf>). The restructured RARMP aims to explain the risk analysis process in greater detail and introduces new terminology to differentiate the steps involved.

Section 1 Application

Title:	Limited and controlled release of insect resistant (VIP) GM cotton*
Applicant:	Deltapine Australia Pty Ltd
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	*Gossypium hirsutum* L.
Modified trait(s):	Insect resistance, antibiotic resistance
Identity of the gene(s) responsible for the modified trait(s):	• *vip3A* gene from the bacterium *Bacillus thuringiensis* (insect resistance) • *aph4* gene from the bacterium *Escherichia coli* (antibiotic resistance)
Proposed location(s):	Shires of Narrabri, NSW and Emerald, QLD
Proposed release size:	1 hectare
Proposed time of release:	September/October 2005–July 2006

*The title of the licence application submitted by Deltapine is *Small scale seed increase of VIP cotton*

Deltapine has applied for a licence to release three insect resistant GM cotton lines (COT102, COT202 and COT203) into the environment. The trial is proposed on one or two sites of up to

half a hectare each, ie a maximum total planting area of 1 hectare, over one summer planting season (2005–06) in the Shires of Narrabri, NSW and Emerald, QLD.

Some details of the gene constructs, including plasmid maps and some of the regulatory sequences, were previously declared Confidential Commercial Information (CCI) under section 185 of the Act, in connection with licence applications DIR 017/2002, DIR 034/2003 and DIR 036/2003. This information was made available to the prescribed expert groups and authorities that were consulted in the preparation of the RARMP.

The purpose of the proposed release is to produce and store pure seed from the GM cotton lines for possible future releases. To obtain pure seed from the trial, the applicant intends to treat the GM cotton plants with insecticide sprays to prevent any insects carrying pollen onto the release sites.

The GM cotton lines have one or two introduced genes. The COT102 line contains both the *vip3A* gene (encoding the Vegetative Insecticidal Protein) and the *aph4* selectable marker gene (encoding the hygromycin resistance protein), while the COT202 and COT203 lines contain only the *vip3A* gene. The insect resistance protein, VIP3A, is toxic to caterpillars of some lepidopterans (butterflies and moths), including *Helicoverpa armigera* and *H. punctigera*, major pests of cotton in Australia.

The applicant intends to transport GM cottonseed to the release sites for planting and harvested GM seed cotton from the release sites for ginning and de-linting. The applicant also intends to sell lint (cellulose fibres removed from the surface cotton seed during ginning) from the cotton plants grown during the release. Processed lint does not contain protein or genetic material.

Section 2 Risk assessment

The risk assessment for Deltapine's application, DIR 058/2005, has been prepared in accordance with the Act and the Regulations. The risk assessment considered information contained in the application, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received during consultation on the application with a wide range of prescribed agencies and authorities (see Appendix B).

The Regulator notified the public that a RARMP had been prepared and invited written submissions in relation to the RARMP. Advice on the RARMP was also sought from those consulted on the application. Issues relating to the assessment of risk to the health and safety of people or the environment raised during the consultation period are summarised in Appendices D and E. These appendices also indicate where the issues were addressed in the RARMP.

In addition, a reference document, *The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia*, is used to inform the risk assessment process for licence applications involving GM cotton plants. This is available from the OGTR or from the website <http://www.ogtr.gov.au>.

The risk assessment first considered what harm to the health and safety of people or the environment could arise due to gene technology, and how it could happen during this release of GMOs into the environment (hazard identification). 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 31 events that describe sets of circumstances (events) by which the proposed release could potentially give rise to adverse outcomes as a result of gene technology.

A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process. The 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 or risk is then estimated using the *Risk Estimate Matrix* (see below and Chapter 2).

		RISK ESTIMATE			
LIKELIHOOD	**Highly Likely**	Low	Moderate	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.

Eight events were identified as requiring further assessment. The potential adverse outcomes associated with these events were: allergic reactions in people, toxicity for non-target invertebrates and weediness. These identified risks were then assessed in further detail in comparison to the parent organism, in the context of the intended agronomic management practices, and the environmental conditions in the regions where the proposed release would occur.

The consequence and likelihood assessments used to derive risk estimates from these eight events are summarised in Table 1 (the detailed risk assessments are in Chapters 3–5). More information on the remaining 23 events is provided in Chapter 2. These 23 events were considered not to give rise to an identified risk.

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 proposed release.

Table 1 Summary table for the risk assessment

Potential adverse outcome	Event that may give rise to the adverse outcome	Consequence assessment	Likelihood assessment	Risk estimate	Risk evaluation
Allergic reactions for people (see Ch 3)	**Event 1** Contact with GM plant materials containing the VIP3A or hygromycin resistance proteins by: • contact with plants damaged during handling • inhalation of pollen • inhalation of cotton dust.	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	**Highly unlikely** • Exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to few people due to low expression of the proteins and the small size and short duration of the proposed release.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.
	Event 2 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	**Highly unlikely** • Exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to few people due to low expression of the proteins and the small size and short duration of the proposed release. • GM cotton volunteers emerging after harvest are expected to be considerably less numerous than the GM cotton plants grown during the release.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.
	Event 3 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	**Highly unlikely** • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • The numbers of recipient plants containing the VIP3A protein as a result of gene transfer are expected to be considerably less than the number of GM cotton plants grown during the release. • The proposed release is limited to a small size and short duration.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.

Potential adverse outcome	Event that may give rise to the adverse outcome	Consequence assessment	Likelihood assessment	Risk estimate	Risk evaluation
Toxicity for non-target invertebrates (see Ch 4)	**Event 4** Direct or indirect ingestion of the VIP3A protein by non-target invertebrates.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Exposure to the VIP3A protein is expected to be restricted to few non-target invertebrates due to low expression of the proteins and the small size and short duration of the proposed release. • Agronomic practices proposed for the release, specifically pesticide use, are expected to have a greater impact on invertebrate survival than the expression of the VIP3A protein in the GM cotton plants.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.
	Event 5 Exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Exposure to the VIP3A protein is expected to be restricted to few non-target invertebrates due to low expression of the proteins and the small size and short duration of the proposed release. • GM volunteers are expected to be considerably less numerous than the GM cotton plants grown during the release.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.
	Event 6 Exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • The numbers of recipient plants containing the VIP3A protein as a result of gene transfer are expected to be considerably less than the number of GM cotton plants grown during the release. • The proposed release is limited to a small size and short duration.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.

Potential adverse outcome	Event that may give rise to the adverse outcome	Consequence assessment	Likelihood assessment	Risk estimate	Risk evaluation
Weediness (see Ch 5)	**Event 7** Expression of the *vip3A* gene increasing spread and persistence of the GM cotton plants through reduced lepidopteran herbivory	**Marginal** • Cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects. • The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release.	**Highly unlikely** • The limited area and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the environment. • The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches, would be no greater than for the non-GM parent.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.
	Event 8 Expression of the *vip3A* gene in other *G. hirsutum* or *G. barbadense* cotton plants as a result of gene transfer	**Minor** • Cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects. • The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release.	**Highly unlikely** • The limited size and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the environment. • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • Following transfer of the *vip3A* gene to any of these cotton plants, the likelihood of it causing weediness in these plants is expected to be the same as for the GM cotton plants.	**Negligible**	No specific treatment options are required, however, some conditions proposed to limit the release in time and space are given below.

Section 3 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 eight events that might lead to risks to the health and safety of people or the environment. The risk estimates for the adverse outcomes associated with all eight events are **negligible** ie insubstantial with no present need to invoke actions for their mitigation.

However, containment measures have been imposed to restrict the release in size, duration and regions to those requested by the applicant, as these were important considerations in establishing the context of the consequence and likelihood assessments used to estimate the level of risk.

3.1 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
- locate the release sites at least 50 m away from natural waterways
- harvest and gin seed cotton from the release separately from other cotton
- prohibit cotton seed and other materials from the release to be used in human food or animal feed
- 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 six consecutive months have passed without any volunteer cotton plants.

To obtain pure seed, the applicant proposes to spray the GM cotton crop with insecticides targeting pollinators, to minimise gene flow from the adjoining non-GM cotton used as a pollen trap. This will further reduce the frequency of gene flow from the GM cotton beyond the release site.

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*; and *Policy on storage of genetically modified organisms, July 2004*). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

3.2 Other regulatory considerations

Some of the dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies. The GM cotton lines proposed for release produce an insecticidal substance and, therefore, could be subject to regulation by the Australian

Pesticides and Veterinary Medicines Authority (APVMA). Deltapine has made an application to the APVMA for a permit to undertake the proposed release.

The possibility of insects developing resistance to the VIP3A protein as a result of this proposed release was considered in the risk assessment. However, no chance of harm to the health and safety of people or the environment was identified. No conditions have been imposed in relation to management of insecticide resistance, as this relates to product efficacy and is considered by the APVMA.

3.3 Identification of issues to be addressed for future releases

The risk assessment identified additional information that may be required to evaluate an application requesting a larger scale release of these GM cotton lines or a reduction in containment conditions. This would include the following:

- expression levels of the VIP3A protein in the COT202 and COT203 GM cotton lines
- the toxicity of the VIP3A protein for a range of organisms in Australia
- Australian field studies addressing the biodiversity of insect populations in unsprayed VIP3A crops compared with conventional cotton crops.

Section 4 Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of insect resistant GM cotton lines into the Shires of Narrabri, NSW and Emerald, QLD poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

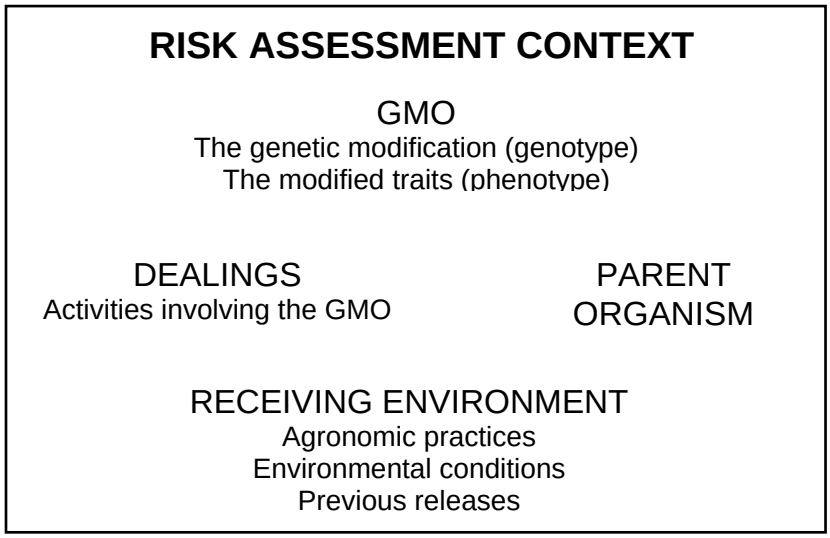
The risk management plan concludes that these negligible risks do not require specific risk treatment measures. Licence conditions have been imposed to contain the release to the proposed size, duration and regions requested by the applicant.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks to the health and safety of people and the environment that may be posed by the proposed release must be assessed. These may include the scope and boundaries for the evaluation process required by the Act, details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. These are summarised in Figure 1.1.

Figure 1.1 Components of the risk context considered during the preparation of the RARMP



2. The *Gene Technology Act 2000* (the Act), Gene Technology Regulations 2001 (the Regulations) and corresponding State and Territory laws govern the process the Gene Technology Regulator (the Regulator) is required to undertake before making a decision on whether or not to issue an intentional release licence. The legislative requirements and the processes for implementing them are outlined in more detail at <http://www.ogtr.gov.au/ir/process.htm> and in the *Risk Analysis Framework* (OGTR 2005).

3. In particular, sections 49 to 51 of the Act outline the matters which the Regulator must take into account, and who she must consult with, in preparing the RARMP.

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

- comparisons with the parent organism
- the nature and effect of the genetic modification
- relevant agricultural practices
- the proposed size, duration and regions requested by the applicant
- the environmental conditions in the regions where the release would occur
- previous approvals for release of these GMOs in Australia and overseas
- any previous releases of these or other GMOs relevant to this application.

5. 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, GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Heritage and local councils where the release may 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.

6. In accordance with section 52 of the Act, the Regulator notified the public that a RARMP had been prepared and invited written submissions in relation to the RARMP. Advice on the RARMP was also sought again from the same expert groups and agencies as mentions above. Issues raised during this consultation period are summarised in Appendices D and E. These appendices also indicate where the issues were addressed in the RARMP.

Section 2 The GMOs and proposed dealings

2.1 The proposed dealings

7. Deltapine proposes to release three insect resistant GM cotton lines (COT102, COT202 and COT203) into the environment under limited and controlled conditions.

8. The proposed dealings include growing the GM cotton lines on one or two sites of 0.5 ha each ie a maximum total planting area of 1 hectare. One site would be at Deltapine's Locharba Research Facility in the Shire of Narrabri, NSW. The location of the second site would be in the Shire of Emerald, QLD.

9. The purpose of the proposed release is to produce pure seed from the GM cotton lines. Therefore, the applicant intends to treat the GM cotton plants with insecticide sprays to prevent any insects carrying pollen onto the release sites from nearby cotton plants. The applicant anticipates using the seed produced during this proposed trial in future larger scale trials that may aim to evaluate the efficacy of the insect resistance trait, undertake ecological assessments and transfer the insect resistance trait to elite Australian cotton cultivars through breeding. These trials would be the subject of future applications and would require separate assessments and approvals.

10. During the proposed release, the applicant intends to transport the GM cottonseed to the release sites and transport the harvested GM seed cotton from the release sites to the Locharba Research Facility for ginning and de-linting. The applicant also proposes to sell lint from the pollen traps and GM cotton lines. Processed lint, which is the cellulose fibres removed from cotton seed during ginning, does not contain protein or genetic material. Following the proposed release, the applicant intends to store the seed harvested from the release.

2.2 The parent organism

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

2.3 The GMOs

12. Three GM cotton lines are proposed for release (COT102, COT202 and COT203). Each line contains the *vip3A* gene derived from *Bacillus thuringiensis*, a common soil bacterium. This gene encodes a Vegetative Insecticidal Protein, VIP3A, which is toxic to some

lepidopteran insects, including two major pests of cotton, *Helicoverpa armigera* and *H. punctigera*.

13. One insect resistant GM cotton line (COT102) also contains a selectable marker gene, *aph4* (also known as *hph*, *hpt* or *hyg*), encoding an antibiotic resistance protein, which was derived from *Escherichia coli*, a common gut bacterium. The *aph4* gene encodes an enzyme, hygromycin phosphotransferase, which confers resistance to the antibiotic hygromycin B.

14. The applicant states that COT102 cotton has shown consistent insecticidal protein expression and efficacy both in the laboratory and in the field. The COT102 cotton line has been approved previously for limited and controlled release in Australia (refer to DIRs 017/2002, 025/2002, 034/2003 and 036/2003).

15. The GM cotton lines COT202 and COT203 contain only the *vip3A* gene and not the *aph4* gene (see Section 4). These lines have been approved previously for a limited and controlled release in Australia (refer to DIR 036/2003).

16. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in the GM cotton lines. These are derived from *Arabidopsis thaliana*, a plant, and from *Agrobacterium tumefaciens*, a common soil bacterium. Although *A. tumefaciens* is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not infectious or capable of causing disease symptoms.

17. Some details of the gene construct, including the plasmid map and some specific regulatory sequences were previously declared Confidential Commercial Information (CCI) under section 185 of the Act, in connection with licence applications DIR 017/2002, DIR 034/2003 and DIR 036/2003. 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.

2.4 The introduced genes and their products

*2.4.1 The insect resistance gene (*vip3A*)*

18. The insect resistance gene, *vip3A*, is derived from *B. thuringiensis*, a common soil bacterium (Martin & Travers 1989). *B. thuringiensis* is readily isolated in association with insects (particularly in dry indoor environments such as warehouses), from leaf surfaces (particularly of deciduous and coniferous trees) or from soil samples (Meadows 1993). One survey found *B. thuringiensis* in 70% of soil samples collected from 30 countries (Martin & Travers 1989). *B. thuringiensis* produces a range of insecticidal proteins, each with specific toxicity for certain groups of insects.

19. The insecticidal genes originally isolated from *B. thuringiensis* were the *cry* genes that express crystalline (Cry) proteins during sporulation of this bacterium. A number of Cry proteins have previously been expressed in genetically modified crop plants to protect them against agricultural pests.

20. The *vip3A* gene encodes a protein that is distinct from the Cry proteins. The encoded protein is secreted into the extracellular environment by *B. thuringiensis* during vegetative growth, and expression continues into the stationary (sporulation) phase (Estruch et al. 1996). Expression of the VIP3A protein in growth stages before sporulation distinguishes it from the Cry proteins, which are mainly expressed during sporulation (Estruch et al. 1996; Schnepf et al. 1998).

21. The VIP3A protein was originally isolated from two strains of *B. thuringiensis* (AB88 and AB424) (Estruch et al. 1996). However, very similar proteins (of the same size and immunological reactivity) are present in commercial Bt sprays (US EPA 2005). In addition, *B. thuringiensis* var. *kurstaki* (Btk) strain HD1 contains a *vip3A* gene (Donovan et al. 2001). Bacteria in which this *vip3A* gene was inactivated showed decreased toxicity to lepidopteran species (in particular, *Agrotis ipsilon* and *Spodoptera exigua*) (Donovan et al. 2001). The Btk HD1 strain is used in commercial Bt sprays in Australia (see APVMA database <http://www.apvma.gov.au/pubcris/subpage_pubcris.shtml>) and elsewhere.

22. The VIP3A protein does not show significant amino acid sequence similarity to the Cry1Ac and Cry2Ab proteins that are produced in Ingard® and/or Bollgard II® cotton. Although the basic mode of action of the VIP3A protein is similar to that of the Cry proteins, the VIP3A protein binds to specific receptor(s) inside the insect gut that are different to those bound by the Cry1Ac protein (Lee et al. 2003). Once bound, the protein inserts into the gut cell membrane and forms ion-specific pores, which disrupt the insect's digestion and subsequently cause it to die. If organisms (eg humans, other mammals, birds, fish or insects) do not have the specific receptor(s) that enable binding of the VIP3A protein, they will not be susceptible to the toxic effects of the protein.

23. More information about the toxicity of the VIP3A protein for a variety of invertebrate species is provided in Chapter 4.

24. The *vip3A* gene was modified for optimum expression in plant cells, using protocols similar to those described by Perlak et al. (1991). During this process, one amino acid was changed in relation to the original sequence due to an error in the published sequence. The amino acid substitution is a substitution of a glutamine residue for a lysine residue at amino acid 284 (information supplied by the applicant).

25. Like the *cry* genes in other GM insect resistant cotton plants (Ingard® and Bollgard II®), the *vip3A* gene encodes a protein that is toxic to some lepidopteran insects, including *H. armigera* and *H. punctigera*, major herbivorous pests of cotton in Australia. Preliminary bioassay data (obtained during a field trial under PR-151) indicated that the COT102 line has more potent insecticidal activity towards *H. armigera* than Ingard® cotton (information provided in CSIRO's application for DIR 036/2003).

26. The ranges of species that are sensitive to the VIP3A and Cry proteins (Cry1Ac and Cry2Ab) overlap (see Chapter 4). However, toxicity studies have used different sets of insect species as subjects, which does not allow complete comparison of the specificities of the three proteins. Nevertheless, one example of a difference in the level of toxicity is for *Spodoptera* species (armyworms). Armyworms were reported to be sensitive to the VIP3A protein but relatively insensitive to Cry1Ac (information from the *Bt Specificity Database* <http://www.glfc.forestry.ca/bacillus>).

