Risk Assessment and Risk Management Plan

Application for licence for dealings involving an intentional release into the environment

DIR 036/2003

Title: Breeding and pre-commercial evaluation of transgenic cotton expressing a vegetative insecticidal protein (VIP) and a herbicide tolerance gene

Applicant: CSIRO

October 2003

Office of the Gene Technology Regulator
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ANZFA</td>
<td>Australia New Zealand Food Authority (now FSANZ)</td>
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<td>APVMA</td>
<td>Australian Pesticides and Veterinary Chemicals Authority</td>
</tr>
<tr>
<td>bar</td>
<td>Bar gene</td>
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<tr>
<td>Bt</td>
<td><em>Bacillus thuringiensis</em></td>
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<tr>
<td>Btk</td>
<td><em>Bacillus thuringiensis</em> variety <em>kurstaki</em></td>
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<tr>
<td>CaMV</td>
<td>cauliflower mosaic virus</td>
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<tr>
<td>CCI</td>
<td>Confidential Commercial Information</td>
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<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
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<td>DIR</td>
<td>dealing involving intentional release</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<td>FAO</td>
<td>Food and Agriculture Organisation of the United Nations</td>
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<td>FSANZ</td>
<td>Food Standards Australia New Zealand (formerly ANZFA)</td>
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<td>g</td>
<td>gram</td>
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<td>GM</td>
<td>genetically modified</td>
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<tr>
<td>GMAC</td>
<td>Genetic Manipulation Advisory Committee</td>
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<td>GMO</td>
<td>genetically modified organism</td>
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<td>GTTAC</td>
<td>Gene Technology Technical Advisory Committee</td>
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<tr>
<td>ha</td>
<td>hectare</td>
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<td>hph</td>
<td>hygromycin resistance gene</td>
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<td>HPT</td>
<td>hygromycin phosphotransferase</td>
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<td>IgE</td>
<td>immunoglobulin E</td>
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<td>IPCS</td>
<td>International Program on Chemical Safety</td>
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<tr>
<td>m</td>
<td>Metre</td>
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<tr>
<td>kDa</td>
<td>KiloDalton</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>ng</td>
<td>nanogram</td>
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<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<td>NICNAS</td>
<td>National Industrial Chemicals Notification and Assessment Scheme</td>
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<tr>
<td>nos</td>
<td>nopaline synthase</td>
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<td>NLRD</td>
<td>Notifiable Low Risk Dealing</td>
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<td>NRA</td>
<td>National Registration Authority for Agricultural and Veterinary Chemicals</td>
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<td>OECD</td>
<td>Organisation for Economic Cooperation and Development</td>
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<td>OGTR</td>
<td>Office of the Gene Technology Regulator</td>
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<tr>
<td>ppm</td>
<td>parts per million</td>
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<tr>
<td>PAT</td>
<td>phosphino N-acetyl transferase protein</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>T-DNA</td>
<td>transfer deoxyribonucleic acid</td>
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<tr>
<td>TGA</td>
<td>Therapeutic Goods Administrations</td>
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<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
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<td>US FDA</td>
<td>United States Food and Drug Administration</td>
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<tr>
<td>vip3A</td>
<td>Vegetative insecticidal protein gene (insecticidal gene)</td>
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<td>VIP3A</td>
<td>Vegetative insecticidal protein</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>------------------------</td>
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<tr>
<td>w/v</td>
<td>weight per volume</td>
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<tr>
<td>µg/g</td>
<td>micrograms per gram</td>
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EXECUTIVE SUMMARY

THE REGULATION OF GENETICALLY MODIFIED ORGANISMS

The Gene Technology Act 2000 (the Act) and the Gene Technology Regulations 2001 (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety and the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under Section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. In accordance with Section 56 of the Act, matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator’s decision on whether, or not, to issue a licence.

THE APPLICATION

CSIRO Plant Industry (CSIRO) has applied for a licence (application number DIR 036/2003) for the intentional release into the environment, under limited and controlled conditions, of three types of genetically modified (GM) cotton. Two types are GM insecticidal cottons and the third type combines the insecticidal trait with herbicide tolerance. CSIRO proposes to carry out a trial over 3 seasons (2003 – 2006) covering a maximum area of 25 hectares in total on fifteen sites in the cotton growing regions of New South Wales and Queensland.