Expression of the VIP3A protein in the GM cotton lines

27. In the COT102 GM cotton line, expression of the *vip3A* gene is controlled by a different plant-derived promoter from that in the COT202 and COT203 GM cotton lines. The COT102 line contains the *actin 2* promoter from *A. thaliana* (including the first exon and intron that occur in the non-translated leader) (An et al. 1996). This is a strong, constitutive promoter. Preliminary expression data indicates that the VIP3A protein is present in the seeds, leaves, squares, roots, bolls and pollen, but could not be detected in nectar or cotton fibre. Levels of VIP3A protein detected in COT102 tissues are presented below in Table 1.1.

Table 1.1 VIP3A protein expression levels in the COT102 GM cotton line.

Seed (fresh weight)	2.0–3.0 μg/g
Leaves (fresh weight)	3.0–22.0 μg/g
Squares (fresh weight)	less than 4.0 μg/g
Roots (fresh weight)	less than 2.5 μg/g
Bolls (fresh weight)	0.3–1.5 μg/g
Pollen (dry weight)	1.1 μg/g

28. The promoter controlling expression of the *vip3A* gene in the COT202 and COT203 GM cotton lines is expected to lead to expression of the VIP3A protein throughout the growing season and in most of the tissues. Preliminary data indicates that the VIP3A protein is detectable in leaves, squares, flowers, bolls and pollen.

29. Details of the origin and construction of the promoter controlling the expression of the *vip3A* gene in the COT202 and COT203 GM cotton lines have been declared CCI under section 185 of the Act.

30. Also required for efficient gene expression in plants is an mRNA termination region, including a polyadenylation signal. The termination signals are provided by the 3'end of the *A. tumefaciens* nopaline synthase gene (*nos*) (Depicker et al. 1982).

*2.4.2 The antibiotic resistance marker gene (*aph4*)*

31. The COT102 insect resistant cotton line also contains the *aph4* antibiotic resistance marker gene located on a plasmid isolated from *Escherichia coli*, a common gut bacterium. The *aph4* gene, which encodes a hygromycin phosphotransferase enzyme, was used in the initial laboratory stages of development of the GM cotton plants, to enable selection of cells containing the desired genetic modification. This enzyme inactivates the antibiotic hygromycin B. The hygromycin resistance protein is in common use as a selectable marker in the production of GM plants (EFSA 2004). The only modification made to the bacterial *aph4* gene was the addition of plant regulatory sequences (promoter and termination regions) to allow efficient expression in plant cells.

32. The termination region is derived from the 3'end of the *nos* gene of *A. tumefaciens* (Depicker et al. 1982). The hygromycin resistance protein is present at low levels or is not detectable in most COT102 tissues tested (information supplied by the applicant).

33. Details of the origin and identity of the promoter controlling expression of the *aph4* gene have been declared CCI under section 185 of the Act.

2.5 Method of genetic modification

34. The COT102, COT202 and COT203 GM cotton lines were each generated by *Agrobacterium*-mediated transformation using protocols similar to those described by Murray et al (1999).

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

36. 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). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing biosafety concerns or any adverse reactions.

37. The COT102 line was generated using a disarmed binary vector, pCOT1, to introduce the insecticidal gene, *vip3A*, and antibiotic resistant marker gene, *aph4*, using standard *Agrobacterium* transformation protocols. Details of the plasmid map have been declared CCI. GM cotton cells were selected through their ability to grow in the presence of the antibiotic hygromycin B, and GM cotton plants were regenerated from these cells. COT102 was derived from a single transformation event.

38. To produce the COT202 and COT203 lines, the insecticidal and antibiotic resistance genes were introduced on separate T-DNAs within a single plasmid vector (p1998), such that the genes were able to insert independently of each other at different sites within the cotton genome. This is the same plasmid previously referred to as pNOV103 in the DIR 036/2003 RARMP. GM cotton cells were selected in the presence of hygromycin and GM plants regenerated from these cells. Subsequent plant breeding led to segregation (separate inheritance) of the two introduced genes. Insect resistant plants without antibiotic resistance (ie without the *aph4* gene) were chosen for further work.

39. COT202 and COT203 were generated from independent transformation events, such that the introduced gene is located at different sites in the cotton genome in each line.

40. All three GM cotton lines have been propagated by self pollination through five generations and have shown stable expression of the *vip3A* gene. Further information about these constructs can also be found in previous RARMPs (DIRs 017/2002, 025/2002, 034/2003 and 036/2003).

Section 3 The receiving environment

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

42. The size and duration of the proposed release are outlined in Section 1.1. The proposed release is to occur in the Shires of Narrabri, NSW and Emerald, QLD. These are both commercial cotton growing regions south of latitude 22° South. Both regions have typical climates for summer cotton growing regions in Australia, with warm summers and higher summer than winter rainfall (see Table 1.2).

Table 1.2 Climatic data for Narrabri and Emerald.

	Narrabri West Post Office	Emerald Post Office
Average daily max/min temperature (summer)	32.3°C/17.3°C	34.2°C/20.3°C
Average daily max/min temperature (winter)	18.9°C/4.5°C	23.3°C/7.8°C
Average monthly rainfall (summer)	72.5 mm	84.4 mm
Average monthly rainfall (winter)	45.7 mm	27.8 mm

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

43. Agronomic management of the release is intended to be similar to non-GM cotton (see end of this section). Because of this, the biotic interactions between the GM cotton plants and these environments are expected to be similar to those occurring already in these environments.

44. The VIP3A protein is produced naturally by strains of *B. thuringiensis*, a common soil bacterium. The hygromycin resistance protein is produced by *E. coli*, a naturally occurring bacterium in soil and in the human gut. This forms part of the baseline data for determining the risks from exposure to these proteins as a result of the proposed release of the GM cotton lines.

45. Resistance to hygromycin B also occurs naturally in the hygromycin B-producing organism *Streptomyces hygroscopicus* (Leboul & Davies 1982). Hygromycin-resistant bacteria have been isolated from human clinical sources in Europe (Salauze et al. 1990) and from bovine and porcine faeces in Japan (Ohmae et al. 1979). In Australia, hygromycin B is not in human clinical use nor is it used in veterinary medicine.

46. A number of releases of GM cotton plants expressing the same and other insect resistance proteins from *B. thuringiensis* have previously been approved (specifically the Cry1Ac and Cry2Ab proteins). Most of these approvals included releases in the Shires proposed in the current application. These releases and their general locations are summarised in Section 3. As a result of the commercial release of Bollgard II® and Bollgard II®/Roundup Ready® cotton in southern Australia (south of latitude 22° South), insect resistant GM cotton plants are widespread in the agricultural environment, comprising about 80% of commercially grown cotton crops. This forms part of the baseline data for estimating the risks that may result from the proposed release of the GM cotton lines.

47. The applicant intends to treat the GM cotton as non-insect resistant cotton. The release will therefore receive applications of water, herbicide, fertilizers, pesticides (including insecticides) and other agronomic management similar to any non-GM cotton growing on the farm during the release. The applicant intends to recycle all irrigation water and maintain a high level of hygiene on the farm (eg all equipment is cleaned on entry and exit to prevent the transfer of disease).

Section 4 Previous Australian and international approvals

4.1 Previous Australian approvals of the same or similar GM cotton lines

4.1.1 Previous releases approved by GMAC or the Regulator

48. Five limited and controlled releases of the COT102 insect resistant GM cotton have been approved previously. These releases were approved for the Shires of Narrabri and/or Emerald (PR-151, DIR 017/2002 and DIR 036/2003). Releases were also approved under DIR 025/2992 and DIR 034/2003 (for WA), however, no plantings occurred under DIR 034/2003. One limited and controlled release (DIR 036/2003) of the COT202 and COT203 insect resistant GM cotton lines was approved for the Shires of Narrabri and Emerald. The licence holders for these releases were CSIRO (PR-151 and DIRs 017/2002, 025/2002 and 036/2003) and Syngenta Australia (DIR 034/2003). The releases ranged in size from 0.05 hectares to 25 hectares.

49. Other insect resistant GM cotton lines expressing the *cry* genes also derived from *B. thuringiensis* have been trialled extensively in southern Australia (eg DIRs 005/2001 and 035/2003) including in the Shires of Narrabri, NSW and Emerald, QLD, as well as commercially released under licences DIR 012/2002 (Bollgard II® and Bollgard II®/Roundup Ready® cotton), DIRs 022/2002 (Ingard® cotton) and 023/2002 (Ingard®/Roundup Ready® cotton). Although the Cry1Ac and Cry2Ab proteins also target lepidopteran pests of cotton, they target different receptors to those of VIP3A and may have a slightly different target range (see Chapter 4).

4.1.2 Approvals by other Australian government agencies

50. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements would also have to be met in respect of release of GMOs, including requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standard Australia New Zealand (FSANZ). This is discussed further in Chapter 6, Risk Management.

51. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Cotton seed oil and linters from the COT102 line have been approved for use in human food. Cotton seed oil and linters from the COT202 and COT203 lines have not been assessed for use in human food. The applicant does not intend to use materials from any of the GM cotton lines proposed for release in food.

4.2 International approvals

52. Field trials were approved for the COT102 line in the USA in 2000 and 2002 and an application for Non-Regulated Status has been submitted to USDA-APHIS in December 2004.

53. Field trials were also approved for the COT102 line in India (2002), Vietnam (2002), Argentina (2000), Brazil (2001) and China (2001) (information supplied by the applicant).

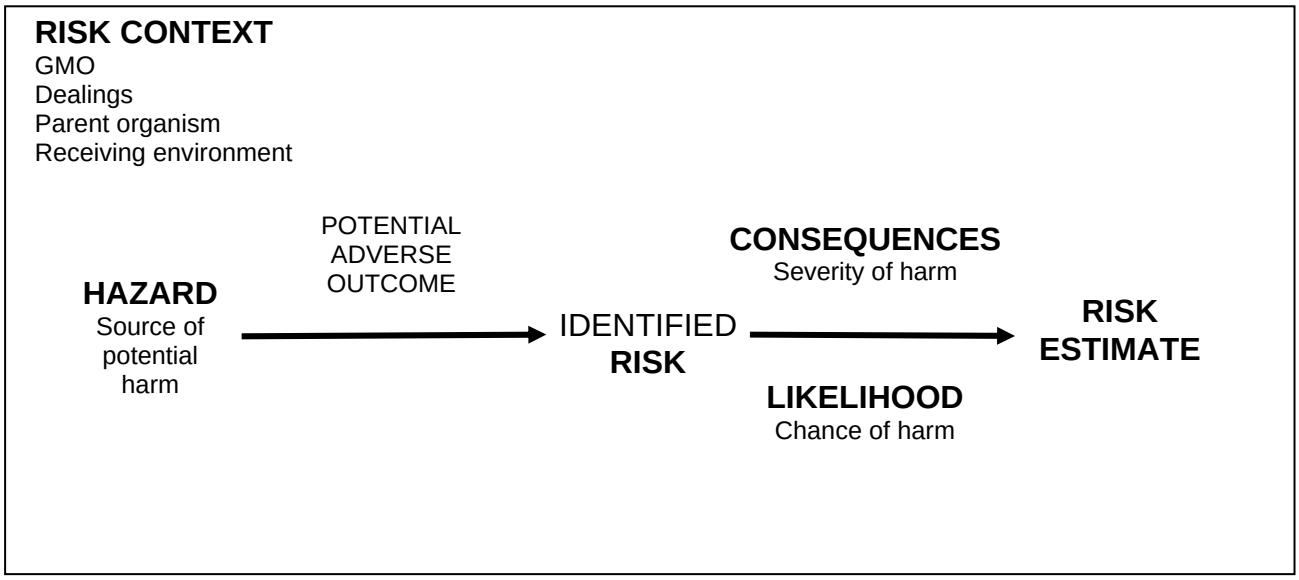
54. The US-EPA approved field trials of the COT202 and COT203 lines in March 2005.

Chapter 2 Risk assessment

Section 1 Introduction

55. 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 2.1) 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 2.1 The risk assessment process.



Note: words in **bold** are defined in Appendix A.

56. Once the risk assessment context has been established the next step is hazard identification to examine what harm could arise and how it could happen during this release of the GMOs into the environment.

57. It is important to note that the word 'hazard' is used in a technical rather than 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).

58. 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.

59. 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 or similar GMOs are also considered.

60. The hazard identification process resulted 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

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

62. 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 constitute 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.

63. 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 receiving environment. The risk assessment process focuses on measurable criteria for determining harm.

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

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- 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 GMOs
- 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 GMOs
- the size, duration and regions of the release.

65. Previous releases of these GM cotton lines containing the *vip3A* insect resistance gene occurred under PR-151 and DIRs 017/2002, 025/2002, and 036/2003. There have been no reports of adverse effects on the health and safety of people or the environment resulting from any of these releases.

66. Events that are discussed in detail later in this section are summarised in Table 2.1. Events that share a number of common features are grouped together in broader hazard categories as indicated in the table. Thirty-one events were characterised, of which eight are considered to lead to an identified risk that requires further assessment.

Table 2.1 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
Production of a substance toxic to people	Ingestion of GM plant materials containing the VIP3A protein.	Toxicity for people	No	Evidence indicates that the VIP3A protein has very low acute oral toxicity for mammals.
	Contact with, or inhalation of, GM plant materials containing the VIP3A protein.	Toxicity for people	No	On the basis of the very low acute oral toxicity of the VIP3A protein, it is also expected that it will have very low acute dermal and inhalation toxicity.
	Ingestion of GM plant materials containing the hygromycin resistance protein.	Toxicity for people	No	Evidence indicates that the hygromycin resistance protein has very low acute oral toxicity for mammals.
	Contact with, or inhalation of, GM plant materials containing the hygromycin resistance protein.	Toxicity for people	No	On the basis of the very low acute oral toxicity of the hygromycin resistance protein, it is also expected that it will have very low acute dermal and inhalation toxicity.
Production of a substance allergenic to people	Deliberate use of GM plant material in food.	Allergic reactions in people	No	No products from these GM plants will be used in food.
	Contact with items containing GM cotton fibre.	Allergic reactions in people	No	Processed lint does not contain detectable amounts of protein.
	Contact with GM plant materials containing the VIP3A or hygromycin resistance proteins.	Allergic reactions in people	Yes	**See Chapter 3, Event 1.**
	Contact with the VIP3A or hygromycin resistance proteins by accidental ingestion of plant material.	Allergic reactions in people	No	People do not deliberately eat cotton plants or any unprocessed material from cotton plants.
Production of a substance toxic to organisms other than people	Direct or indirect ingestion of the VIP3A protein by vertebrates as a result of this release.	Toxicity for vertebrates	No	Evidence indicates that the VIP3A protein is not toxic to vertebrates.
	Direct or indirect ingestion of the hygromycin resistance protein by vertebrates as a result of this release.	Toxicity for vertebrates	No	There is no evidence suggesting that the hygromycin resistance protein is toxic to any organism. Vertebrates are exposed to hygromycin resistance proteins through natural sources.
	Direct or indirect ingestion of the VIP3A protein by non-target invertebrates as a result of this release.	Toxicity for non-target invertebrates	Yes	**See Chapter 4, Event 4.**
	Direct or indirect ingestion of the hygromycin resistance protein by invertebrates as a result of this release.	Toxicity for invertebrates	No	There is no evidence suggesting that the hygromycin resistance protein is toxic to any organism. Invertebrates are exposed to hygromycin resistance proteins through natural sources.
	Contact with the VIP3A protein by microorganisms as a result of this release.	Toxicity for microorganisms	No	There is no evidence suggesting that the VIP3A protein is toxic to any organism. Microorganisms are exposed to the VIP3A protein through natural sources.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	Contact with the hygromycin resistance protein by microorganisms as a result of this release.	Toxicity for microorganisms	No	There is no evidence suggesting that the hygromycin resistance protein is toxic to any organism. Microorganisms are exposed to hygromycin resistance proteins through natural sources.
Spread and persistence of the GM cotton in the environment	Expression of the *vip3A* gene increasing spread and persistence of the GM cotton plants through reduced lepidopteran herbivory.	Weediness	Yes	**See Chapter 5, Event 7.**
	Exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment.	Allergic reactions in people	Yes	**See Chapter 3, Event 2.**
	Exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment.	Toxicity for non-target invertebrates	Yes	**See Chapter 4, Event 5.**
	Expression of the *aph4* gene or the presence of the regulatory sequences in the GM cotton plants.	Weediness or toxicity	No	The *aph4* gene is not expected to have any influence on the spread and persistence of the GM cotton plants.
	Spillage of seed during transport or storage	Weediness, allergic reactions, toxicity	No	The applicant proposes to transport and store seed according to OGTR guidelines.
Gene flow by vertical gene transfer	Gene transfer to native *Gossypium* species	Weediness	No	Well established genetic incompatibility prevents vertical gene transfer to native *Gossypium* species.
	Expression of the *vip3A* gene in other *G. hirsutum* or *G. barbadense* cotton plants reducing lepidopteran herbivory.	Weediness	Yes	**See Chapter 5, Event 8.**
	Exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants.	Allergic reactions in people	Yes	**See Chapter 3, Event 3.**
	Exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants**.**	Toxicity for non-target invertebrates	Yes	**See Chapter 4, Event 6.**
	Presence of the introduced regulatory sequences in *G. hirsutum* or *G. barbadense* plants.	Unpredictable effects	No	The introduced regulatory sequences do not behave any differently than endogenous regulatory sequences in plants.
Gene flow by horizontal gene transfer	Presence of the *vip3A*, *aph4* genes or the introduced regulatory sequences in other organisms.	Toxicity, weediness, increased pathogenicity or decreased effectiveness of antibiotic medicines	No	Horizontal gene transfer of the introduced sequences to other organisms is not expected to result in any adverse outcomes during this release.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Unintended changes in toxicity	Random insertion of the gene construct into the GM cotton lines changing in the level of innate toxic or anti-nutritional compounds.	Toxicity for people and other organisms	No	COT102 cotton seed has no compositional changes in comparison to the parent cotton line. The composition of the COT202 and COT203 cotton seed is expected to be similar but data would be required for assessments of commercial releases involving use of GM cotton seed oil and linters in human food or animal feed.
Unintended changes in biochemistry or physiology	Altered biochemistry or physiology of the GM cotton lines resulting from the introduction and/or expression of the introduced genes.	Toxicity for people and/or other organisms or weediness	No	COT102 cotton seed has no compositional changes in comparison to the parent cotton line. The composition of the COT202 and COT203 lines is expected to be similar but data would be required for assessments of commercial releases involving use of GM cotton seed oil and linters in human food or animal feed. There is no evidence of altered physiology of the cotton lines proposed for release.
Unintended effects on other insects	Decreased use of insecticide sprays on the GM cotton resulting in the increased prevalence of other insect pests.	Increased non-lepidopteran insect herbivory	No	The release is small in area, short in duration and insecticide sprays will be used on the plants as if they were non-GM.
Secondary impacts	Development of VIP3A insecticide resistance.	Loss of GM trait efficacy	No	The release is small in area and short in duration. Resistance is not expected to develop during this release. However, this is an issue under consideration by the APVMA.
	Secondary effects on populations of organisms that interact with lepidopteran insects.	Potential to impact biodiversity	No	The release is small in area and insecticide sprays will be used on the plants as if they were non-GM.
Unauthorised activities	Use of GMOs outside the proposed licence conditions (non-compliance).	Weediness, allergic reactions, toxicity	No	The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires a test 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

67. 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 LD_{50}. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

68. 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 (Konig et al. 2004;OECD 1998). The use of purified toxin also increases the reproducibility of the assays.