All three types of GM cottons proposed for release contain an insecticidal gene (vip3A), derived from a common soil bacterium, Bacillus thuringiensis. This gene encodes a vegetative insecticidal protein (VIP3A) that is toxic to caterpillar pests of cotton. The VIP3A protein is different from the insecticidal proteins (Cry1Ac and Cry2Ab), derived from the same bacterium, that are present in other types of GM insecticidal cotton that are currently being trialed or grown commercially in Australia. The applicant expects that the new insecticidal gene may provide additional options to manage the risk of development of insects resistant to Cry1Ac, Cry2Ab or other insecticidal proteins.

One type of insecticidal GM cotton, described as transformation event COT102, also contains an antibiotic resistance marker gene (hph), conferring resistance to hygromycin. This gene is derived from the common gut bacterium, Escherichia coli, and was used as a selectable marker in the initial laboratory stages of developing the GM cotton.

The second type of insecticidal GM cotton, described as the COT200 series (cotton lines containing transformation events 202 and 203), contains the same insecticidal gene used in the first type but its expression is governed by a different control sequence to COT102. This cotton does not contain the antibiotic resistance gene.
The third type of GM cotton is produced by conventional breeding of COT102 GM cotton with a herbicide tolerant GM cotton (Liberty® cotton LL25 event containing the bar gene). Therefore, the third type of GM cotton contains three new genes (vip3A, hph and bar). The herbicide tolerance gene is derived from the common soil bacterium Streptomyces hygroscopicus and produces an enzyme (phosphinothricin N-acetyltransferase, PAT) that detoxifies glufosinate ammonium (the active ingredient of Liberty® or Basta® herbicides), rendering the plant tolerant to the herbicide.

It is intended that addition of the Liberty® herbicide tolerance trait will allow more effective weed control in insect resistant cotton crops by allowing the crop to be sprayed with Liberty® herbicide to kill problem weeds without damaging the crop itself.

In accordance with Section 185 of the Act, details of the gene construct including the plasmid map and regulatory sequences for the COT102 event GM insecticidal cotton were declared as Confidential Commercial Information (CCI) under a previous CSIRO application, DIR 017/2002. Details of the gene construct including the plasmid map and regulatory sequences of the GM cotton lines containing the COT200 series have also been declared as CCI. However, all CCI materials were made available to the prescribed expert groups, which were consulted in the preparation of the risk assessment and risk management plan.

The proposed field trial is part of an ongoing breeding program to develop GM cotton varieties that are suitable for commercial release (subject to future applications and approvals) in Australian conditions. The applicant’s stated aims for the field trials are: to transfer the insecticidal trait (transformation events COT102 and COT200 series) to elite Australian cotton varieties and evaluate their agronomic performance, along with cotton lines containing the combined insecticidal and Liberty® herbicide tolerance traits (COT102 x LL25). The applicant also proposes to assess the efficacy of the insecticidal protein, the combined insecticidal/herbicide tolerance traits, and to produce seed for possible future releases subject to further approvals.

None of the cotton plants from the release, or their by-products, will be used for animal feed or human food, and seed not required for future plantings will be destroyed. However, the applicant proposes to sell lint from the release. Processed lint contains no genetic material or protein. Transport of any GM material would be in accordance with the transport guidelines issued by the Regulator.

Three limited and controlled releases of COT102 GM insecticidal cotton were approved previously (refer PR-151, DIR 017/2002 and DIR25/2002). These field trials were conducted by CSIRO in New South Wales, Queensland and northern Western Australia and the size of the releases ranged from 0.05 to 3 hectares.

Six limited and controlled releases of Liberty® herbicide tolerant cotton lines containing the bar gene were also previously approved in Australia (PR-82, PR-82X, PR-124, PR-124X, PR124X 2 and DIR 015/2002). These trials were conducted in NSW and the size of the releases ranged from 0.04 to 2 hectares.