2.1.1 Ingestion of GM plant materials containing the VIP3A protein

69. The applicant has provided data on the level of acute oral toxicity of the VIP3A protein for mammals. The results of two studies addressing the acute mammalian toxicity of the VIP3A protein were provided. No signs of toxicity were observed for any of the mice in the two studies, each of which lasted for 14 days. It was concluded that the oral LD_{50} was more than 2700 mg VIP3A/kg body weight (Study No. 1) and more than 3675 mg/kg body weight (Study No. 2). These were the maximum doses used in the studies. Both studies used protein extracted from GM *E. coli*. The protein used in Study No. 2 was identical to that expressed in the GM cotton plants proposed for release, while the protein used in Study No. 1 was shown to be equivalent in terms of biochemistry and function.

70. Two additional acute mammalian toxicity studies were submitted to FSANZ by Syngenta Australia for consideration during assessment of the safety of products derived from the COT102 GM cotton lines (FSANZ 2004). These studies also concluded that there was no evidence of acute oral toxicity for mammals from exposure to the VIP3A protein. One of these studies used VIP3A protein isolated from GM corn plants while other studies used VIP3A protein isolated from GM *E. coli*. Western blotting and mass spectrometry experiments were submitted to demonstrate that the VIP3A protein was equivalent, in terms of post-translational modifications, from GM corn, GM cotton and GM bacteria (FSANZ 2004).

71. In some countries, including the USA (but not Australia), cotton seed meal is approved for use in human diets (OGTR 2002). Levels of VIP3A protein in cotton seed from the COT102 line have been measured at 4 μg/g dry weight. A 70 kg person would need to consume approximately 64 tonnes of dried cotton seed at one sitting in order to be acutely exposed to 3675 mg/kg body weight. As indicated above, no toxic effects were observed in mice at this level. In addition, the applicant does not intend to use cotton seed oil or linters from the proposed release in human food. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of the VIP3A protein will not be assessed further.

2.1.2 Contact with, or inhalation of, GM plant materials containing the VIP3A protein

72. Dermal and inhalation toxicity studies have not been conducted with the VIP3A protein. However, on the basis of its very low acute oral toxicity, it is also expected to be of very low acute dermal and inhalation toxicity. Since the introduced protein is retained within the GM plant cells, dermal and inhalation contact may occur only when the plant cells have been damaged or broken. Therefore, only people who work with damaged GM cotton plants may come into contact with the introduced protein during handling and/or processing the GM cotton or its products that contain the protein. The concentration of protein available for contact via dermal and inhalation routes is expected to be low since the concentration of protein in GM cotton cells is low. Furthermore, since the proposed release is to be conducted on only two sites with a maximum total size of 1 hectare for a single growing season, 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 cotton seed oil or linters from the proposed release in human food. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, the VIP3A protein will not be assessed further.

2.1.3 Ingestion of GM plant materials containing the hygromycin resistance protein

73. A study of the toxicity of the hygromycin resistance protein, using protein purified from *E. coli*, indicated that the hygromycin resistance protein has very low acute oral toxicity for mice (FSANZ 2004). The oral LD_{50} was estimated to be greater than the maximum oral dose of 779 mg/kg body weight.

74. The *aph4* gene is not present in the COT202 and COT203 GM cotton lines and is expressed at levels too low to quantify in the COT102 line. Therefore, **no risk is identified** and the potential for toxicity for people as a result of ingestion of the hygromycin resistance protein will not be assessed further.

2.1.4 Contact with, or inhalation of, GM plant materials containing the hygromycin resistance protein

75. Dermal and inhalation toxicity studies have not been conducted with the hygromycin resistance protein. However, on the basis of its very low acute oral toxicity, it is also expected to be of very low acute dermal and inhalation toxicity. Since the introduced protein is retained within the GM plant cells, dermal and inhalation contact may occur only when the plant cells have been damaged or broken. Therefore, only people who work with damaged GM cotton plants may come into contact with the introduced protein during handling and/or processing the GM cotton or its products that contain the protein. The concentration of protein available for contact via dermal and inhalation routes is expected to be low since the concentration of protein in GM cotton cells is low. Furthermore, since the proposed release is to be conducted on only two sites with a maximum total size of 1 hectare for a single growing season, the frequency and duration of contact with the introduced proteins is expected to be very limited. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, the hygromycin resistance protein will not be assessed further.

2.2 Production of a substance allergenic to people

76. The possibility that exposure of people to proteins expressed from the introduced genes or their enzymatic products in the GM cotton plants may result in an allergic reaction is considered. Routes of exposure to the VIP3A or hygromycin resistance proteins could include consumption of food containing cotton products, accidental ingestion of material from the cotton plants, contact with clothing or household items containing cotton, or contact with material from cotton plants, either as a result of occupational exposure or from living near the proposed release.

2.2.1 Deliberate use of GM plant material in food

77. Cotton seed oil and linters derived from the COT102 GM cotton line have recently been approved for human consumption (FSANZ 2004). The COT102 GM cotton line contains both the *vip3A* and *aph4* genes while lines from the COT202 and COT203 GM cotton lines contain only the *vip3A* gene. The applicant does not intend to use cotton seed oil or linters derived from any of the GM cotton plants from this release in human food or animal feed. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through food will not be assessed further.

2.2.2 Contact with items containing GM cotton fibre

Fibres are removed from the surface of cotton seed in two separate processes (Gregory et al. 1999). During ginning, the long fibres are removed from the seed. These fibres are called cotton lint. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from cotton lint. Following the ginning process, the seeds can be delinted, which involves the removal of the shorter fibres (linters) still attached to the seed. These cotton

linters are used in a variety of products including medical dressings, felt, fine quality paper (including banknotes in many countries), twine and mops. Cellulose derivatives produced from the linters may be used in pharmaceuticals, cosmetics, toothpaste, lacquers, paints, toothpaste, ice cream, salad dressing and a variety of plastics (FSANZ 2004; Gregory et al. 1999). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity.

78. A study of the accumulation of mineral nutrients in cotton fruit found no detectable nitrogen in fibre fractions (Leffler & Tubertini 1976). Using more sensitive methods, specific proteins were detected at very low levels in raw, but not processed, linters and lint (Sims et al. 1996). Therefore the safety of wearing cotton clothing or using other products made from cotton is not expected to be affected by the genetic make-up of the cotton plants from which these components have been derived, that is, whether or not it is derived from GM or non-GM cotton plants. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through cotton fibre will not be assessed further.

2.2.3 Contact with GM plant materials containing the VIP3A or hygromycin resistance proteins

79. People working with cotton plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein. However, dermal exposure to proteins (including the VIP3A and hygromycin resistance proteins) or to other cellular components of the cotton plants may occur if damage to the plants during handling results in rupture of plant cells.

80. Inhalation of pollen by workers or people living near cotton crops could potentially result in allergic reactions if either of these proteins were allergenic and were expressed in the pollen.

81. The primary processing of cotton at cotton gins, and the bulk handling of cotton seed and cotton fibre, can create and stir up fine dust and lint particles. Use of personal protective equipment by exposed workers is commonplace in such facilities to prevent respiratory irritations.

82. Therefore, **a risk is identified** and the potential for allergic reactions in people resulting from exposure through contact with the VIP3A or hygromycin resistance proteins will be assessed further in **Chapter 3, Event 1**.

2.2.4 Contact with the VIP3A or hygromycin resistance proteins by accidental ingestion of GM plant material

83. Exposure could also occur as a result of accidental ingestion of material from the GM cotton plants. However, people do not deliberately eat cotton plants or any unprocessed material from cotton plants. Cotton seed oil and linters of the GM cotton plants from the proposed release will not be used in human food. Any accidental ingestion of material from GM cotton plants is expected to be very limited and result in extremely low exposure to the introduced proteins. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from exposure through accidental ingestion of plant material will not be assessed further.

2.3 Production of a substance toxic to organisms other than people

84. A range of organisms may be exposed directly, through feeding on the GM cotton plants, or indirectly through eating organisms that feed on GM cotton plants. These organisms include vertebrates, invertebrates and microorganisms.

85. 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).

86. Mammals generally avoid feeding on cotton plants. 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, except for cattle, which are less affected by these components. 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.

87. Neither cotton trash nor stubble are used as animal feed, due to the possible presence of pesticide residues.

2.3.1 Direct or indirect ingestion of the VIP3A protein by vertebrates as a result of this release

88. The VIP3A protein is produced by some strains of *Bacillus thuringiensis*, a soil bacterium commonly found in agricultural environments, including in the HD1 strain used in commercial Bt sprays. It is therefore likely that many vertebrates will have been exposed to the VIP3A protein through natural sources, without evidence of toxic effects.

89. The VIP3A protein is known to bind to a specific receptor located in the gut of the larvae of some insects (Lee et al. 2003). Current evidence indicates that only lepidopteran insects are affected (see Chapter 4). Although it is possible that insect species from some other orders may be sensitive, it is unlikely that this protein will interact with receptors in vertebrates.

90. GM cotton seed and pollen from the release are not expected to enter aquatic habitats in any significant quantities. Therefore, the level of exposure of aquatic organisms to the GM cotton lines will be low. Irrigation practices used by cotton growers in Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways.

91. As discussed in Section 2.1.1, a number of studies aimed at determining the level of acute oral toxicity of the VIP3A protein for mammals, have been performed. These studies did not find any evidence of acute toxicity.

92. In addition, the applicant has also provided the results of a bobwhite quail feeding study using VIP3A isolated from GM bacteria. Ten birds, approximately 9 weeks old, were dosed orally with gelatine capsules containing the equivalent of 400 mg pure VIP3A/kg body weight. The birds were monitored for 14 days, during which no clinical signs of acute toxicity were noted. No deaths occurred during this time. At the end of the study, post-mortem examinations of the gastro-intestinal tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat revealed no abnormal findings. The oral LD_{50} was estimated to be greater than 400 mg/kg body weight.

93. The above evidence indicates that the VIP3A protein has very low acute oral toxic for vertebrates. Therefore, **no risk is identified** and the potential for toxicity for vertebrates resulting from the expression of the *vip3A* gene in the GM cotton lines will not be assessed further.

2.3.2 Direct or indirect ingestion of the hygromycin resistance protein by vertebrates as a result of this release

94. The function of the hygromycin resistance protein is the inactivation of the antibiotic hygromycin through phosphorylation. A study submitted to FSANZ during assessment of the safety of cotton seed oil and linters derived from the COT102 line indicated that the hygromycin resistance protein has very low acute oral toxic to mice (FSANZ 2004). The oral LD_{50} was estimated to be greater than 779 mg/kg body weight (the highest dose given to the test animals).

95. Since similar proteins are produced by bacteria that are widespread in the environment, the hygromycin resistance protein is not expected to be a novel source of harm for vertebrates. In addition, the hygromycin resistance protein is expressed at levels too low to quantify in the COT102 line. Therefore, **no risk is identified** and the potential for toxicity for vertebrates resulting from the expression of the *aph4* gene in the COT102 GM cotton line will not be assessed further.

96. The COT202 and COT203 lines do not contain the *aph4* gene.

2.3.3 Direct or indirect ingestion of the VIP3A protein by non-target invertebrates as a result of this release

97. The VIP3A protein is known to be toxic to a range of lepidopteran species other than the two major pests of cotton plants, which are the primary targets of the VIP3A protein (*H. armigera* and *H. punctigera*) in the GM cotton lines (information supplied by the applicant). Therefore, **a risk is identified** and the potential for toxicity for non-target invertebrates resulting from exposure to the GM cotton lines will be assessed further in **Chapter 4, Event 4**

2.3.4 Direct or indirect ingestion of the hygromycin resistance protein by invertebrates as a result of this release

98. There is no direct evidence to indicate whether or not the hygromycin resistance protein and other similar proteins are toxic to invertebrates. However, these proteins are produced by common soil bacteria. Invertebrates are therefore expected to be exposed to these proteins in the agricultural environment already without evidence of toxic effects. Any additional exposure resulting from the very low level of expression of the hygromycin resistance protein in the COT102 line is not expected to result in significant toxic effects. Therefore, **no risk is identified** and the potential of toxicity for invertebrates as a result of the expression of the *aph4* gene in the COT102 GM cotton line will not be assessed further.

2.3.5 Contact with the VIP3A protein by microorganisms as a result of this release

99. The production of the VIP3A protein by bacteria suggests that this protein does not kill bacteria. The bacterial species for which this is known are the gram positive *B. thuringiensis*, which produces the protein naturally, and the gram negative *E. coli*, which has been used to produce large quantities of the protein for research. Microorganisms (including bacteria, nematodes, and fungi) are also already exposed to VIP3A and other similar proteins in the agricultural environment, as *B. thuringiensis* excretes the VIP3A protein during vegetative growth. Currently, there is no evidence to indicate whether or not the VIP3A protein accumulates in soil, however, most proteins in the soil can be degraded by microorganisms. Any additional exposure resulting from the low level expression of VIP3A in the GM cotton plants is not expected to result in significant toxic effects. Therefore, **no risk is identified** and the potential for toxicity for microorganisms as a result of the expression of the *vip3A* gene will not be assessed further.

2.3.6 Contact with the hygromycin resistance protein by microorganisms as a result of this release

100. There is no direct evidence to indicate whether or not the hygromycin resistance protein or other similar proteins may be toxic to some microorganisms. However, these proteins are produced by common soil bacteria to protect them against exposure to the antibiotic hygromycin B. Microorganisms are therefore expected to be exposed to these proteins in the natural and agricultural environment already. Any additional exposure resulting from the low level expression of hygromycin resistance protein in the COT102 line is not expected to result in significant toxic effects. Therefore, **no risk is identified** and the potential for toxicity for microorganisms as a result of the expression of the *aph4* gene will not be assessed further.

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

2.4.1 Expression of the vip3A *gene increasing spread and persistence of the GM cotton plants through reduced lepidopteran herbivory*

101. The GM cotton lines produce the VIP3A protein, which is toxic to some lepidopteran insects, including major lepidopteran pests of cotton crops. In environments where lepidopteran herbivory is a significant limit on the spread and persistence of cotton plants, this could lead to weediness of the GM cotton lines. Therefore, **a risk is identified** and the potential for weediness of the GM cotton lines as a result of the presence of the *vip3A* gene will be assessed further in **Chapter 5, Event 7**.

2.4.2 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment

102. If expression of the VIP3A protein in the GM cotton lines resulted in their increased spread and persistence in the environment, this could provide an opportunity for increased numbers of people to come into contact with the GM cotton plants. This might increase the potential for allergenicity for people. Therefore, **a risk is identified** and the potential for allergic reactions in people as a result of increased spread and persistence of the GM cotton lines will be assessed further in **Chapter 3, Event 2**.

2.4.3 Exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment

103. If expression of the VIP3A protein in the GM cotton lines resulted in their increased spread and persistence in the environment, this could provide an opportunity for non-target organisms to come into contact with the GM cotton plants. This might increase the potential for toxicity for non-target organisms. Therefore, **a risk is identified** and the potential for toxicity to non-target invertebrates as a result of increased spread and persistence of the GM cotton lines will be assessed further in **Chapter 4, Event 5**.

2.4.4 Expression of the aph4 *gene or the presence of the regulatory sequences in the GM cotton plants*

104. In contrast, neither the *aph4* gene nor the introduced regulatory sequences in the GM cotton lines are expected to have any impact on the spread or persistence of the GM cotton lines. Hygromycin B is not applied to plants outside of the laboratory, nor does expression by microorganisms result in high levels of the antibiotic in the environment. The introduced regulatory sequences behave no differently to endogenous regulatory sequences in cotton. Therefore, **no risk is identified** and the potential for weediness of the GM cotton lines as a result of the presence of the *aph4* gene and/or the introduced regulatory sequences will not be assessed further.

2.4.5 Spillage of seed during transport or storage

105. In the course of the proposed dealings the applicant proposes to transport seed to and from the release sites, and to store all GM cottonseed harvested from the crop. Any accidental spillage of seed during transport or storage could allow the GM cotton plants to spread and persist in the environment.

106. 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*; and *Policy on storage of genetically modified organisms, July 2004*). The applicant proposes to transport and store seed according to OGTR guidelines. Therefore, any spillage of seed during transport to and from the release sites or while in storage would be rare. Any incident involving spillage of GM seed is expected to be readily controlled through cleaning and monitoring of the site of the spill. In addition, the opportunity for any adverse outcome from any such rare occurrence is further diminished by the need for appropriate environmental conditions for germination, survival and persistence of any few escaped seeds. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of spillage of seed during transport or storage will not be assessed further.

2.5 Gene flow by vertical gene transfer

107. Transfer of genetic material to offspring by reproduction, either asexual or sexual (vertical gene transfer) could result in the transfer of the *vip3A* or *aph4* genes or their associated regulatory elements to other plants. The only sexually compatible species present in Australia that could receive genes from the GM cotton lines are *G. hirsutum* and *G. barbadense* (including both cultivated (GM and non-GM) and naturalised cotton populations).

2.5.1 Gene transfer to native Gossypium *species*

108. 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.

109. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from cultivated cotton fields. Thus, gene transfer from the GM cotton lines to native cotton plants is prevented not only by genetic incompatibility but also by geographic constraints on cross-pollination (OGTR 2002). Therefore, **no risk is identified** and the potential for weediness in these sexually incompatible species as a result of gene transfer will not be assessed further.

2.5.2 Expression of the vip3A *gene in other* G. hirsutum *or* G. barbadense *cotton plants as a result of gene transfer*

110. Sexually compatible plants (ie other cultivated cotton species, including other GM cotton plants) expressing the *vip3A* gene as a result of vertical gene transfer could become resistant to lepidopteran insects. This could confer a fitness advantage on plants normally controlled by these insects and could result in weediness. Therefore, **a risk is identified** and the potential for weediness as a result of vertical gene transfer of the *vip3A* gene into other cultivated cotton species will be assessed further in **Chapter 5, Event 8**.

2.5.3 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to G. hirsutum *or* G. barbadense *plants*

111. Expression of either of the introduced genes in sexually compatible plants (ie other cultivated cotton species, including other GM cotton plants) could lead to increased exposure of people to the expressed proteins. This could result in allergic reactions. Therefore, **a risk is identified** and the potential for allergic reactions in people as a result of vertical gene transfer of the *vip3A* gene into other cultivated cotton species will be assessed further in **Chapter 3, Event 3**.

2.5.4 Exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to G. hirsutum *or* G. barbadense *plants*

112. There could also be adverse impacts on lepidopteran insect populations, or secondary impacts on specialist predators and parasitoids that feed on them and consequently other organisms linked to these organisms through food webs. Therefore, **a risk is identified** and the potential for toxicity for non-target invertebrates as a result of vertical gene transfer of the *vip3A* gene into other cultivated cotton species will be assessed further in **Chapter 4, Event 6**.

2.5.5 Presence of the introduced regulatory sequences in G. hirsutum *or* G. barbadense *plants*

113. 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. 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 vip3A *gene,* aph4 *gene or the introduced regulatory sequences in other organisms*

114. Transfer of the *vip3A* gene, *aph4* gene or the introduced regulatory sequences to sexually incompatible plants, animals or microorganisms can occur without reproduction or human intervention (horizontal gene transfer).

115. Transfer of the *vip3A* gene to other organisms could cause the spread of this gene in the environment, leading to increased exposure of lepidopteran and other insects to the toxic protein outside of the agricultural environment, and could also have adverse effects on populations of organisms that interact with lepidopteran insects in the food web.

116. Transfer of the *aph4* gene to other organisms could result in antibiotic medicines containing hygromycin becoming less effective due to spread of resistance to hygromycin B in the environment. However, there are no products containing hygromycin B currently registered for human medicinal or veterinary use in Australia (TGA, MIMS Online database; and APVMA, Pubcris database). Furthermore, other genes conferring resistance to hygromycin are already naturally present in bacterial species (Zalacain et al. 1987; Penaloza-Vazquez et al. 1995), so any transfer of this gene is much more likely to occur between bacteria than between plant and bacteria.

117. 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 unpredictable effects.

As there is no difference between the two events, this does not represent a novel adverse outcome as a result of the genetic modification.

118. Horizontal gene transfer has been examined in detail in a number of other RARMPs (most recently DIR 057/2004), which are available from the OGTR website <http://www.ogtr.gov.au> or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to 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 relied on the use of highly similar sequences to allow homologous recombination to occur, and conditions designed to enhance the selective advantage of gene transfer events (Nielsen et al. 1998; Mercer et al. 1999; Nielsen et al. 2000; Gebhard & Smalla 1998; De Vries et al. 2001). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed release. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of horizontal gene transfer will not be assessed further.

2.7 Unintended changes in toxicity

2.7.1 Random insertion of the gene construct into the GM cotton lines changing the level of innate toxic or anti-nutritional compounds

119. 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). 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 genetic manipulation. A detailed compositional analysis of the COT102 line in comparison to the parental Coker 312 line has been assessed by FSANZ during application for approval to use cotton seed oil and linters derived from this line in human food. There were no significant differences in any of the toxic or anti-nutritional compounds listed above and FSANZ concluded that COT102 cotton seeds are compositionally equivalent to non-GM cotton seeds (FSANZ 2004). Similar data are not available for the COT202 and COT203 GM cotton lines and it has been assumed during this assessment that these lines will be similar to the COT102 lines. Therefore, **no risk is identified** and the potential for toxicity as a result of unintended changes in toxicity will not be assessed further.

2.8 Unintended changes in endogenous gene expression

2.8.1 Altered biochemistry or physiology of the GM cotton lines resulting from expression of the introduced genes

120. Genetic modification has the potential for unintended effects due to the insertion process or by producing a product that affects multiple traits. Consideration is given to such unintended effects, which 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, transcriptional read-through etc
- increased metabolic burden associated with high level expression of the introduced gene
- 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.

121. Such unintended effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional or agronomic 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).

122. Unintended changes in gene expression could alter either the biochemistry or the physiology of the GM cotton plants. Biochemical or physiological changes to the GM cotton lines 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).

123. Experience from two decades of research on, and commercialisation of, GM crops, and expanding knowledge of plant genome structure and dynamics all indicate that the genetic modification process itself provides little potential for unexpected adverse outcomes that would not be eliminated during the process of selecting modified plants in the laboratory or contained environment and again during the variety development process. The GM cotton lines proposed for release were selected from a large number of initial transformants and were selected on the basis that they did not show any altered agronomic properties beyond expression of the VIP3A protein (information supplied by the applicant).

124. These same GM cotton lines were also assessed in the RARMPs prepared for DIR licences 017/2002, 025/2002, 034/2003 and 036/2003. Information provided by the applicants for these assessments indicated that the COT102 line was field tested in the USA between 2000 and 2002 and displayed agronomic characteristics within the normal range for the parental variety. Previous releases of this line in Australia (performed under PR-151 and DIR 017/2002) did not show any unintended secondary effects (DIR 034/2003 RARMP). Preliminary field trials of the COT202 and COT203 lines in the USA also indicated that their agronomic characteristics are within the normal range for the parental cotton variety (DIR 036/2003 RARMP).

125. The VIP3A and hygromycin resistance proteins act independently of endogenous metabolic pathways in cotton and are therefore not expected to alter other biochemical or physiological characteristics of the GM cotton plants. Data provided to FSANZ during their assessment of the safety of cotton seed oil and linters derived from the COT102 line (FSANZ 2004) indicate that there are no significant compositional differences between the parental line (Coker 312) and the COT102 line. Therefore, **no risk is identified** and the potential for toxicity as a result of unintended changes in gene expression will not be assessed further.

2.9 Unintended effects on other insects

2.9.1 Decreased use of insecticide sprays on the GM cotton resulting in the increased prevalence of other insects

126. The GM cotton is resistant to the major lepidopteran pests of cotton crops, thus it is expected to require fewer applications of pesticide sprays than non-GM cotton crops. This has the potential to allow populations of other insects, normally controlled by pesticide sprays, to increase to problematic levels. This could not only result in an increased pest burden in the environment but may also increase the incidence of gene flow if pollinator species became more abundant. However this is only a small trial with a maximum size at each site of 0.5 ha and is limited to a single season. The applicant also intends to treat these small trial plantings in the same way as non-GM cotton, so similar levels of pesticides will be applied to these sites as are applied to non-GM cotton crops. Therefore, **no risk is identified** and the potential

for increased non-lepidopteran herbivory as a result of unintended effects on existing pests will not be assessed further.

2.10 Secondary impacts

2.10.1 Development of VIP3A insecticide resistance

127. The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complementary regulatory role in respect to this application due to its responsibility for agricultural chemical use in Australia, including insecticides and herbicides, under the *Agricultural and Veterinary Chemicals Code Act 1994*.

128. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an insecticide 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 Gene Technology Regulator's assessment, such as the efficacy of an insecticide or herbicide, and insecticide and herbicide resistance management and, if necessary, imposes conditions in relation to these. The APVMA can impose conditions on both registrations and permits.

129. Widespread and long-term use of the insect-resistant GM cotton lines expressing the *vip3A* gene could result in lepidopteran insects becoming resistant to this toxin, decreasing the efficacy of this GM trait. Although efficacy is not covered by the Act, this issue has been considered. A release of this area and duration (two sites of 0.5 ha for a single season) is not expected to lead to the development of resistance.

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

2.10.2 Secondary effects on populations of organisms that interact with lepidopteran insects

131. Widespread and long-term use of the insect-resistant GM cotton lines expressing the *vip3A* gene could result in other organisms that interact with lepidopteran insects in the food web being adversely affected, which could result in reduced biodiversity. However, as the proposed trial will be treated in the same manner as non-GM cotton, insecticides will be used to decrease insect numbers in the crop. There will be little opportunity for the VIP3A protein to affect lepidopteran insects and the wider food web. Therefore, **no risk is identified** and the potential for an adverse outcome for organisms interacting with lepidopteran insects, as a result of the expression of the *vip3A* gene in the GM cotton lines, will not be assessed further.

2.11 Unauthorised activities

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

132. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM cotton lines outside of the proposed release area. The adverse outcomes that this event could cause are discussed in Section 2.4. 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. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unauthorised activities will not be assessed further.

Section 3 Risk estimate process for identified risks

133. Eight events from the hazard identification process (Events 1–8 in Table 2.1) are considered to lead to identified risks for the adverse outcomes of allergic reactions in people, toxicity for non-target invertebrates and weediness. The risks from this proposed release of GMOs into the environment are assessed in further detail in the following chapters to obtain estimates of the level of risk.

134. Chapters 3–5 give detailed consideration to the consequences and likelihood of these eight events. The risks are 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).

135. Information contained in the application (including information required by the Act and the Regulations on the GMOs, 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.

136. 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 then combined to give an overall risk estimate using the Risk Estimate Matrix (Figure 2.2). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty that arise from a lack of knowledge.

Figure 2.2 The OGTR Risk Estimate Matrix (OGTR 2005)

		RISK ESTIMATE			
LIKELIHOOD	**Highly Likely**	Low	Moderate	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.

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

138. 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 6).

Chapter 3 Risk estimates for allergic reactions in people

139. This chapter estimates the risks associated with three events that could lead to the adverse outcome of allergic reactions in people arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

Section 1 Background

140. Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies (known as IgE) or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO & WHO 2000). Allergy has a well-defined aetiology (ie biochemical cause) that is distinct from toxicity.

141. The biochemical events associated with allergic reactions, as well as the kinds of proteins that cause these reactions, are well characterised (Metcalfe et al. 1996; Taylor & Lehrer 1996). Sequence, structural and biochemical comparisons with known allergens have been used to predict the allergenicity of uncharacterised proteins (Astwood et al. 1996; Flavell et al. 1992; Fuchs & Astwood 1996; Metcalfe et al. 1996; Taylor & Lehrer 1996; Kimber et al. 1999).

142. Some characteristics common to many allergens are:

- molecular weight ranges between 15,000–70,000 M_r
- presence of conserved 5–8 amino acid signature sequences
- typically glycosylated
- stable in the mammalian digestive system
- stable in high temperatures involved in cooking or processing
- present as the major protein component in the specific foods.

143. However, many non-allergenic proteins also share some of the characteristics common to many allergens.

144. The GM cotton lines proposed for release express one or two proteins from the introduced gene(s). Events that may give rise to toxicity and allergic reactions in people as a result of the proposed release are considered in Chapter 2. Evidence indicates that neither the VIP3A nor hygromycin resistance proteins produced by the GM cotton lines proposed for release is acutely toxic to people. Studies of the COT102 line show no increased levels of innate toxic or anti-nutritional compounds in cotton seed in comparison to the parent organism (FSANZ 2004). Therefore the discussion in this chapter will be limited to assessing the risk of allergic reaction from the GM cotton lines proposed for release.

Section 2 Consequence and likelihood assessments

145. Consideration is given to events 1–3 identified in Chapter 2 (Hazard identification) that may give rise to allergic reactions in people. 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).

146. The Regulator is required to consider risks posed by, or as a result of, gene technology. For this reason, the level of risk from the proposed dealings with the GMOs is considered relative to the baselines of the low allergenicity of the non-GM parent for people and

characteristics of the environment in which the GM cotton plants would be released. Therefore, other sources of the same or similar proteins in the environment (such as from naturally occurring bacteria) are relevant to the risk estimate.

2.1 Allergenicity of non-GM cotton

147. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton lines 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, including information on its lack of allergenicity, is provided in the document *The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia* (OGTR 2002).

148. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore, its potential to act as an airborne allergen is extremely low. However, inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition.

2.2 Event 1: Contact with GM plant materials containing the VIP3A or hygromycin resistance proteins

149. There are a number of different routes by which people living or working near cotton crops could be exposed to the VIP3A or hygromycin resistance proteins expressed by the introduced genes in the GM cotton lines proposed for release. These are by:

- contact with plants damaged during handling
- inhalation of pollen
- inhalation of cotton dust.

150. The risk of allergic reactions in people from contact with GM plant materials containing the VIP3A or hygromycin resistance proteins would depend upon the allergenicity, if any, of the proteins (consequence assessment) and the chance that people experience an allergic response through exposure to the proteins (likelihood assessment). The risk is assessed against the baseline of low allergenicity of the non-GM parent organism and allergenicity due to the presence of the introduced genes in the environment in strains of common bacteria.

2.2.1 Consequence assessment

151. Prediction of allergenicity is currently based on comparisons with known allergens and their properties. The VIP3A protein has a high M_r of about 88,000. In insect guts this is processed to a protein with a M_r of about 62,000. This is the form that is toxic for lepidopteran insects. However, people exposed to the GM cotton lines proposed for release would be exposed to the larger protein. This is outside the typical size range of known allergens.

152. The potential allergenicity of the VIP3A protein has been assessed through sequence comparisons to a database of allergens to determine whether the protein shows sequence similarity to allergenic proteins. No significant amino acid sequence similarity was found.

153. Mass spectral analyses of the VIP3A protein isolated from GM *E. coli*, GM corn and the COT102 GM cotton line have been performed and have found no evidence of post-translational modifications such as glycosylation (FSANZ 2004).

154. The digestibility of the VIP3A protein was studied in simulated mammalian gastric fluid containing pepsin. Two different sources of the VIP3A protein were used in this study, GM *E. coli* and GM corn. No VIP3A protein was detected upon immediate sampling of the

reaction mixture. When the pepsin concentration was diluted 1000-fold, no VIP3A protein was detected after 10 minutes.

155. The stability of the VIP3A protein during cooking or processing has not been assessed. The VIP3A protein is not the major component of any tissue in the GM cotton lines.

156. The VIP3A protein is derived from *B. thuringiensis* (Bt), a common soil bacterium. It is produced during vegetative growth, and its expression continues into sporulation (Rice 1999; Donovan et al. 2001). Data submitted to the US-EPA indicate that commercial Bt microbial formulations contain similar proteins (proteins of the same size that react with antibodies raised against the VIP3A protein) (US EPA 2005). These microbial formulations have been used in commercial sprays over the past 30 years to protect crops from insect attack. Thus, there are already VIPs in the environment to which people have been exposed without evidence of allergic reactions.

157. The size of the hygromycin resistance protein is within the typical size range of known allergens.

158. The potential allergenicity of the hygromycin resistance protein was similarly assessed by amino acid sequence comparison to a database of known allergens with no significant sequence similarity revealed. The allergenicity of the hygromycin resistance protein has also been assessed in previous RARMPs, most recently DIRs 049/2004 and 052/2004.

159. Sequence analysis of the hygromycin resistance protein indicates that it is not targeted for glycosylation.

160. The *in vitro* digestibility of the hygromycin resistance protein was studied in both simulated mammalian gastric fluid (SGF) and simulated mammalian intestinal fluid (SIF). Immediate sampling of both reaction mixtures found only degradation products remaining. These were completely digested within two minutes and five minutes in SGF and SIF respectively (FSANZ 2004).

161. The stability of the hygromycin resistance protein during cooking or processing has not been assessed. The hygromycin resistance protein is not the major component of any tissue in the COT102 GM cotton line.

162. The hygromycin resistance protein is produced by strains of *Escherichia coli*, a common gut bacterium. Proteins with the same biochemical function are also produced by other bacterial species (see Chapter 1 for more details). Thus there are already hygromycin resistance proteins in the environment to which people have been exposed without evidence of allergic reactions.

163. The experimental evidence suggests that both the VIP3A and hygromycin resistance proteins have limited potential for allergenicity. In addition, the VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens and exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions. Therefore the consequences of contact with GM plant materials containing the VIP3A or hygromycin resistance proteins are assessed as **marginal**.

2.2.2 Likelihood assessment

164. Due to the limited size and duration of the release, few people would have the opportunity to come into direct contact with the GM plants. Those that did, would need to damage the plants to be exposed to the VIP3A or hygromycin resistance proteins. Both the VIP3A and hygromycin resistance proteins are expressed at low levels in tissues from the COT102 GM cotton plants. Levels are also expected to be low in the COT202 and COT203 GM cotton plants.

165. Due to the physical characteristics of cotton pollen and flowers (OGTR 2002), it is expected that people would be exposed to very small quantities of pollen. The VIP3A protein is detectable in pollen at low levels (COT102 data only, see Section 2.4 of Chapter 1) while the hygromycin resistance protein is not detectable in pollen from the COT102 line. The COT202 and COT203 lines do not have the introduced hygromycin resistance gene.

166. Significant quantities of cotton dust are generated during ginning of cotton seed. Cotton dust contains many contaminants including material from the cotton plants and microorganisms from the field (Salvaggio et al. 1986). The relative amount of the VIP3A and hygromycin resistance proteins in this mixture would be extremely low. People working in cotton gins routinely take precautions against inhalation of cotton dust as a way of decreasing the risk of developing byssinosis.

167. In summary, exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to a few people due to low expression of the proteins and the small area and short duration of the proposed release. Therefore the likelihood of allergic reactions in people resulting from event 1 is assessed as **highly unlikely**.

2.3 Event 2: Exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment

168. The risk of allergic reactions in people from exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment would depend upon the allergenicity, if any, of the proteins (consequence assessment) and the chance that people experience an allergic response through exposure to the proteins by contact with GM cotton volunteers (likelihood assessment). The risk is assessed in the context of the limited scale of the proposed release and against the baseline of low allergenicity of the non-GM parent organism.

2.3.1 Consequence assessment

169. The *vip3A* gene could potentially increase the spread and persistence of volunteer GM cotton plants by reducing lepidopteran herbivory. Increased spread and persistence of the GM cotton plants would increase the number of people who might have contact with the VIP3A and hygromycin resistance proteins. Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions. However, the consequences of this contact would be the same as for contact with the introduced proteins in the GM cotton plants proposed for release.

170. As discussed in section 2.2.1, the VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. Therefore the consequences of exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment are assessed as **marginal**.

2.3.2 Likelihood assessment

171. Expression of the *vip3A* gene in the GM cotton lines is not expected to increase the spread and persistence of the GM cotton plants in the environments in which it is proposed for release (see Chapter 5).

172. As discussed in section 2.2.2, exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to a few people due to low expression of the proteins and the small area and short duration of the proposed release. Furthermore, GM volunteers are expected to be considerably less numerous than the GM cotton plants grown during the release and would not significantly increase the exposure of people to either VIP3A or

hygromycin resistance proteins. Therefore, the likelihood of allergic reactions in people from event 2 is assessed as **highly unlikely**.

2.4 Event 3: Exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants

173. The risk of allergic reactions in people from exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants would depend upon the allergenicity, if any, of the proteins (consequence assessment) and the chance that people experience an allergic response through exposure to the proteins following gene transfer (likelihood assessment). The risk is assessed in the context of the limited scale of the proposed release, other cultivated or naturalised cotton plants growing in the vicinity of the release sites and the conditions necessary for cross-pollination.

2.4.1 Consequence assessment

174. About 80% of the area currently planted to *G. hirsutum* in Australia is GM (Bollgard II® and Roundup Ready®). Gene transfer to GM cotton plants previously approved for commercial release may result in new combinations of proteins. None of these combinations are expected to provide any additional risks for people.

175. Gene transfer to sexually compatible cotton species, *G. hirsutum* or *G. barbadense*, could result in the expression of the VIP3A protein in these plants. This could encompass gene transfer to both non-GM cotton plants and commercially released GM cotton plants that may be growing in the same area (Ingard®, Bollgard II® and Roundup Ready®). This could further increase the spread of the genes in the environment, resulting in a greater number of people who might come into direct contact with the VIP3A or hygromycin resistance proteins. However, as the VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens and exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions, the consequences of exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants are assessed as **marginal**.

2.4.2 Likelihood assessment

176. The adverse outcome of allergic reactions in people resulting from gene transfer to other cotton plants is contingent on both of the following steps:

- transfer of the introduced genes to other cotton plants
- allergenicity of the recipient plants for people as a result of expression of the introduced genes.

177. The frequency of gene transfer to a small number of other *G. hirsutum* and *G. barbadense* cotton plants is discussed in detail in Chapter 5. Briefly, gene transfer to other *G. hirsutum* and *G. barbadense* cotton plants occurs at a low frequency in southern Australia.

178. The presence of the introduced genes in other *G. hirsutum* and *G. barbadense* cotton plants would not alter the likelihood of allergic reactions in people in comparison to that of the original GM cotton plants. Therefore the likelihood of allergic reactions in people from event 3 is assessed as **highly unlikely**.

2.6 Uncertainty

179. There is limited compositional data available for the COT202 and COT203 lines, including levels of expression of the VIP3A protein in different parts of the plants. These data are not required for assessing the risk of harm to people from this proposed release due to the

limited area and duration of the release and because no products would be used in human food. More detailed compositional data would be required for risk assessments of an application for release that proposed the use of products from these GM cotton lines in human food or animal feed.