There have been no reports of adverse effects on human health and safety or the environment resulting from any of these releases.
GM insecticidal cotton containing the COT102 event was considered in detail for the issuing of a licence for a limited and controlled release in response to application DIR 034/2003. In addition, an application for the limited and controlled release of GM Liberty® cotton containing the bar gene is being considered separately under application DIR 038/2003. More information on both is available at www.ogtr.gov.au.

THE EVALUATION PROCESS

The RARMP DIR 036/2003 from CSIRO Plant Industry has been prepared using the OGTR Risk Analysis Framework. This framework was developed by the Regulator in consultation with the public and State, Territory and Australian government stakeholders and the Gene Technology Technical Advisory Committee, and is available at www.ogtr.gov.au/pdf/public/raffinal.pdf.

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that must be considered in preparing a RARMP, are set out in Appendix 8 of the RARMP. The complete RARMP can be obtained from the OGTR or from the OGTR’s web site at www.ogtr.gov.au.

The risk assessment considered information relevant to the evaluation of potential impacts on human health and safety and the environment contained in the application (including information required by Act and the Regulations on the GMO, the parent organism, the proposed dealings and containment measures), submissions received during consultation with expert groups and authorities, and current scientific knowledge.

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of GM insecticidal cotton lines (containing the COT102 and COT200 series transformation events) and GM insecticidal (COT102)/herbicide tolerant (Liberty®) cotton lines were identified. These have been evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised.

The identified potential hazards relate to:

- toxicity and allergenicity for humans: could COT102, COT200 series or COT102/Liberty® cotton lines be more toxic or allergenic than non-GM cotton, as a result of the novel gene products or because of unintended effects?
- toxicity for non-target organisms: could COT102, COT200 series or COT102/Liberty® cotton lines be harmful to non-target organisms as a result of the novel gene products or because of unintended effects?
- weediness: could the genetic modifications be harmful to the environment by increasing the potential for the COT102, COT200 series or COT102/Liberty® cotton lines to establish as problem weeds?
- transfer of introduced genes to other organisms: could there be adverse consequences from potential transfer of the introduced genes to non-GM cotton crops, feral or native cottons, or to other organisms? and
- herbicide and insecticide resistance: could weeds develop resistance to glufosinate ammonium if the Liberty® crop-herbicide combination is used inappropriately and could
target insects develop resistance to the insecticidal proteins produced by the introduced insecticidal gene in COT102, COT200 series or COT102/Liberty® cotton lines?

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complimentary regulatory role in respect of this application due to its responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the *Agricultural and Veterinary Chemicals (Code) Act 1994*. Further information about the APVMA’s assessment and approval processes is contained in Chapter 2 and Appendix 6 of the RARMP.

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. The APVMA can impose conditions on the use of herbicides or insecticides in registrations and permits.

CSIRO has submitted an application to the APVMA for a research permit for the use of the insecticidal gene and the use of the herbicide Liberty® (a formulation of glufosinate ammonium) on all the types of GM cotton that are proposed for release. The APVMA and the OGTR are working closely to ensure the thorough coordinated assessment of these parallel proposals.

**CONCLUSIONS OF THE RISK ASSESSMENT**

The Regulator considers that the limited and controlled release of COT102, COT200 series or COT102/Liberty® cotton lines will not pose any significant risk to public health and safety, or to the Australian environment, that cannot be managed. The assessment of each potential hazard identified above is summarised under a separate heading below.

**Toxicity or allergenicity to humans**

The COT102, COT200 series and COT102/Liberty® cotton lines are unlikely to prove more toxic or allergenic to humans via occupational exposure than conventional cotton. Humans are commonly exposed to the proteins produced by the introduced genes as the organisms from which they are derived are naturally widespread in the environment. None of the proteins have any known toxicity or allergenicity and there have been no reports of toxic or allergic effects from previous releases of COT102 and Liberty® cotton lines. Cottonseed from the proposed release would not be permitted for human food use. However, Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval will be needed before products from these GM cottons could be used in human food. However, lint from the release would be sold commercially for use in fabric, upholstery and other non-food products, as processed lint contains no DNA or protein and is not used in food.