Section 3 Risk estimates

180. 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).

181. The risk estimates for allergic reactions in people as a result of contact with the VIP3A or hygromycin resistance proteins expressed in the GM cotton lines have been made relative to the baselines of allergenicity of non-GM cotton and allergenicity due to the presence of the introduced genes in the environment in strains of common bacteria.

182. The consequences of contact with GM plant materials containing the VIP3A or hygromycin resistance proteins have been assessed as **marginal**, and the likelihood of this resulting in allergic reactions in people as **highly unlikely**. Therefore the risk estimate for event 1 is **negligible**.

183. The consequences of exposure of people to the VIP3A or hygromycin resistance proteins as a result of spread and persistence of the GM cotton in the environment have been assessed as **marginal**, and the likelihood of this resulting in allergic reactions as **highly unlikely**. Therefore the risk estimate for event 2 is **negligible**.

184. The consequences of exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants have been assessed as **marginal**, and the likelihood of this resulting in allergic reactions as **highly unlikely**. Therefore the risk estimate for event 3 is **negligible**.

185. As the risks of all events that may lead to allergic reactions in people are estimated to be **negligible**, there is no present need to invoke actions for mitigation (OGTR 2005). Therefore, no risk treatment measures are proposed.

Table 3.1 Summary of risk assessment

Event that may give rise to allergic reactions in people	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 1 Contact with GM plant materials containing the VIP3A or hygromycin resistance proteins by: • contact with plants damaged during handling • inhalation of pollen • inhalation of cotton dust.	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	**Highly unlikely** • Exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to a few people due to low expression of the proteins and the small size and short duration of the proposed release.	**Negligible**	**No**
Event 2 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of	**Highly unlikely** • Exposure to the VIP3A and hygromycin resistance proteins is expected to be restricted to a few people	**Negligible**	**No**

Event that may give rise to allergic reactions in people	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
spread and persistence of the GM cotton in the environment	allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	due to low expression of the proteins and the small size and short duration of the proposed release. • GM cotton volunteers emerging after harvest are expected to be considerably less numerous than the GM cotton plants grown during the release.		
Event 3 Exposure of people to the VIP3A or hygromycin resistance proteins as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants	**Marginal** • The VIP3A and hygromycin resistance proteins lack several characteristics typical of allergens. • Exposure to natural sources of the introduced proteins already occurs without evidence of allergic reactions.	**Highly unlikely** • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • The numbers of recipient plants containing the VIP3A and/or hygromycin resistance proteins as a result of gene transfer are expected to be considerably less than the number of GM cotton plants grown during the release. • The proposed release is limited to a small size and short duration.	**Negligible**	**No**

Chapter 4 Risk estimates for toxicity for non-target invertebrates

186. This chapter estimates the risks associated with three events that could lead to the adverse outcome of toxicity for non-target invertebrates arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

Section 1 Background

187. 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).

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

189. The GM cotton lines proposed for release express one or 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 proposed release were considered in Chapter 2. Expression of the hygromycin resistance protein is not expected to be a novel source of harm to non-target organisms as it and similar proteins are naturally expressed by common bacterial species without any indication of toxicity for any organism. Evidence indicates that the VIP3A protein is not toxic to vertebrates or microorganisms and is expected to pose a risk only to non-target invertebrates. Therefore this chapter will be limited to assessing the risk of toxicity for non-target invertebrates as a result of expression of the VIP3A protein by the GM cotton lines. The toxicity of VIP3A for insect pests of cotton, in particular the targets *H. armigera* and *H. punctigera*, is not considered to be an adverse outcome.

Section 2 Consequence and likelihood assessments

190. Consideration is given to events 4–6 identified in Chapter 2 (Hazard identification) that may give rise to allergic reactions in people. 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).

191. 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 GMOs is considered relative to the baselines of toxicity for invertebrates 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 from naturally occurring bacteria) and the anticipated use of pesticide sprays on the GM cotton plants to control insect pests are relevant to the risk estimate.

2.1 Toxicity of non-GM cotton

192. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton lines 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, including information on its lack of allergenicity, is provided in the document *The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia* (OGTR 2002).

193. 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) (referenced in Percival et al.

1999) showed that high levels of gossypol, even in combination with morphological characteristics that discourage insect infestations, such as okra or laciniate leaf forms, were not sufficient to provide protection against the pink bollworm (*Pectinophora gossypiella*), a major pest of cotton in the USA.

194. 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.

2.2 Event 4: Direct or indirect ingestion of the VIP3A protein by non-target invertebrates

195. The VIP3A protein is known to be toxic to the two major lepidopteran pests of cotton in Australia, namely *H. armigera* and *H. punctigera*. However, the toxicity range of the VIP3A protein has not been fully investigated in regard to Australian invertebrates.

196. In addition, toxicity for non-target lepidopteran insects may also result in indirect impacts that may give rise to harm by:

- effects on populations of specialist parasitoids and predators that feed on lepidopteran insects affected by the Bt toxin; and
- effects on populations of other organisms that interact with lepidopterans affected by the Bt toxins.

197. The risk of toxicity for non-target invertebrates from direct or indirect ingestion of the VIP3A protein would depend upon the level of toxicity of the VIP3A 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 insects, toxicity due to the presence of the introduced genes in the environment in strains of common bacteria and the toxicity of the intended pesticide regime.

2.2.1 Consequence assessment

198. The VIP3A protein is derived from *B. thuringiensis* (Bt), a common soil bacterium. It is produced during vegetative growth, and its expression continues into sporulation (Estruch et al. 1996). Data submitted to the US-EPA indicate that commercial Bt microbial formulations contain similar proteins (proteins of the same size that react with antibodies raised against the VIP3A protein) (US EPA 2005). These microbial formulations have been used in commercial sprays over the past 30 years to protect crops from insect attack, without evidence of toxicity for non-target invertebrates.

199. A preliminary field survey conducted as part of DIR 017/2002 indicated that non-lepidopteran insect numbers were not affected by the insecticidal protein VIP3A.

200. Two additional surveys conducted under DIR 025/2002 compared numbers of invertebrates collected from unsprayed GM cotton (COT102) and non-GM cotton plants. The composition of the invertebrate populations was reported to be more strongly correlated with the survey period than with whether or not the crop was GM. Further analysis of the data indicated that some invertebrate species were found more frequently on one type of cotton than on the other type. For instance, mirids, jassids and thrips were more abundant on the COT102 plants than on the non-GM plants. This was hypothesised to be due to the GM crop being taller, lusher and having more squares, and therefore nectar to feed on, than the unsprayed, non-GM crop. In contrast, lepidopteran species were more common on the non-GM crop as they are the target species of the COT102 plants. However, these differences explained only 2% of the variation found in population composition.

201. The effect of the VIP3A protein on soil invertebrates is not known. The VIP3A protein could enter the soil through root exudations or incorporation of material from the GM cotton plants. However, *B. thuringiensis* is a common soil bacterium and the VIPs are known to be released into the environment during the vegetative growth of the bacteria (Estruch et al. 1996). Therefore, it is expected that soil invertebrates would be naturally exposed to similar proteins in environments containing *B. thuringiensis* bacteria.

202. Preliminary US data, supplied by the applicant, indicate that the insects listed in Table 4.1 that have been tested for sensitivity to the VIP3A protein.

Table 4.1 Species tested for sensitivity to the VIP3A protein

Order	Species	Common name	Sensitive?
Coleoptera	*Coleomegilla maculate*	Pink spotted ladybeetle	No
	Diabrotica longicornis	Northern corn rootworm	No
	Diabrotica undecimpunctata	Southern corn rootworm	No
	Diabrotica virgifera	Western corn rootworm	No
	Leptinotarsa decemlineata	Colorado potato beetle	No
	Popillia japonica	Japanese beetle	No
	Tenebrio molitor	Yellow mealworm	No
Diptera	*Culex pipiens*	Northern house mosquito	No
	Drosophila melanogaster	Vinegar fly	No
	Musca domestica	House fly	No
Homoptera	*Myzus persicae*	Green peach aphid	No
Hymenoptera	*Apis mellifera*	Honey bee	No
Isotomidae	*Folsomia candida*	Springtails	No
Lepidoptera	*Agrotis ipsilon*	Black cutworm	**Yes**
	Danaus plexippus	Monarch butterfly	No
	Diatraea grandiosella	Southwestern corn borer	**Yes**
	Diatraea saccharalis	Sugarcane borer	**Yes**
	Helicoverpa zea	Corn earworm	**Yes**
	Heliothis virescens	Tobacco budworm	**Yes**
	Homoeosoma electellum	Sunflower moth	**Yes**
	Hyphantria cunea	Fall webworm	No
	Manduca sexta	Tobacco hornworm	**Yes**
	Ostrinia furnicalis	Asian corn borer	**Yes**
	Ostrinia nubilalis	European corn borer	No
	Pectinophora gossypiella	Pink bollworm	**Yes**
	Plodia interpunctella	Indian meal moth	No
	Plutella xylostella	Diamondback moth	No
	Pseudoplusia includens	Soybean looper	**Yes**
	Sesamia nonagioides	Mediterranean corn borer	**Yes**
	Spodoptera exigua	Beet armyworm	**Yes**
	Spodoptera frugiperda	Fall armyworm	**Yes**
	Spodoptera ornithogalli	Yellow-striped armyworm	**Yes**
	Trichoplusia ni	Cabbage looper	**Yes**
Neuroptera	*Chrysoperla carnea*	Green lacewings	No
Thysanoptera	*Frankliniella occidentalis*	Western flower thrips	No

203. Of the 35 species tested, from eight Orders, 15 were found to be sensitive to the VIP3A protein. All of these were lepidopterans. A search of the Bt specificity database, <http://www.glfc.forestry.ca/bacillus/>, found one additional species that is not sensitive to

the VIP3A protein. This is the dipteran, *Culex quinquefacisciatus*. Literature and database searches did not find any evidence of toxicity for non-lepidopteran species.

204. Toxicity of the VIP3A protein is specific to some lepidopteran insects. Therefore, expression of VIP3A in the GM cotton lines has the potential to be toxic to some Australian lepidopteran species as a result of coming into contact with the VIP3A protein in the GM cotton lines and the consequences of direct or indirect ingestion of the VIP3A protein by non-target invertebrates are assessed as **minor**.

2.2.2 Likelihood assessment

205. Invertebrates may be exposed directly, through feeding on the GM cotton plants, or indirectly through eating other organisms, including the lepidopteran target organisms, that have fed on the GM cotton plants. However, the VIP3A protein is produced at low levels (COT102 data only, refer to Chapter 1 for details). Relative exposure will be greatest for herbivorous species feeding on the GM cotton plants.

206. Pollinator species and various insects that feed on pollen, and species feeding on lepidopteran larvae, may also be exposed to low levels of the VIP3A protein. However, sap feeders, such as aphids, will have minimal exposure, as the sap is composed primarily of sugars and mineral salts dissolved in water. Studies have investigated whether proteins expressed from introduced genes could be detected in phloem sap from GM maize or tobacco plants. No Cry1Ab protein could be detected in phloem sap from GM maize plants (Raps et al. 2001), however, the snowdrop lectin toxin was present in the honeydew of aphids feeding on GM tobacco plants expressing this protein, indicating that this protein is present in the phloem sap (Shi et al. 1994). The size of the protein may determine whether it is present in phloem sap or not: the snowdrop lectin is 12,500 M_r while Cry1Ab is over 60,000 M_r in size (Raps et al. 2001). The large size of the VIP3A protein of about 88,000 M_r (Estruch et al. 1996) suggests that it may not be present in phloem sap.

207. In addition, the trial would be limited to a total of two 0.5 ha sites for a single season and the applicant intends to manage the GM cotton plants as if they were non-GM cotton plants ie multiple insecticide sprays throughout the season. These factors would reduce the numbers of insects that would be exposed to the VIP3A protein.

208. Exposure to the VIP3A protein is expected to be restricted to a small number of non-target invertebrates due to low expression of the proteins and the small area and short duration of the proposed release. Typical agronomic practices for the release, specifically pesticide use, are expected to have a greater impact on invertebrate survival than the expression of the VIP3A protein in the GM cotton plants. The likelihood of toxicity for non-target invertebrates resulting from event 4 is assessed as **highly unlikely**.

2.3 Event 5: Exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment

209. The risk of toxicity for non-target invertebrates from exposure to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment, would depend on the toxicity of the VIP3A protein (consequence assessment) and the level of exposure to the proteins during this release (likelihood assessment). The risk is assessed in the context of the limited scale of the proposed release and against the baseline of the toxicity of the non-GM parent organism for insects.

2.3.1 Consequence assessment

210. The *vip3A* gene could potentially increase the spread and persistence of the GM cotton plants by reducing insect herbivory in regions where this would otherwise limit the growth of cotton plants. Spread and persistence of the GM cotton plants could result in the deaths of a greater number of non-target invertebrates. However, toxicity of the VIP3A protein is specific to a subset of lepidopteran insects. Therefore the consequences of exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment are assessed as **minor**.

2.3.2 Likelihood assessment

211. Exposure to the VIP3A protein is expected to be restricted a small number of non-target invertebrates due to low expression of the proteins and the small area and short duration of the proposed release. GM volunteers are expected to be considerably less numerous than the GM cotton plants grown during the release and would not significantly increase the exposure of non-target invertebrates to the VIP3A protein. Therefore the likelihood of toxicity for non-target invertebrates resulting from event 5 is assessed as **highly unlikely**.

2.4 Event 6: Exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants

212. The risk of toxicity for non-target invertebrates from exposure to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants would depend upon the toxicity of the proteins (consequence assessment) and the level of exposure to the proteins during this release (likelihood assessment). The risk is assessed in the context of the limited scale of the proposed release, other cultivated or naturalised cotton plants growing in the vicinity of the releases sites and the conditions necessary for cross-pollination.

2.4.1 Consequence assessment

213. About 80% of the area currently planted to *G. hirsutum* in Australia is GM (Bollgard II® and Roundup Ready®). Gene transfer to GM cotton plants previously approved for commercial release may result in new combinations of proteins. For a Roundup Ready®/VIP3A combination, there are not expected to be any additional risks for non-target invertebrates. For Ingard® or Bollgard II®, the combination of Cry1Ac and/or Cry2Ab proteins with the VIP3A protein may increase the range of non-target invertebrates affected by the GM cotton plant. This is discussed further below.

214. The Cry1Ac protein is active against some lepidopteran insects but has also shown toxicity against at least one dipteran insect, *Gossinia mortisans* (adult Tsetse fly) (van Frankenhuyzen & Nystrom 2002). The Cry2Ab toxin present in Bollgard II® cotton is generally considered to have specific toxicity for lepidopterans (Widner & Whiteley 1990; Widner & Whiteley 1989; Dankocsik et al. 1990). However, there is some evidence that Cry2Ab is active against at least one dipteran insect, *Anopheles gambiae* (African malarial mosquito) (Ahmad et al. 1989).

215. As questions were raised over the potential impact of Cry2Ab expressed in insecticidal GM cottons on species of Diptera, the Regulator commissioned additional work by CSIRO Entomology to investigate the toxicity of Bt cotton plants on a range of dipteran insects. Three dipteran species were tested (*Culex quinquefasciatus*, mosquito; *Musca domestica*, house-fly; and *Chironomus tepperi*, bloodworm) using a freeze-dried powder of cotton leaves containing the Cry2Ab protein. The study was recently completed and found no evidence of toxicity at exposure levels that were equivalent to or higher than would occur in fresh GM cotton leaves (unpublished CSIRO report).

216. The specificities of the VIP3A, Cry1Ac and Cry2Ab proteins appear to be largely restricted to overlapping subsets of lepidopterans. Therefore, any increase in the target range as a result of the expression of all three of these proteins is expected to be confined to lepidopteran species because all are specific for a limited range of lepidopteran insects (see Chapter 4).

217. The possibility of synergistic, additive or antagonistic effects exists (Schnepf et al. 1998; del Rincon-Castro et al. 1999). Synergistic or additive effects could be expected to occur between toxins isolated from the same or different strains of bacteria (Schnepf et al. 1998), particularly where different receptor molecules are involved. Such effects could increase the potency of the resulting insecticide.

218. Therefore, a stacked GM cotton plant might prove to be more toxic for lepidopteran pests of cotton than either of the parent GM cotton plants. However, it is worth noting that these three proteins are all present in the microbial formulations in commercial sprays that are approved for use in Australia (see Section 2.4.1, Chapter 1) and it is not expected that the target range would increase beyond the target range of these sprays.

219. It has been observed that the potency of the Bt spores produced by strains of Bt bacteria is greater than the potency observed in combinations of the purified proteins (Schnepf et al. 1998). This suggests that there may be additional insecticidal factors in the spores that have not yet been isolated.

220. Therefore, it is possible that a stacked GM cotton plant would have less toxicity for lepidopteran pests of cotton than the commercial Bt sprays.

221. Antagonistic reactions are not expected to occur between toxins present in the same strain of bacteria as these toxins would have evolved together. Antagonistic effects could occur between toxins isolated from different strains of bacteria (del Rincon-Castro et al. 1999), particularly where the same receptor molecule is targeted. Such effects could act to decrease the efficacy of the resulting insecticide.

222. Therefore, a stacked GM cotton plant is not expected to show decreased efficacy for lepidopteran pests of cotton as all three proteins occur naturally in the same strain of *B. thuringiensis*.

223. Gene transfer of *vip3A* to *G. hirsutum* or *G. barbadense* plants could result in the deaths of a greater number of non-target lepidopterans. Due to the potential toxicity of this protein for some non-target invertebrates (particularly lepidopterans) the consequences of expression of the *vip3A* gene in *G. hirsutum* or *G. barbadense* plants are assessed as **minor**.

2.4.2 Likelihood assessment

224. The adverse outcome of toxicity for non-target invertebrates resulting from gene transfer to other cotton plants is contingent on both of the following steps:

- transfer of the introduced *vip3A* gene to other cotton plants
- toxicity of the recipient plants for non-target invertebrates as a result of expression of the introduced gene.

225. Gene transfer to sexually compatible cotton species, *G. hirsutum* or *G. barbadense*, could result in the expression of the VIP3A protein in these plants. This could encompass gene transfer to both non-GM cotton plants and commercially released GM cotton plants that may be growing in the same area (Ingard®, Bollgard II® and Roundup Ready®). This could further increase the spread of the genes in the environment, leading to exposure of more invertebrates to the VIP3A protein.

226. The frequency of gene transfer to other *G. hirsutum* and *G. barbadense* cotton plants is discussed in detail in Chapter 5. Briefly, gene transfer to other *G. hirsutum* and *G. barbadense* cotton plants occurs at a low frequency in southern Australia.

227. Gene transfer to other cotton plants is expected to occur at low levels naturally and will be further decreased by the applicant's intended use of insecticide sprays on the release.

228. The number of recipient plants containing the VIP3A protein as a result of gene transfer are expected to be considerably lower than the number of GM cotton plants grown during the release.

229. The presence of the VIP3A protein in any other *G. hirsutum* or *G. barbadense* cotton plants would increase the toxicity of these plants for some non-target invertebrates (particularly lepidopterans). However, gene transfer to other cotton plants is expected to occur at low levels. Consequently, the numbers of recipient plants containing the VIP3A protein as a result of gene transfer are expected to be considerably less than the number of GM cotton plants grown during the release. Furthermore, the proposed release is limited to a small area and short duration. Therefore the likelihood of toxicity for non-target invertebrates from event 6 is assessed as **highly unlikely**.

2.6 Uncertainty

230. The toxicity of the VIP3A protein for invertebrates in Australia has not been studied in detail. Data on toxicity for a range of invertebrates in Australia would be required for risk assessments of applications for large scale releases of these GM cotton lines. This information is not required for this proposed release 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 lines.

231. More detailed expression data would be required for the COT202 and COT203 GM cotton lines for risk assessments of applications for large scale releases of these GM cotton lines, to determine the likely level of exposure to the VIP3A protein.

232. The effect of stacking of a Bollgard II® GM cotton plant with a VIP3A GM cotton plant is not known, however stacking is not expected to occur during this limited release. Information may be required before a commercial release of the VIP3A cotton could be assessed.

Section 3 Risk estimates

233. 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).

234. The risk estimates for toxicity for non-target invertebrates as a result of the proposed release of these GM cotton plants have been made relative to the baseline of the toxicity of cotton for invertebrates and in the context of the proposed management conditions of the release, including spraying with pesticides to control insect pests in the crop.

235. The consequences of direct or indirect ingestion of the VIP3A protein in the GM cotton plants have been assessed as **marginal**, and the likelihood of this resulting in toxicity to non-target invertebrates as **highly unlikely**. Therefore the risk estimate for event 4 is **negligible**.

236. The toxicity of the VIP3A protein is more specifically targeted at a limited range of lepidopteran pests than the typical chemical pesticide sprays that are used on non-GM cotton. Such pesticides are expected to have a greater impact on invertebrate populations than the

GM cotton lines. Therefore the risk of toxicity for non-target invertebrates would remain **negligible** in the absence of the proposed spraying regime.

237. The consequences of exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment have been assessed as **minor**, and the likelihood of this resulting in toxicity to non-target invertebrates as **highly unlikely**. Therefore the risk estimate for event 5 is **negligible**.

238. The consequences of exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants have been assessed as **minor**, and the likelihood of this resulting in toxicity to non-target invertebrates as **highly unlikely**. Therefore the risk estimate for event 6 is **negligible**.

239. As the risks of all events that may lead to toxicity for non-target invertebrates are estimated to be **negligible**, there is no present need to invoke actions for mitigation (OGTR 2005). Therefore, no risk treatment measures for toxicity for non-target invertebrate organisms are proposed.

Table 4.3 Summary of risk assessment

Event that may give rise to allergic reactions in people	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 4 Direct or indirect ingestion of the VIP3A protein by non-target invertebrates.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Exposure to the VIP3A protein is expected to be restricted to few non-target invertebrates due to low expression of the proteins and the small size and short duration of the proposed release. • Agronomic practices proposed for the release, specifically pesticide use, are expected to have a greater impact on invertebrate survival than the expression of the VIP3A protein in the GM cotton plants.	**Negligible**	**No**
Event 5 Exposure of non-target invertebrates to the VIP3A protein as a result of spread and persistence of the GM cotton in the environment.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Exposure to the VIP3A protein is expected to be restricted to few non-target invertebrates due to low expression of the proteins and the small size and short duration of the proposed release. • GM volunteers are expected to be considerably less numerous than the GM cotton plants grown during the release.	**Negligible**	**No**

Event that may give rise to allergic reactions in people	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 6 Exposure of non-target invertebrates to the VIP3A protein as a result of gene transfer to *G. hirsutum* or *G. barbadense* plants.	**Minor** • Toxicity of the VIP3A protein is specific to a subset of lepidopteran insects.	**Highly unlikely** • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • The numbers of recipient plants containing the VIP3A protein as a result of gene transfer are expected to be considerably less than the number of GM cotton plants grown during the release. • The proposed release is limited to a small size and short duration.	**Negligible**	**No**

Chapter 5 Risk estimates for weediness

240. This chapter estimates the risks associated with two events that could lead to the adverse outcome of weediness arising from this proposed release. The risk estimates are based on the consequence and likelihood assessments of each event.

Section 1 Background

241. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. In addition, plants may also be considered weeds if they are simply growing where they are not wanted.

242. Weediness in Australia is often correlated with weediness of the species, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999; Pheloung 2001). The chance of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for the plants to establish and spread into new environments (eg escapes of commonly used garden plants) (Mulvaney 2001; Groves et al. 2005).

243. Negative characteristics of weeds may include spread and persistence, competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. In addition, the spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative environmental impacts such as:

- reduced biodiversity (including genetic, species and ecosystem diversity) that results from lower abundance of desirable species, reduced species richness, or undesirable changes in species composition
- interference with the intended use of the land they occupy
- degradation of landscape/ecosystems, such as altered water or nutrient availability.

244. Complex interactions between a plant and its environment (including availability of water, nutrients and light) determine the degree to which that plant can spread and persist in the environment. A number of measurable characteristics of plants that may influence spread and persistence are listed below. All of these factors have the potential to affect their competitiveness:

- germination, survival and reproduction under a wider range of environmental conditions
- rates of seedling growth
- rates of growth to reproductive stage
- degree of self-pollination
- use of non-specialist pollinators or wind when out-crossing
- period of seed production
- seed output
- degree of seed dispersal
- longevity of seed and degree of dormancy
- allelopathy (affect on the germination and/or growth of neighbouring plants through chemical exudates)
- resistance to pests or pathogens.

245. In the risk assessment, consideration is given to characteristics that may be expected to be altered as a result of the genetic modification and that may increase the spread and persistence of the GMOs, or of sexually compatible relatives that may receive the introduced gene(s). Alterations in these characteristics may indicate potential for weediness.

246. The GM cotton lines proposed for release express one or two proteins as a result of the genetic modification. Events that may give rise to weediness were considered in Chapter 2. Expression of the hygromycin resistance protein is not expected to have any impact on the weediness of the GM cotton. The toxicity of the VIP3A protein for lepidopteran pests of cotton may lead to weediness of the GM cotton plants. Therefore this chapter will be limited to assessing the risk of weediness as a result of expression of the *vip3A* gene in the GM cotton lines.

Section 2 Consequence and likelihood assessments

247. Consideration is given to events 7–8 identified in Chapter 2 (Hazard identification) that may give rise to allergic reactions in people. 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).

248. 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 GMOs is considered relative to the baselines of weediness of the non-GM parent and the environment in which the GM cotton plants are proposed for release. Therefore, the widespread planting of the insect resistant Bollgard II® and Bollgard II®/Roundup Ready® GM cotton in commercial cotton growing regions in NSW and QLD is considered when assessing the risks posed by the proposed release of these insect resistant GM cotton lines.

2.1 Weediness of non-GM cotton

249. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton lines being considered in this risk assessment. Attributes of non-GM cotton associated with potential weediness are discussed in the document *The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia* (OGTR 2002). This document concludes that non-GM cotton is not a serious weed in Australia, because environmental factors including temperature, soil moisture, nutrient limitation and roadside management practices limit the establishment and/or persistence of cotton seedlings outside of agricultural and other disturbed environments.

250. Small, persistent cotton populations have been observed, mainly in northern Australia. It has been noted by scientists over many years that the morphology of many of these naturalised cotton populations is distinct from that of the cultivated cotton varieties. When grown in a glasshouse, they tend to have poor architecture and produce small bolls and seed with sparse, grey lint. They also produce mainly tufted rather than fuzzy seeds, which is a strong indication that they are not derived from modern cultivars which are all fuzzy seeded cotton plants (Curt Brubaker and Lyn Craven, CSIRO, pers. comm.).

251. Tufted seeded cotton plants were originally used when hand delinting was required, before the advent of mechanical saw gins in the late 18th century. Tufted seeded cotton plants were subsequently replaced by fuzzy seeded varieties with better lint characteristics and disease resistance. It seems likely, therefore, that many naturalised cotton populations result from attempts in the early 19th century to establish cotton industries in northern Queensland and the Northern Territory (Curt Brubaker and Lyn Craven, CSIRO, pers. comm.).

252. Other cotton plants appear to be of more recent origin (eg Eastick 2002) but these are confined to areas of disturbed land with at least a seasonal water supply: typical locations are above the high tide mark on beaches and near river banks.

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

254. The weed status of cotton has also been considered previously in many of the RARMPs produced during the assessment of a variety of GM cotton lines (eg DIRs 012/2002, 022/2002, 036/2003, 055/2004). In addition to the information in the Biology and Ecology document (OGTR 2002), these RARMPs have considered new data that has been collected during previous releases of GM cotton lines in Australia.

255. A survey for cotton volunteers was performed along transport routes around Narrabri and from Emerald to the Atherton Tablelands (Farrell & Roberts 2002). This report concluded that volunteer cotton plants had established in roadside environments only rarely during 12 years of transporting ginned cotton seed for stockfeed. Cotton plants were not observed in undisturbed habitats.

256. Small quantities of *G. barbadense* (Pima cotton) are also commercially grown in Australia. Herbarium records for *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in northern, central and south eastern Queensland and in the northern regions of the Northern Territory and Western Australia (OGTR 2002). The presence of remnants of some of these populations has not been confirmed.

2.2 Event 7: Expression of the *vip3A* gene increasing spread and persistence of the GM cotton plants through reduced lepidopteran herbivory.

257. The risk of weediness developing in the GM cotton plants as a result of the expression of the *vip3A* gene would depend on the weediness of non-GM cotton plants, the importance of lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the scale of the release and the chance of progeny establishing as weeds (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism and in the context of the limited scale of the proposed release and the receiving environment for this proposed release.

258. Detailed discussions of the risk of insect resistance proteins increasing the weediness of cotton plants in Australia is provided in the risk assessment documents for DIRs 012/2002 and 022/2002, available at <http://www.ogtr.gov.au>. These risk assessments concluded that GM cotton plants expressing insect resistance proteins targeting lepidopteran pests do not enhance the weediness potential of these GM cotton plants (in comparison to non-GM cotton plants) in the southern cotton growing regions of Australia south of latitude 22° South. These assessments related to GM cotton expressing the Cry1Ac and Cry2Ab proteins, also derived from *B. thuringiensis*, but the conclusions should be applicable to cotton expressing the VIP3A protein, which has a similar mode of action and also targets some of the same lepidopteran insects (see Chapters 1 and 4).

2.2.1 Consequence assessment

259. The *vip3A* gene could confer a selective advantage in regions where lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in spread and persistence of the GM cotton lines in the environment.

260. As described in Section 1, the spread and persistence of a plant may have a number of negative environmental impacts including reduced biodiversity, interference with the intended use of the occupied land or degradation of the landscape/ecosystem.

261. Cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects.

262. Although it is possible that GM cotton plants protected against lepidopteran herbivory could produce higher numbers of seeds compared to cotton plants that are not protected (eg non-GM cotton without insecticidal sprays), the germination and establishment of any GM cotton seedlings in the environment would remain limited by temperature, water, nutrients, light, frost and herbivory by non-lepidopteran insects.

263. The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release. Water in particular is known to be a major limitation in the proposed release regions.

264. Therefore, the consequences of the expression of the *vip3A* gene in the GM cotton plants increasing the spread and persistence of GM cotton through reduced lepidopteran herbivory are assessed as **marginal**.

2.2.2 Likelihood assessment

265. The limited area and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the Shires of Narrabri, NSW and Emerald, QLD, in comparison to the numbers of GM insect resistant cotton plants already grown in these regions.

266. A limited number of GM cotton seeds may spread from the release sites, germinate and persist in the environment following the release. However, the chance of volunteer GM plants establishing as weeds by finding suitable ecological niches, would be no greater than for the non-GM parent organism. Therefore, the likelihood of weediness as a result of event 7 is assessed as **highly unlikely**.

2.3 Event 8: Expression of the *vip3A* gene in other *G. hirsutum* or *G. barbadense* cotton plants reducing lepidopteran herbivory

267. The risk of weediness developing in other cotton plants as a result of the gene transfer to *G. hirsutum* or *G. barbadense* plants would depend on the importance of lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The risk is assessed in the context of the limited scale of the proposed release, other cultivated or naturalised cotton plants growing in the vicinity of the release sites and the conditions necessary for cross-pollination.

2.3.1 Consequence assessment

268. Transfer of the introduced *vip3A* gene to other sexually compatible plants such as *G. hirsutum* and *G. barbadense* could result in the expression of the VIP3A protein in these plants. These plants could be GM cotton plants previously approved for commercial release or non-GM cotton plants. The *vip3A* gene could confer some selective advantage as discussed in

Section 2.2.1. This could result in spread and persistence of these cotton plants in environments where lepidopteran herbivory is the major constraint on cotton survival.

269. It is not expected that transfer of the *vip3A* gene to a Roundup Ready® cotton plant would result in any increase in weediness in comparison to the original GM cotton plants.

270. Transfer of the *vip3A* gene to Ingard® or Bollgard II® plants (containing the *cry1Ac* and *cry2Ab* genes) might increase the degree of toxicity and the range of lepidopteran species affected by the resulting plants in comparison to the original GM cotton plants (see Chapters 1, 3 and 4). Non-lepidopteran herbivores are not expected to be affected by a combination of these three insect resistance proteins due to the overlapping, but restricted, specificities of the individual proteins. The possibility of synergistic, additive and antagonistic effects exists and this is discussed in more detail in Chapter 4 (Section 2.4.1).

271. Transfer of the *vip3A* gene to other cotton plants is not expected to alter the fact that cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects. The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release. Therefore, the consequences of expression of the *vip3A* gene transferred to other *G. hirsutum* or *G. barbadense* cotton plants reducing lepidopteran herbivory are assessed as **minor**.

2.3.2 Likelihood assessment

272. The adverse outcome of weediness resulting from an increase in the spread and persistence of other cotton plants is contingent on both of the following steps:

- transfer of the introduced *vip3A* gene to other cotton plants
- weediness of the recipient plants as a result of expression of the introduced gene.

273. Cotton is primarily self-pollinating with pollen that is large, sticky and heavy and not easily dispersed by wind (Jenkins 1992; OGTR 2002). Overseas studies have shown that insect pollinators can transfer pollen to other nearby cotton plants at rates up to 80% (eg Oosterhuis & Jernstedt 1999). However, cotton pollen dispersal studies consistently show that outcrossing is localised around the pollen source and decreases significantly with distance (OGTR 2002 and references therein). For vertical gene transfer to occur, the GM cotton lines would need to be planted within pollination distance of other *G. hirsutum* or *G. barbadense* plants.

274. The use of pesticide sprays on the GM cotton plants should ensure that pollination rates were low, as the sprays would also kill pollinator species.

275. *G. barbadense* is the closest relative of *G. hirsutum* occurring in Australia (OGTR 2002). It is commercially grown on a small scale in Australia. Hybridisation can occur naturally between these two species (Brubaker et al. 1999). Hybrid progeny exhibit characteristics intermediate to the parents but typically with a lower capacity to produce fruit. *G. barbadense* and hybrids are not weedier or more difficult to control than is *G. hirsutum* (Warwick Stiller & Greg Constable, CSIRO, pers. comm.).

276. Transfer of the *vip3A* gene to naturalised cotton populations could also occur. It is not known if the herbarium records for *G. hirsutum* and *G. barbadense* indicate current naturalised populations of these plants. A comparison of shires where cotton is cultivated and shires where feral cotton populations occur (in Queensland) indicates that feral populations occur in only three cotton production shires, and one shire adjacent to a cotton production shire. However, it is very difficult to determine the exact distances between feral and

cultivated populations without physically locating the populations in question. Where significant geographic distances between naturalised populations and the cotton growing regions of NSW and Queensland exist, this will decrease the frequency of gene transfers.

277. Lepidopteran herbivory is not known to be a significant limiting factor on the growth of uncultivated cotton (either *G. hirsutum* or *G. barbadense*), particularly in the southern cotton growing regions of Australia (OGTR 2002).

278. The limited area and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the environment, which would reduce the occurrence of gene transfer events. Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. Following transfer of the *vip3A* gene to any of these cotton plants, the likelihood of it causing weediness in these plants is expected to be the same as for the GM cotton plants. Therefore, the likelihood of weediness as a result of event 8 is assessed as **highly unlikely**.

279. The OGTR has developed a gene flow research program, involving various GM cotton licensees, to collect data on pollen-mediated gene flow. Research will be conducted in a range of current and potential cotton growing regions, therefore no additional research requirements relating to gene flow would be imposed if a licence were to be issued.

2.4 Uncertainty

280. There is limited data on the existence and proximity of naturalised cotton populations to commercial cotton growing regions. This information would be useful for determining the opportunity for, and possible frequency of, gene transfer between GM cotton crops and these populations. However, this information is not required for assessing the risks of this proposed release of insect resistant GM cotton lines as cross-pollination between the GM cotton lines and any naturalised cotton populations will be minimised by the pesticides sprays proposed for use and other measures proposed to limit and control the release (see Chapter 6).

Section 3 Risk estimates

281. 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).

282. The risk estimates for weediness of the GM cotton lines as a result of the expression of the *vip3A* gene have been made relative to the baselines of the weediness of non-GM cotton growing in Australia and the current widespread use of the Bollgard II® cotton varieties (containing the *cry1Ac* and *cry2Ab* genes) in commercial cotton crops.

283. The consequences of increased spread and persistence of cotton resulting from the presence of the *vip3A* gene in the GM cotton lines have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore the risk estimate is **negligible**.

284. The consequences of increased spread and persistence resulting from the presence of the *vip3A* gene in other *G. hirsutum* and *G. barbadense* cotton plants, as a result of gene transfer, have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore the risk estimate is **negligible**.

285. As the risks of both events that may lead to weediness are estimated to be **negligible**, there is no present need to invoke actions for mitigation (OGTR 2005). Therefore, no risk treatment measures for weediness are proposed.

Table 5.1 Summary of risk assessment

Event that may give rise to allergic reactions in people	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Event 7 Expression of the *vip3A* gene increasing spread and persistence of the GM cotton plants through reduced lepidopteran herbivory	**Marginal** • Cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects. • The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release.	**Highly unlikely** • The limited size and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the environment. • The chance of volunteer GM plants establishing as weeds by finding suitable ecological niches, would be no greater than for the non-GM parent.	**Negligible**	**No**
Event 8 Expression of the *vip3A* gene in other *G. hirsutum* or *G. barbadense* cotton plants as a result of gene transfer	**Minor** • Cotton is not a serious weed in southern Australia because of limitations placed on it by temperature, water, nutrients, light, frost and herbivory by a range of insects. • The expression of the *vip3A* gene is not expected to alter the environmental limitations on the spread and persistence of cotton in the regions proposed for the release.	**Highly unlikely** • The limited size and duration of this proposed release would result in the introduction of a relatively small number of GM cotton lines into the environment. • Cotton is primarily in-breeding and gene transfer to other cotton plants is expected to occur at low frequencies. • Following transfer of the *vip3A* gene to any of these cotton plants, the likelihood of it causing weediness in these plants is expected to be the same as for the GM cotton plants.	**Negligible**	**No**

Chapter 6 Risk management

286. This chapter evaluates the risks assessed in Chapters 3–5 to determine whether or not specific treatments are required to mitigate harm that may arise during the proposed limited release. Other risk management considerations required under the Act are also addressed in this chapter.

Section 1 Background

287. 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.

288. Under section 62 of the Act, the Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. 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

289. 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 agencies.

290. FSANZ is responsible for human food safety assessment. FSANZ may approve the use of products derived from GM food crops approved for release overseas, prior to their general release in Australia. FSANZ has approved the use of cotton seed oil and linters from the COT102 GM cotton line in human food (FSANZ 2004). Approval has not been sought for the use of products derived from the COT202 or COT203 GM cotton lines. Deltapine does not intend to use any products derived from this proposed release in human food.