**Toxicity to non-target organisms**

The COT102, COT200 series and COT102/Liberty® cotton lines are unlikely to prove more toxic to non-target organisms than conventional cotton. Toxicity to the vegetative insecticidal protein (VIP3A) is specific to lepidopteran caterpillars. The introduced proteins are naturally widespread in the environment and have no known toxicity to mammals, birds and fish. The proposed release is limited in scale and controls would be imposed to limit the movement of the GMOs and the introduced genes, and none of the material from the release would be allowed to be used in animal feed.
Preliminary results from Australian and US field trials indicate that GM insecticidal cottons proposed for release are not toxic to non-target invertebrates and soil microorganisms. However, the applicant would be required to provide information on the effect of VIP3A protein to non-target invertebrates and soil biota under Australian field conditions before any application for a large scale or commercial release of these GM cottons could be evaluated.

**Weediness**

The risk of the COT102, COT200 series and COT102/Liberty® cotton lines establishing as problematic weeds in the southern cotton growing areas of Australia is low and not likely to be greater than that of non-GM cotton. This is largely due to cotton reproducing primarily by self-pollination and not exhibiting seed dormancy. Hence the germination and persistence of cotton volunteers in southern Australia are limited by the availability of adequate soil moisture and nutrients, plant competition and/or frosts. It is highly unlikely that the genetic modifications would affect the response of the GM cottons to these variables and, thereby, alter the weediness of the GM cottons.

Results from the previous field trials of the COT102 and Liberty® cotton lines showed no unintended or secondary effects on the GM cotton plants including agronomic characteristics relating to weediness. As part of the field trial, the applicant proposes to further evaluate agronomic characteristics of these GM cotton lines. However, licence conditions have been imposed to minimise the spread and persistence of GM cottons in the environment.

**Transfer of introduced genes to other organisms**

Some gene transfer from the GM cottons to other cultivated cottons would be likely under uncontrolled conditions, even though the overall frequency of out-crossing would be very low as cotton is primarily self-pollinating. Transfer of introduced genes to other cultivated cotton would pose the same risks as the low risks posed by the COT102, COT200 series and COT102/Liberty® cotton lines themselves. However, licence conditions have been imposed to minimise the risk of transfer of introduced genes to other cotton crops (refer to key licence conditions below).

Transfer of genes to feral/naturalised cotton is unlikely due to geographic isolation and the risk of transferring the introduced genes to native cotton is negligible, because of genetic incompatibility. Similarly, the likelihood of transfer of the introduced genes to other organisms is negligible because of even greater genetic incompatibility. Even if such transfer occurred it would be unlikely to pose any hazard to human health and safety and the environment.

As part of the OGTR’s ongoing commitment to the review of data, specific research conditions have been imposed in the licence. The research is intended to confirm research on gene flow undertaken prior to the implementation of the Act and commercial release of GM cottons and validate the containment measures imposed during the field trials of GM cottons. In order to facilitate this review and data collection, the Regulator has imposed conditions to establish 400 m research zones surrounding the proposed GM cotton trials comprising plantings in excess of one hectare. This research program will be developed in consultation with the OGTR.

**Insecticide and herbicide resistance**

This hazard will be assessed by the APVMA in considering CSIRO’s permit application for the use of the insecticidal gene as an insecticide in the insecticidal GM cotton lines and the use of Liberty®
herbicide on the glufosinate ammonium tolerant cotton lines, respectively. Details of APVMA’s responsibilities are given above.

**Additional data**

The risk assessment identified a range of data requirements that, while not necessary for managing the risks posed by the release, would be required before future applications for significantly larger scale releases of these GM cotton lines or requests for reduced containment conditions could be evaluated. The required data includes information on expression levels of the introduced proteins, molecular characterisation of the inserted genetic material, characterisation of the molecular basis and specificity of the VIP3A protein, and the potential for accumulation and persistence of the introduced proteins in the soil and water.

**THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)**

As part of the evaluation process for this licence application, a risk management plan has been developed to address the risks identified (refer to Conclusions of the risk assessment, above). This plan is given effect by the proposed licence conditions. The key licence conditions are outlined below.

**Toxicity or allergenicity to humans**

Licence conditions have been imposed which require the applicant to:
- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- limit the scale of the release;
- destroy all seed not required for future trials;
- securely transport and store the GMOs; and
- report adverse impacts.

**Toxicity to non-target organisms**

Licence conditions have been imposed which require the applicant to:
- prevent cottonseed being used as stockfeed;
- limit the scale of the release;
- destroy all seed not required for future trials; and
- securely transport and store the GMOs.

**Weediness**

Licence conditions have been imposed which require the applicant to:
- limit the scale of the release;
- surround the GM cotton by a 20 m pollen trap of non-GM cotton;
- surround trial sites in excess of one hectare with a 400 metre wide research zone for the purpose of conducting research for the ongoing review of the data on spread and persistence of the GMOs and to validate previous research on containment measures;
securely transport and store the GMOs;
prevent cottonseed being used as stockfeed;
clean equipment used at release sites; and
monitor release sites after harvest and destroy volunteers.

**Transfer of introduced genes to other organisms**
Licence conditions have been imposed which require the applicant to:
- limit the scale of the release;
- surround the GM cotton by a 20 m pollen trap of non-GM cotton;
- surround trial sites in excess of one hectare with a 400 metre wide research zone for the purpose of conducting research for the ongoing review of the data on spread and persistence of the GMOs and to validate previous research on containment measures;
- securely transport and store the GMOs;
- clean equipment used at release sites; and
- monitor release sites after harvest and destroy volunteers.

**Insecticide and herbicide resistance**
No conditions have been imposed in relation to management of insecticide or herbicide resistance management, as this is the responsibility of the APVMA. If the APVMA determines there is a risk that requires management, the applicant’s obligation to comply with any conditions imposed by the APVMA has been noted in the licence.

**General conditions**
The licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example:
- identification of the persons or classes of person covered by the licence;
- a requirement that the applicant allow access to the release sites by the Regulator, or persons authorised by the Regulator, for the purposes of monitoring or auditing; and
- a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

Chapter 2 of the risk assessment and the risk management plan provides a tabulated summary of assessment conclusions and corresponding management conditions. Full details of the licence conditions are provided in Appendix 7.

**Monitoring and enforcement of compliance by the OGTR**
As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. 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. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all
field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling (which takes into account biological, seasonal, geographical and ecological risk factors), to determine whether licence holders are complying with the licence conditions, or whether there are any unintended effects.

**FURTHER INFORMATION**

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 036/2003).
CHAPTER 1 BACKGROUND

1. This chapter provides background information about the application and previous releases of relevant genetically modified organisms (GMOs) into the environment.

2. The OGTR has received an application (licence application number DIR 036/2003) from CSIRO for the intentional release of genetically modified (GM) insecticidal and insecticidal/herbicide tolerant cottons into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

SECTION 1 THE APPLICATION

Project Title: Breeding and pre-commercial evaluation of transgenic cotton expressing a vegetative insecticidal protein (VIP) gene and a herbicide tolerance gene

Applicant: CSIRO
GPO Box 225
Dickson ACT 2602

Common name of the parent organism: Cotton
Scientific name of the parent organism: Gossypium hirsutum L.

Modified trait(s): Insect resistance, herbicide tolerance, antibiotic resistance

Identity of the gene(s) responsible for the modified trait(s):
• vip3A gene from the bacterium Bacillus thuringiensis (insect resistance)
• bar gene from Streptomyces hygroscopicus (herbicide tolerance)
• hph gene from Escherichia coli (antibiotic resistance)

Proposed Location

Proposed Release Size: 15 sites covering a maximum of 25 hectares in total over 3 years


3. In accordance with Section 185 of the Act, details of the gene construct including the plasmid map and regulatory sequences for the GM insecticidal cotton, transformation event COT102 was declared as Commercial Confidential Information (CCI) under CSIRO’s previous application DIR17/2002. CSIRO has also received approval for details of the gene construct including the plasmid map and regulatory sequences of the GM insecticidal cotton, COT200 series (transformation events COT 202 and 203) as CCI. However, all CCI was made available to the prescribed expert groups which were consulted in the preparation of the risk assessment and risk management plan.
Section 1.1 The proposed dealings

4. CSIRO seeks approval for the limited and controlled release of three types of GM cottons on fifteen sites in cotton growing regions of New South Wales (NSW) and Queensland (Qld) covering a maximum of 25 hectares in total for three seasons (2003 - 2006).