291. The *Gene Technology (Consequential Amendments) Act 2000* requires the APVMA to consult the Regulator for the purposes of making certain decisions regarding registration or issuing a permit for an agricultural chemical product that is, or contains a product from, a GMO.

292. The GM cotton lines proposed for release meet the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to their production of an insecticidal substance, and therefore they are subject to regulation by the APVMA. Deltapine has made an application to the APVMA for a permit to undertake the proposed release.

Section 3 Risk treatment measures for identified risks

293. The detailed risk assessment of events 1–8 contained in Chapters 3–5 concluded that the risk estimates for all eight events are **negligible**. These events were considered in the context of the proposed release of one hectare for one season in the Shires of Narrabri, NSW and Emerald, QLD.

294. 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. However, containment measures are required to limit the release to a maximum total area of 1 ha and the duration to the summer season of 2005–06 as proposed by the applicant.

Section 4 General risk management

4.1 Licence conditions associated with managing limited and controlled releases

4.1.1 Measures to limit and control the proposed release

295. A number of 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 and gin seed cotton from the release separately from other cotton
- not permit cotton seed or other material from the release to be used in human food or animal feed
- 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 six consecutive months have passed without any volunteer cotton plants.

296. In addition, the applicant proposes to spray the GM cotton crop with insecticides targeting pollinators, to preserve the purity of seed against gene flow from the adjoining non-GM cotton used as a pollen trap.

4.1.2 Measures to control other activities associated with the release

297. 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* and *Policy on storage of genetically modified organisms, July 2004*). Licence conditions would also be imposed 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

298. All DIR licences issued by the Regulator contain a number of general conditions that also 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

299. 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.

300. Before making the decision to issue a licence for this application (DIR 058/2005), the Regulator determined that Deltapine Australia Pty Ltd is suitable to hold a licence.

301. 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.

302. 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

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

304. Deltapine 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 lines outside of the permitted areas.

305. Deltapine is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic material in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.2.3 Reporting structures

306. The licence obliges the licence holder to report immediately 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.

307. 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.

308. A number of written notices would also be required under the licence in relation to:

- expected and actual dates of planting

- expected and actual dates of harvest and cleaning
- dates and locations of any burial of GM seed (when used as a method of destruction).

Section 5 Monitoring and Compliance

309. 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 site.

310. 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.

311. In cases of non-compliance with licence conditions, the Regulator may also instigate an investigation to determine the nature and extent of non-compliance. The Act provides the Regulator with extensive powers of enforcement to ensure 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 the health and safety of people or the environment could result.

Section 6 Issues to be addressed for future releases

312. The risk assessment identified additional information that may be required if the applicant were to submit an application for a large scale release of these GM cotton lines or to justify a reduction in the containment conditions. This would include the following:

- expression levels of the VIP3A protein in the COT202 and COT203 GM cotton lines
- the toxicity of the VIP3A protein for a range of organisms in Australia
- Australian field studies addressing the biodiversity of insect populations in VIP3A crops compared with conventional cotton crops.

Section 7 Conclusions of the RARMP

313. The risk assessment concludes that this limited and controlled release of insect resistant GM cotton lines into the Shires of Narrabri, NSW and Emerald, QLD poses negligible risks to the health and safety of people and the environment as a result of gene technology.

314. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, if a licence were to be issued, conditions have been imposed to contain the release to the proposed size, duration and regions requested by the applicant.

Chapter 7 Licence conditions

Section 1 Interpretations and Definitions

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

In this licence:

(a) words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;

(b) words importing a gender include any other gender;

(c) words in the singular include the plural and words in the plural include the singular;

(d) words importing persons include a partnership and a body whether corporate or otherwise;

(e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

(f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

(g) specific conditions prevail over standard conditions to the extent of any inconsistency.

In this licence:

'Act' means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law.

'Annual Report' means a written report provided to the Regulator within 90 days of each anniversary of this licence containing all the information required by this licence to be provided in the Annual Report.

'Clean' (or **'Cleaned'**), as the case requires, means:

(a) in relation to a Location or other area, the Destruction of the GMOs, Pollen Trap Plants and Plant Material in that Location or area, to the reasonable satisfaction of the Regulator; or

(b) in relation to Equipment, the removal and Destruction of the GMOs, Pollen Trap Plants and Plant Material from the Equipment, to the reasonable satisfaction of the Regulator.

'Cotton' means plants of the species *Gossypium hirsutum* L.

'Destroy', (or **'Destroyed'** or **'Destruction'**) means, as the case requires, killed by one or more of the following methods:

(a) stalk pulling; or

(b) uprooting by ploughing; or

(c) burning; or

(d) treatment with herbicide; or

(e) hand weeding; or

(f) autoclaving.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of killing the remains of harvest of the GMOs, treatment of post harvest remains by herbicide would not be a sufficient mechanism.

'Equipment' includes harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), clothing and tools.

'GM' means genetically modified.

'GMOs' means the genetically modified organisms listed in Attachment B and authorised for release by this licence.

'Location' means an area of land where the GMOs or other plants are planted and grown pursuant to this licence but does not include a Pollen Trap.

'Natural Waterways' means waterways other than irrigation channels, holding dams or storage ponds used to collect water runoff from irrigated areas.

'OGTR' means the Office of the Gene Technology Regulator.

'Plant Material' means viable parts of the GMOs, Pollen Trap Plants or non-GM cotton grown at the Location including seed, stubble and pollen, whether from the plant itself or derived from or produced by the plant.

'Pollen Trap' means an area of land, extending at least 20 metres in all directions from the outside edge of a Location

'Pollen Trap Plant' means non-GM Cotton or GM Bollgard II® Cotton from a Pollen Trap.

'Regulator' means the Gene Technology Regulator.

'Sign-off' means a notice in writing from the Regulator, in respect of a place or a Location, whichever applies, that post harvest inspection conditions no longer apply in respect of that place or Location.

'Volunteer Plants' means progeny of the GMOs, Pollen Trap Plants or non-GM cotton grown at the Location, or regrowth of previous GM or non-GM cotton plants.

Section 2 General conditions

Duration of Licence

1. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with GMOs are authorised during any period of suspension.

Holder of Licence

2. The holder of this licence ('the licence holder') is Deltapine Australia Pty Ltd.

Project Supervisor

3. The Project Supervisor in respect of this licence is identified at Attachment A.
4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

No dealings with GMOs except as authorised by this licence

5. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

Persons covered by this GMO licence

6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs grown in a Location pursuant to this licence.

Informing people of their obligations

7. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:
   (a) the particular condition (including any variations of it);
   (b) the cancellation or suspension of the licence;
   (c) the surrender of the licence.
8. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

Applicant to notify of circumstances that might affect suitability

9. The licence holder must immediately, by notice in writing, inform the Regulator of:
   (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
   (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;
   (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it.

Licence holder must provide information on matters related to suitability

10. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Additional information to be given to the Regulator

11. It is a condition of a licence that the licence holder inform the Regulator if the licence holder:

    (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    (b) becomes aware of any contraventions of the licence by a person covered by the licence; or

    (c) becomes aware of any unintended effects of the dealings authorised by the licence.

12. The licence holder must provide the information required by paragraphs (a) (b) and (c) of the immediately preceding condition to the Regulator as soon as practically and reasonably possible and must also include the information in the Annual Report.

People dealing with GMOs must allow auditing and monitoring of the dealing

13. If a person is authorised by this licence to deal with GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Remaining an Accredited organisation

14. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

Section 3 Specific conditions

GMOs covered by this licence

1. The GMOs covered by this licence are described at Attachment B.

Permitted dealings

2. The permitted dealings with the GMOs are to plant, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings.
3. Non-GM Cotton may be planted and grown at the Location, but if planted, must be handled and controlled as if it were a GMO for purposes of this licence.

Locations and size of trial

4. The permitted dealings with the GMOs may be undertaken during the cotton growing seasons in Summer 2005/2006 within the Shires of Narrabri, NSW and Emerald, QLD.
5. The maximum number of Locations for this release is two. The maximum combined area of both Locations is limited to 1 hectare,
6. The maximum size of any individual Location must not exceed 0.5 of a hectare.
7. The licence holder must be able to access and control a Location to the extent necessary to comply with this licence, for the duration of the life of the licence.

Notice of intention to plant and of planting

8. The licence holder must provide a notice in writing to the Regulator which contains:
   (a) the date on which planting of the GMOs is intended to commence;
   (b) the date on which planting of any non-GMOs on a Location is intended to commence;
   (c) details of the Location where the GMOs will be planted, including a street address and GPS coordinates for the Location;
   (d) the period during which the licence holder considers the GMOs are likely to flower;
   (e) the period during which the licence holder considers the GMOs are likely to be harvested (or Destroyed in lieu of harvest); and
   (f) if GMOs have previously been planted at a site, a history indicating how the site has been used in the preceding two years, including details of previous GMOs and post-harvest crops planted at the Location.
9. The notice must be provided to the Regulator at least 7 days prior to the date on which planting of the GMOs is intended to commence.
10. Notice of the actual date of planting must be provided to the Regulator if it differs from the forecasted/intended date of planting.

Note: Information about where the GMOs are being planted pursuant to this licence, including the exact site details, will be made available at the beginning of the summer 2005/2006.

Notice of harvest and Cleaning following harvest

11. The licence holder must provide the Regulator with a notice of intention to harvest GMOs at the Location. This notice must be provided at least 7 days, and not more than 21 days, in advance of the intended date of harvest set out in the notice. Any change of intention prior to the intended harvest date must be notified to the Regulator as soon as is reasonably and practically possible.
12. The licence holder must provide the actual date or dates of commencement of harvesting of GMOs at the Location. This notice must be provided within 7 days of commencement of harvesting of the GMOs at the Location.

Note: There are 2 relevant notices with respect to a forecast of harvest. One is a long-term forecast provided under Condition 8 and the other is a short-term forecast under Condition 10.

13. The licence holder must provide a notice in writing to the Regulator when a Location is Cleaned following harvest. The notice must be provided to the Regulator within 14 days of the date on which Cleaning of the Location concluded.

Measures to manage Gene Flow

14. Each Location must be surrounded by a Pollen Trap.
15. Each Pollen Trap must contain only Pollen Trap Plants grown in such a way as to reasonably promote a dense and vigorous growth and in such a way as to ensure that the Pollen Trap Plants flower at the same time and for the same period of time as the GMOs.
16. The edge of every Pollen Trap that is farthest from the GMOs (the 'outer edge of the Pollen Trap') must not be within 50 metres of a Natural Waterway.
17. Pollen Trap Plants must be handled and controlled as if they are the GMOs (ie subject to other applicable conditions elsewhere in this licence) and Plant Material must be handled and controlled as if it is the GMOs (ie subject to other applicable conditions elsewhere in this licence).
18. A Pollen Trap must be able to be accessed and controlled by the licence holder to an extent that is commensurate with the licence holder's rights to access and control the Location within it.

Material from the GMOs may be collected

19. Any material from the GMOs, including Plant Material, may be collected from a Location for the purpose of conducting experiments on it.
20. Any material from the GMOs, including Plant Material, that is collected may be transported off the Location to:

    (a) a facility certified by the Regulator to physical containment level 2 (PC2); or

    (b) a facility approved in writing by the Regulator and signed so as to indicate that GM Plant Material is stored within the facility,

    and may be experimented on and stored in any one or more of these facilities.
21. After any experiments with the material from the GMOs are completed, the Plant Material must be Destroyed.

Note: the above conditions do not permit experiments involving any genetically modified organisms other than those covered by this licence to be performed in these facilities.

Harvest and post-harvest procedures

22. If the GMOs or Pollen Trap Plants are harvested, they must be harvested separately from any other crop.
23. If seed Cotton harvested from the GMOs or from Pollen Trap Plants is ginned, it must be ginned separately from any other crop.
24. Following ginning, seed from the GMOs and Pollen Trap Plants must be:
    (a) stored in a sealed container, within a locked facility that is signed so as to indicate that GM Cotton seed is stored within the facility;
    (b) exported; or
    (c) Destroyed by burning.
25. Any GM Cotton seed obtained from ginning may only be transported to the extent necessary to store them, export them, Destroy them by burning, or take them to a facility certified by the Regulator to physical containment level 2 (PC2).
26. Cotton lint obtained from ginning of seed Cotton harvested from the GMOs or Pollen Trap Plants may be sold.

Cleaning – post harvest and generally

27. Equipment, a Location or other area (including a gin) used pursuant to this licence in respect of GMOs, Pollen Trap Plants or Plant Material must be Cleaned.
28. For each Location, either within 14 days of harvest of the GMOs or within 9 months of planting of the GMOs, whichever occurs first, the Location must be Cleaned.
29. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned. (For the sake of clarity, it is not necessary for Equipment to be Cleaned only at a Location.)
30. Cleaning must occur immediately or as soon as practicable after use and before it is used for any other purpose. (For example, if GM seed is ginned, the gin must be Cleaned immediately following its use and before any other crop is ginned.)
31. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with the Cleaning conditions in this licence.

Inspection

32. Following Cleaning of a Location or other area, the following places must be inspected for the existence of Volunteer Plants:
    (a) the Location;
    (b) the Pollen Trap in respect of the Location;
    (c) irrigation channels and drains through which water flows to and from the Location and the Pollen Trap;
    (d) any areas used to Clean Equipment used in connection with the GMOs at the Location or to Destroy the GMOs, Pollen Trap Plants or Plant Material.
33. Inspection must be performed by a person who is able to recognise Volunteer Plants.

34. For each Location, all the places required to be inspected must be inspected at least once every 2 months, commencing on the last day of Cleaning of the Location and continuing until the Regulator has issued a Sign-off.

35. The results of inspection activities must be recorded in a logbook or paper file. The findings of the inspections as recorded in the logbook or paper file must be forwarded to the Regulator within 14 days of inspection taking place and must also be included in the licence holder's Annual Report to the Regulator. The logbook or paper file must contain at least the following:

    (a) details of the areas inspected;

    (b) details of the date of inspection;

    (c) the names of the person or persons who undertook the monitoring and details of the experience, training or qualification that enabled them to recognise Volunteer Plants;

    (d) the means of inspection used;

    (e) the number of Volunteer Plants observed, if any;

    (f) details of the development stages reached by the Volunteer Plants, if any; and

    (g) details of methods used to Destroy Volunteer Plants, if any.

36. Any Volunteer plant must be Destroyed prior to the plant flowering.

37. For each Location, and all places in respect of that particular Location as listed in condition 31,

    (a) if inspections have been routinely completed in that Location and all those places for a period of at least 12 months, and,

    (b) if inspection records for that Location and all those places show that no Volunteers have been observed in the most recent 6 month inspection period,

    the licence holder may make written application to the Regulator that these inspection conditions no longer apply to that particular Location and the places in respect of that particular Location as listed in condition 31.

38. These inspection conditions do not apply in respect of a place if the licence holder has received a Sign-off.

General conditions on use of Locations post-harvest

39. If the GMOs are grown at a Location, no plants may be planted at the Location, Pollen Trap in respect of the Location until inspection obligations are completed unless:

    (a) the plants are grasses (grass pastures), cereals (cereal crops); or

    (b) the plants are plants agreed to in writing by the Regulator.

Transportation of the GMOs, Pollen Trap Plants and Plant Material

40. Subject to the conditions immediately below in respect of transportation, the GMOs, Pollen Trap Plants and Plant Material must be transported in accordance with the OGTR *Guidelines for the Transport of GMOs June 2001* issued by the Regulator.

41. Harvested GMOs, Pollen Trap Plants and Plant Material may be transported to a ginning facility in a cotton module that is:

    (a) completely enclosed within 2 layers of tarpaulin ('double wrapped in tarpaulin');

(b) completely enclosed within a layer of tarpaulin and a layer of shade cloth ('double wrapped in tarpaulin and shade cloth'); or

(c) contained within an enclosed chain-bed truck specifically designed for the purpose of transporting cotton modules.

42. Cotton lint derived from GMOs and Pollen Trap Plants from ginning is not subject to transportation conditions.

43. Every container used to transport the GMOs, Pollen Trap Plants and Plant Material must be labelled:

(a) to indicate that it contains genetically modified Cotton; and

(b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.

44. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs, Pollen Trap Plants or Plant Material sent is delivered and must document routes, methods and procedures used for transportation of GMOs, Pollen Trap Plants and Plant Material.

Contingency Plans

45. Within 30 days of the date of issue of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs, Pollen Trap Plants or Plant Material, outside an area that must be inspected.

46. The Contingency Plan must include details of procedures to:

(a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;

(b) destroy any of the GMOs, Pollen Trap Plants or Plant Material; and

(c) inspect for and Destroy any Volunteer Plants that may exist as a result of the event.

47. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs, Pollen Trap Plants and Plant Material is discovered outside an area that must be inspected.

Compliance Management Plan

48. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with these conditions and document that compliance.

Reporting

49. The licence holder must provide an Annual Report to the Regulator.

Testing methodology

50. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence (at Attachment B) in a recipient organism. The instrument must be provided within 30 days of the issuing of this licence.

Use of GMOs, Pollen Trap Plants and Plant Material

51. The GMOs, Pollen Trap Plants and Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in their use as food for animals or humans.

References

Ahmad, W., Nicholls, C., Ellar, D.J. (1989). Cloning and expression of an entomocidal protein gene from *Bacillus thuringiensis* galleriae toxic to both lepidoptera and diptera. *FEMS Microbiology letters - Federation of European Microbiological Societies,* **59**: 198-202.

An, W.Q., McDowell, J.M., Huang, S., McKinney, E.C., Chambliss, S., Meagher, R.B. (1996). Strong, constitutive expression of the *Arabidopsis ACT2/ACT8* actin subclass in vegetative tissues. *Plant Journal* **10**: 107-121.

Astwood, J.D., Leach, J.N., Fuchs, R.L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotechnology* **14**: 1269-1273.

Bergelson, J., Purrington, C.B., Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* **395**: 25.

Bevan, M. (1984). Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research* **12**: 8711-8721.

Bradford, K.J., van Deynze, A., Gutterson, N., Parrot, W., Strauss, S.H. (2005). Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. Nature Biotechnology 23[4], 439-444.

Brubaker, C.L., Brown, A.H.D., Stewart, J.M., Kilby, M.J., Grace, J.P. (1999). Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* **108**: 199-213.

Craven, L. A., Stewart, J. M., Brown, A. H. D., and Grace, J. P. (1994). Challenging the future; the Australian wild species of *Gossypium*. In "*Proceedings of the 1st World Cotton Research Conference"*, pp. 278-281.

Dankocsik, C., Donovan, W.P., Jany, C.S. (1990). Activation of a cryptic crystal protein gene of *Bacillus thuringiensis* subspecies *kurstaki* by gene fusion and determination of the crystal protein insecticidal specificity. *Molecular Microbiology* **4**: 2087-2094.

De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.

del Rincon-Castro, M.C., Barajas-Huerta, J., Ibarra, J.E. (1999). Antagonism between Cry1Ac1 and Cyt1A1 toxins of *Bacillus thuringiensis*. *Applied and Environmental Microbiology* **65**: 2049-2053.

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., Goodman, H.M. (1982). Nopaline synthase: transcript mapping and DNA sequence. *Journal of Molecular and Applied Genetics* **1**: 561-573.

Donovan, W.P., Donovan, J.C., Engleman, J.T. (2001). Gene knockout demonstrates that *vip3A* contributes to the pathogenesis of *Bacillus thuringiensis* toward *Agrotis ipsilon* and *Spodoptera exigua*. *Journal of Invertebrate Pathology* **78**: 45-51.