5. The proposed release involves three types of GM cottons carrying the insecticidal transformation events COT102, the COT200 series (transformation events 202 or 203) and COT102 crossed with Liberty<sup>®</sup> cotton which incorporates the herbicide tolerance LL25 transformation event.

6. The aims of the proposed field trial are: to evaluate the agronomic performance of GM cotton lines derived from conventional crosses between elite Australian varieties with lines containing the insecticidal trait (transformation event COT102, COT200 series lines containing transformation events 202 or 203 and lines containing the combined insecticidal and Liberty herbicide tolerant traits (COT102 x LL25). The applicant also proposes to assess efficacy of the insecticidal protein, the combined insecticidal/herbicide tolerance traits, and to produce seed for future possible releases (which would require further licence applications and separate assessment processes).

7. None of the cotton plants from the release, or their by-products, will be used for animal feed or human food. However, the applicant proposes to sell lint from the release. Processed lint does not contain genetic material or protein.

Section 1.2 Parent organism

8. The parent organism is cultivated cotton (Gossypium hirsutum L.), which is exotic to Australia and is grown as an agricultural crop in NSW and Qld and on a trial basis in WA and the NT. More detailed information on cotton can be found in a review document ‘The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document is available at www.ogtr.gov.au.

Section 1.3 Genetic modification and its effect

9. All three types of GM cottons proposed for release contain an insecticidal gene, vip3A, derived from the common soil bacterium Bacillus thuringiensis (Bt). This gene encodes a vegetative insecticidal protein (VIP3A) that is toxic to two important caterpillar pests of cotton, Helicoverpa armigera and H. punctigera. The VIP3A protein is different from the Cry1Ac and Cry2Ab insecticidal proteins, derived from the same bacterium, that are present in other types of GM insecticidal cotton available commercially or currently being trialed in Australia. The applicant expects that the new gene may provide additional options to manage the risk of development of insects resistant to Cry1Ac and Cry2Ab or other insecticidal proteins.

10. One type of insecticidal GM cotton, transformation event COT102, also contains an antibiotic resistance marker gene, hph (also known as aph4) derived from the common gut bacterium, Escherichia coli. This gene encodes a hygromycin phosphotransferase enzyme (HPT) which confers resistance to the antibiotic hygromycin. The marker gene was only used to enable selection...
of plant cells in which the insecticidal gene was also present. The COT102 GM cotton lines have previously been trialed in Australia (See Section 2.1).

11. The second insecticidal GM cotton, the COT200 series GM cotton lines containing events COT202 or COT203, also contain the vip3A insecticidal gene, but under the control of a different plant promoter and they do not contain the antibiotic resistance gene.

12. The third GM cotton was produced by conventional breeding of COT102 cotton with herbicide tolerant GM cotton (Liberty® cotton LL25 event), which contains the herbicide tolerance bar gene (also known as the pat gene). This herbicide tolerant gene is derived from the common soil bacterium Streptomyces hygroscopicus, and encodes the phosphinothricin N-acetyltransferase (PAT) enzyme that detoxifies glufosinate ammonium (the active ingredient of Liberty® and Basta® herbicides), thus rendering the plant tolerant to the herbicide. Hence, this GM cotton contains the insecticidal gene, vip3A, the antibiotic resistance gene, hph, and the herbicide tolerant gene, bar.

13. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in the GM cottons. These are derived from a plant and a common soil bacterium Agrobacterium tumefaciens. Although A. tumefaciens is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not in themselves capable of causing disease.

14. Detailed information on the vip3A, bar and hph genes, the characterisation of the inserted genetic materials, and the new proteins expressed by the GM cotton lines are provided in Appendix 1.