Eastick, R. (2002). Evaluation of the potential weediness of transgenic cotton in northern Australia. Report No. Technical Bulletin no. 305, Northern Territory Government and Australian Cotton Cooperative Research Centre,

EFSA (2004). Opinion of the scientific panel on genetically modified organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal* **48**: 1-18.

Estruch, J.J., Warren, G.W., Mullins, M.A., Nye, G.J., Craig, J.A., Koziel, M.G. (1996). Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences of the United States of America* **93**: 5389-5394.

FAO and WHO (2000). Safety aspects of genetically modified foods of plant origin. <http://www.who.int/fsf/GMfood/FAO-WHO_Consultation_report_2000.pdf>.

Farrell, T. and Roberts, G. (2002). Survey of cotton volunteers north of latitude 22º south. Australian Cotton CRC and CSIRO Plant Industry, Narrabri.

Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992). Selectable marker genes: safe for plants? *Bio/Technology* **10**: 141-144.

Fryxell, P.A. (1992). A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). *Rheedea* **2**: 108-165.

FSANZ (2004). Final assessment report - Application A509: Food derived from insect protected cotton line COT102.

Fuchs, R.L., Astwood, J.D. (1996). Allergenicity assessment of foods derived from genetically modified plants. *Food Technology* **50**: 83-88.

Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.

Gregory, S.R., Hernandez, E., Savoy, B.R. (1999). Cottonseed processing. Chapter 4.5. In: CW Smith, JT Cothren, eds. *Cotton: Origin, History, Technology and Production*. John Wiley & Sons, New York. pp 793-819.

Groves, R.H., Boden, R., and Lonsdale, W.M. (2005). Jumping the garden fence: Invasive garden plants in Australia and their environmental and agricultural impacts. Report No. CSIRO Report prepared for WWF Australia, WWF-Australia,

Groves, R.H., Hosking, J.R., Batianoff, D.A., Cooke, D.A., Cowie, I.D., Keighery, B.J., Rozefelds, A.C., and Walsh, N.G. (2000). The naturalised non-native flora of Australia: its categorisation and threat to native plant biodiversity. Unpublished report to Environment Australia by the CRC for Weed Management Systems.

Groves, R.H., Hosking, J.R., Batianoff, G.N., Cooke, D.A., Cowie, I.D., Johnson, R.W., Keighery, G.J., Lepschi, B.J., Mitchell, A.A., Moerkerk, M., Randall, R.P., Rozefelds, A.C.,

Walsh, N.G., Waterhouse, B.M. (2003). *Weed categories for natural and agricultural ecosystem management.* Bureau of Rural Sciences, Canberra.

Groves, R.H., Hosking, J.R., Cooke, D.A., Johnson, R.W., Lepschi, B.J., Mitchell, A.A., Moerkerk, M., Randall, R.P., Rozefelds, A.C., and Waterhouse, B.M. (2002). The naturalised non-native flora of Australia: its categorisation and threat to agricultural ecosystems. Unpublished report to Agriculture, Fisheries and Forestry Australia by the CRC for Weed Management Systems.

Holm, L., Doll, J., Holm, E., Pancho, J., Herberger, J. (1997). *World weeds. Natural histories and distribution.* John Wiley and Sons, Inc, USA.

Holm, L., Pancho, J., V, Herberger, J.P., Plucknett, D.L. (1979). *A geographical atlas of world weeds.* John Wiley and Sons, Brisbane, Australia. pp 471-04393.

Jenkins, J.N. (1992). Cotton. In: *OECD Historical Review of Traditional Crop Breeding*

Kimber, I., Kerkvliet, N.L., Taylor, S.L., Astwood, J.D., Sarlo, K., Dearman, R.J. (1999). Toxicology of protein allergenicity: prediction and characterization. *Toxicological Sciences* **48**: 157-162.

Klee, H.J., Rogers, S.G. (1989). Plant gene vectors and genetic transformation: plant transformation systems based on the use of *Agrobacterium tumefaciens*. *Cell Culture and Somatic Cell Genetics of Plants* **6**: 1-23.

Konig, A., Cockburn, A., Crevel, R.W., Debruyne, E., Grafstroem, R., Hammerling, U., Kimber, I., Knudsen, I., Kuiper, H.A., Peijnenburg, A.A., Penninks, A.H., Poulsen, M., Schauzu, M., Wal, J.M. (2004). Assessment of the safety of foods derived from genetically modified (GM) crops. *Food and Chemical Toxicology* **42**: 1047-1088.

Kurland, C.G., Canback, B., Berg, O.G. (2003). Horizontal gene transfer: a critical view. *Proceedings of the National Academy of Science of the United States of America* **100**: 9658-9662.

Leboul, J., Davies, J. (1982). Enzymatic modification of hygromycin B in *Streptomyces hygroscopicus*. *The Journal of Antibiotics (Tokyo)* **35**: 527-528.

Lee, M.K., Walters, F.S., Hart, H., Palekar, N., Chen, J.-S. (2003). The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. *Applied and Environmental Microbiology* **69**: 4648-4657.

Leffler, H.R., Tubertini, B.S. (1976). Development of cotton fruit: accumulation and distribution of mineral nutrients. *Agronomy Journal* **68**: 858-861.

Llewellyn, D., Fitt, G. (1996). Pollen dispersal from two field trials of transgenic cotton in the Namoi valley, Australia. *Molecular Breeding* **2**: 157-166.

Martin, P.A.W., Travers, R.S. (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Applied and Environmental Microbiology* **55**: 2437-2442.

Meadows, M.P. (1993). *Bacillus thuringiensis* in the environment: ecology and risk assessment. Chapter 9. In: PF Entwistle, JS Cory, MJ Bailey, S Higgs, eds. *Bacillus*

thuringiensis, an environmental biopesticide: theory and practice. John Wiley and sons, Chichester, UK. pp 193-220.

Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews in Food Science and Nutrition* **36(S)**: S165-S186.

Mulvaney, M. (2001). The effect of introduction pressure on the naturalization of ornamental woody plants in south-eastern Australia. Chapter 15. In: RH Groves, FD Panetta, JG Virtue, eds. *Weed Risk Assessment*. CSIRO Publishing, Melbourne. pp 186-193.

Murray, F., Llewellyn, D., McFadden, H., Last, D., Dennis, E.S., Peacock, W.J. (1999). Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. *Molecular Breeding* **5**: 219-232.

Nielsen, K.M., Bones, A.M., Smalla, K., van Elsas, J.D. (1998) Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? FEMS Microbiol Rev 22 (2): 79-103.

Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.

OECD (1998). *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Cooperation and Development, Paris

OGTR (2002). *The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia*.

OGTR (2005). *Risk Analysis Framework*. Australian Government, Canberra, ACT.

Ohmae, K., Nakamura, M., Yonezawa, S., Terakado, N. (1979). Distribution of destomycin A and hygromycin B resistance and R plasmids in *Escherichia coli* strains isolated from the faeces of calves and pigs. *Annual Report of the National Veterinary Assay Laboratory* **16**: 36-46.

Oosterhuis, D.M., Jernstedt, J. (1999). Morphology and anatomy of the cotton plant. Chapter 2.1. In: CW Smith, JT Cothren, eds. *Cotton: Origin, History, Technology and Production*. John Wiley & Sons, New York. pp 175-206.

Panetta, F.D. (1993). A system of assessing proposed plant introductions for weed potential. *Plant Protection Quarterly* **8**: 10-14.

Penaloza-Vazquez, A., Mena, G.L., Herrera-Estrella, L., Bailey, A.M. (1995). Cloning and sequencing of the genes involved in glyphosate utilization by *Pseudomonas pseudomallei*. *Applied and Environmental Microbiology* **61**: 538-543.

Percival, A.E., Wendel, J.F., Stewart, J.M. (1999). Taxonomy and Germplasm Resources. Chapter 1.2. In: CW Smith, JT Cothren, eds. *Cotton: origin, history, technology, and production*. John Wiley & Sons, New York, USA. pp 33-63.

Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L., Fischhoff, D.A. (1991). Modifications of the coding sequence enhances plant expression of insect control protein genes. *Proceedings of the National Academy of Sciences of the United States of America* **88**: 3324-3328.

Pheloung, P.C. (1995). Determining the weed potential of new plant introductions in Australia. Department of Agriculture, Perth, Australia.

Pheloung, P.C. (2001). Weed risk assessment for plant introductions to Australia. Chapter 7. In: RH Groves, FD Panetta, JG Virtue, eds. *Weed Risk Assessment*. CSIRO Publishing, Melbourne. pp 83-92.

Pheloung, P.C., Williams, P.A., Halloy, S.R. (1999). A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management* **57**: 239-251.

Randall, R.P. (2002). *A global compendium of weeds.* R.G. & F.J. Richardson, Meredith, Victoria. pp 1-905.

Raps, A., Kehr, J., Gugerli, P., Moar, W.J., Bigler, F., Hilbeck, A. (2001). Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Molecular Ecology* **10**: 525-533.

Rice, W.C. (1999). Specific primers for the detection of *vip3A* insecticidal gene within a *Bacillus thuringiensis* collection. *Letters in Applied Microbiology* **28**: 378-382.

Salauze, D., Otal, I., Gomez-Lus, R., Davies, J. (1990). Aminoglycoside acetyltransferase 3-IV (aacC4) and hygromycin B 4-I phosphotransferase (hphB) in bacteria isolated from human and animal sources. *Antimicrobial Agents and Chemotherapy* **34**: 1915-1920.

Salvaggio, J.E., O'Neil, C.E., Butcher, B.T. (1986). Immunologic responses to inhaled cotton dust. *Environmental Health Perspectives* **66**: 17-23.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews* **62**: 775-806.

Shi, Y., Wang, M.-B., Powell, K. (1994). Use of rice sucrose synthase-1 promoter to direct phloem-specific expression of beta-glucuronidase and snowdrop lectin genes in transgenic tobacco plants. *Journal of Experimental Botany* **45**: 623-631.

Sims, S.R., Berberich, S.A., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C., Fuchs, R.L. (1996). Analysis of expressed proteins in fibre fractions from insect-protected and glyphosate-tolerant cotton varieties. *Crop Science* **36**: 1212-1216.

Sjoblad, R.D., McClintock, J.T., Engler, R. (1992). Toxicological considerations for protein components of biological pesticide products. *Regulatory Toxicology and Pharmacology* **15**: 3-9.

Taylor, S.L., Lehrer, S.B. (1996). Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition* **36**: S91-S118.

US EPA (2005). *Bacillus thuringiensis* VIP3A protein and the genetic material necessary for its production; Temporary exemption from the requirement of a tolerance. <http://www.epa.gov/EPA-PEST/2005/April?Day-28/p8530.htm>.

van Frankenhuyzen, K. and Nystrom, C. (2002). The Bacillus thuringiensis toxin specificity database. <http://www.glfc.cfs.nrcan.gc.ca/bacillus>.

Wang, K., Herrera-Estrella, L., Van Montagu, M., Zambryski, P. (1984). Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from *Agrobacterium* to the plant genome. *Cell* **38**: 455-462.

Widner, W.R., Whiteley, H.R. (1990). Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from *Bacillus thuringiensis*. *Journal of Bacteriology* **172**: 2826-2832.

Widner, W.R., Whiteley, H.R. (1989). Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. *Journal of Bacteriology* **171**: 965-974.

Zalacain, M., Malpartida, F., Pulido, D., Jimenez, A. (1987). Cloning and expression in *Escherichia coli* of a hygromycin B phosphotransferase gene from *Streptomyces hygroscopicus*. *European Journal of Biochemistry* **162**: 413-418.

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 agencies and authorities[1] on application DIR 58/2005

Issues raised relating to the Risk Assessment and where they are considered in the RARMP:

- risk of weediness (see Chapters 2 and 5)
- risk of the development of resistance to the VIP3A protein (see Chapter 2)
- risk of toxicity to non-target organisms (including people) (see Chapters 2, 3 and 4)
- risk of unintended effects on insect pests not controlled by VIP3A, as a result of altered pesticide regimes (see Chapter 2)
- risk resulting from any gene flow (both vertical and horizontal) (see Chapters 2, 3, 4 and 5)
- risk resulting from dissemination of the GM cotton/pollen beyond the intended areas (see Chapters 2, 3, 4 and 5)
- risk resulting from persistence and/or accumulation of the VIP3A and hygromycin resistance proteins in soils (see Chapters 2, 3 and 4)
- risk resulting from occupational exposure (see Chapters 2 and 3).

Issues raised relating to the Risk Management Plan:

- standard licence conditions for limiting and controlling the release (see Chapter 6)
- lint should not be sold unless it can be guaranteed to be free from viable seed
    - *The ginning and lint cleaning processes are designed to remove as much trash (small particles of leaf and seed coat) from the lint as possible in order to create a saleable product. Contamination by viable seeds is not likely to occur.*
- an insect resistance management plan (see Chapter 2).

Suggested data requirements for possible future releases:

- more complete characterisation of the VIP3A protein (similar to data provided or Cry proteins), including details of mode of action and specificity
- more detailed molecular characterisation of the genetic modification
- toxicity studies for a diverse range of Australian species (avian, mammalian, and invertebrate). These studies should include both terrestrial and aquatic organisms
- further data regarding the amino acid substitution and its effect on toxicity

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

- expression levels of the VIP3A protein in all cotton tissues under Australian field conditions
- data on accumulation of the VIP3A protein in soil around the GM cotton plants
- field studies on insects in VIP3A cotton crops compared with conventional cotton crops, grown under typical commercial conditions including required pesticide spray regimes
- evidence of whether or not VIP3A protein expression will increase the survival of cultivated cotton off-farm and therefore its weediness in non-agricultural areas, especially in northern Australia
- information on any occurrence of the development of insect resistance to the VIP3A protein and development of an insect resistance management plan

Appendix C Summary of issues raised in submissions received from the public on application DIR 058/2005

Two submissions were received that raised issues that are outside the scope of the assessment that the Regulator is required to conduct under the Act.

Submission 1:

No GMOs should be released into the environment. The public does not support GM foods.

Submission 2:

A philosophical objection to the release of insecticidal cotton:

- GM pushes the bounds of nature, overcoming the natural boundaries between species
- There are unknowable risks to human health and safety and the environment, which will prove to be unmanageable in the future.

Appendix D Summary of issues raised in submissions received from prescribed agencies and authorities on the consultation RARMP for DIR 058/2005

Issues raised relating to the Risk Assessment:

- Concern about the possible future development of insect resistance to the VIP3A protein (see Chapter 2)
- Clarification sought of whether any non-lepidopterans have been found to be sensitive to the VIP3A protein (see Chapter 4).

Suggested data requirements for possible future releases:

- Increased data should be provided on the COT202 and COT203 GM cotton lines in any future applications releases
- The exudation of the VIP3A toxin into the soil from the roots and from decomposition of the GM cotton material
- The accumulation, persistence and fate of the VIP3A toxin in various Australian soil types and the effects on non-target soil-organisms
- Data should be provided on toxicity of the VIP3A protein for a representative range of insects found in Australian cotton growing conditions.
    - GM plant material should be used to test this toxicity.

Appendix E Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 058/2005

Abbreviations used:
Issues raised: E: Ethical concerns; **G**: Gene transfer; **H**: Health concerns; **HR**: Herbicide resistance; **IR**: insecticide resistance; **M**: Market and trade concerns; **OSA**:. Outside the scope of the assessment
[a] **Submission from: A**: agricultural/industry organisation; **I**: Individual; **LGA**: Local Government Authority

Sub.No.	Date	Type[a]	Summary of issues raised	Issue	Consideration of issue
1	05/09/05	I	The health of soil organisms , animals fed cotton waste and humans fed cottonseed oil, may be at risk. All avenues of contamination cannot be identified.	EN, H,	Evidence indicates that the proteins expressed by the GM cotton plants are not toxic to mammals, birds or bacteria Cottonseed oil will not be produced from this trial , cotton waste will not be fed to animals. See Chapter 2 and 6
			Some people have ethical reasons for wishing to avoid contact with genetically engineered organisms and their derivatives and they will not be able to do so with the derivatives of the cotton industry. These people suffer from the denial of their civil liberties—ie their right to live in a genetically natural environment.	E	OSA
			The loss of genetic integrity causes profound anguish and health problems (depression)	H, E	While the Regulator acknowledges that the release of GM crops into the environment causes concern for some people, the risk assessment has used current scientific knowledge to determine that risks to the health and safety of people and the environment as a result of this release are negligible.
2	02/09/05	LGA	As advised in previous correspondence, Council holds grave concerns over the introduction of genetically modified organisms into the environment of the Shire. These concerns relate to the following:		The current application proposes the limited and controlled release of GM cotton lines on one or two sites in the Shires of Narrabri, NSW and Emerald, QLD. The release will not occur in this Shire.
			The provision of insufficient buffer areas between areas planted with genetically modified plants and normal crops, as pollen is capable to travelling several kilometres should the wind conditions be right.	G	Cotton pollen is dispersed by insects rather than wind. The applicant intends to spray the crop to ensure pure seed production during this release See Chapter 1

			Potentially breeding a poison-resistant weed.	HR	This release does not involve the use of GM herbicide tolerant cotton plants See Executive Summary and Chapter 1
			Potentially breeding pesticide-resistant bugs.	IR	The a small scale, limited duration and the planned use of pesticide sprays on the GM cotton plants will to prevent the development of insect resistance to the VIP3A protein during this release See Chapters 1 and 2
			The potential health concerns generally stemming from what is not known of the effect on the environment and the food chain from these newly developed plants.	H	This is a small scale, short term release and risks to the health and safety of the people and the environment due to this release have been assessed as negligible. See Executive summary and Chapters 2, 3, 4 and 5
			The detrimental effect of any cross breeding of cross-contamination of crops on existing and future export markets.	M	OSA
			There are ethical consequences of biotechnology that Council feels have yet to be addressed by industry or government.	E	OSA
			As such, Council's position is simply that it does not wish to see trials of genetically modified plants occurring in the Shire.		Noted
3	07/10/05	A	The introduction of GM insecticidal cotton has shown a dramatic reduction in their pesticide use requirements compared to conventional cotton. There has also been a change in the types of pesticides that are applied, leading to less use of older broad-spectrum disruptive chemistry. The two factors combined have a significant impact on the reducing the risks to the environment and human health from topically applied insecticides. Combining this technology with the Cotton Industry Best Management Practice Program has had and continues to have a significant impact on the environment performance of the Australian Cotton Industry.	None	Noted
			The biggest risk to sustainability of growing insecticidal cotton is the threat of insect resistance to the genetically introduced toxin….The introduction of additional unrelated toxins to those commercially in use in the current stacks of Bollgard II® such as this application for the toxin produced by the *vip3A* gene, will allow for toxin rotation and hence reduced selection pressure on the pest. This will allow for the maintenance of relaxed area constraints, the reduced risk of resistance development and aid in the sustainability of this important technology to our industry.	None	Noted

			After 40 years of cotton growing and processing through the north-western regions of New South Wales, cotton has not been able to establish itself as a weed. This is despite the spills of seed cotton and fuzzy seed that occurred on farms and on the rural road networks prior to modern hygiene requirements on product transportation. The introduction of insecticide-tolerant and/or herbicide-tolerant traits has also not altered that status to date, hence our agreement with the assessments relating to weediness in the RARMP for this release. The likelihood of the GM cotton becoming a weed is absolutely minimal.	None	Noted
			Strongly support the proposed field plantings by Deltapine of the GM cotton expressing the vip3A trait for seed increase. They are satisfied that the risk management plan supporting the limited and controlled release sufficiently covers the minimal risks attached to this technology.	None	Noted