Section 1.4 Method of gene transfer

15. The vip3A, hph and bar genes were introduced into cotton on plasmid vectors carried by Agrobacterium tumefaciens. The vectors were ‘disarmed’ since they lacked the genes that encode the tumour-inducing functions of A. tumefaciens. These genetic modifications generated three types of GM cotton including transformation event COT102 (vip3A and hph genes), COT200 series lines containing transformation event 202 or 203 (vip3A gene only, the hph gene was removed by further breeding and selection of plants containing only the vip3A gene) and the event LL25 (bar gene). These transformation events were transferred into elite Australian cotton cultivars by conventional breeding.

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian Releases

16. Three limited and controlled releases (PR-151, DIR 017/2002 and DIR25/2002) of the COT102 GM insecticidal cotton were approved previously. These field trials were conducted by CSIRO in NSW and Qld and the release size ranged from 0.05 to 3 hectares.

17. Other GM insecticidal cottons (containing cry genes derived from the same bacterium as vip3A gene, but which target different receptors in lepidopterans) have been trialed extensively in Australia (eg. DIRs 005/2001, 006/2001, 008/2001 and 009/2001), as well as commercially released under licences DIR 012/2001 (Bollgard® II cotton) and DIR 022/2002 (INGARD® cotton).
18. Glufosinate ammonium tolerant Liberty® cottons containing the bar gene have also been trialed in Australia, six limited and controlled releases (PR-82, PR-82X, PR-124, PR-124X, PR124X (2) and DIR 015/2002) were previously approved. These trials were conducted in NSW and the size of the releases ranged from 0.04 to 2 hectares.

19. There have been no reports of adverse effects on human health and safety or the environment resulting from any of these trials.

Section 2.2 Approvals by other Australian government agencies

20. The OGTR is responsible for assessing the biosafety risks to human health and the environment associated with development and use of GMOs. Other government regulatory requirements must also be met in respect of the release of the GMOs, and the use of products of the GMOs, including the requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standard Australia New Zealand (FSANZ).

2.2.1 Australian Pesticides and Veterinary Medicines Authority

21. The APVMA has a complimentary regulatory role in respect of this application due to its responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the Agricultural and Veterinary Chemicals (Code) Act 1994. 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.

22. In considering applications for registration or permits, the APVMA considers a number of issues that are outside the scope of the Gene Technology Regulator’s assessment, such as the efficacy of herbicides and insecticides, and herbicide and insect resistance management. The APVMA can impose conditions on the use of herbicides or insecticides in both registrations and permits.

23. CSIRO has submitted an application to the APVMA for a research permit for the use of the insecticidal gene and the use of the herbicide Liberty® (formulation of glufosinate ammonium) on all the types of GM cotton that are for the proposed release. The APVMA and the OGTR work closely together to ensure thorough coordinated assessments are undertaken and, wherever possible, that timing of assessments and decisions by both agencies coincide.

24. The APVMA and the OGTR work closely together to ensure thorough, coordinated assessments are undertaken and, wherever possible, that timing of assessments and decisions by both agencies coincide. Further information about the regulation of insecticides and herbicides and the management of insecticide and herbicide resistance is available from the APVMA:

Australian Pesticides and Veterinary Medicines Authority
PO Box E240
KINGSTON ACT 2604
Phone: (02) 6272 5852
Fax: (02) 6272 4753
Email: contact@apvma.gov.au
http://www.apvma.gov.au
Food Standards Australia New Zealand

25. FSANZ is responsible for human food safety assessment and labelling, including GM foods. Recently, the applicant has applied to FSANZ for approval of material from the COT102 GM cotton lines for use in human food. FSANZ approval would need to be obtained before any of the GM cotton proposed for release under this application could be used in human food. Further information about food safety and food labelling are available from FSANZ:

Food Standards Australia New Zealand
PO Box 7186
Canberra Mail Centre ACT 2610
Phone: (02) 6271 2222
Fax: (02) 6271 2278
E-mail: info@foodstandards.gov.au
http://www.foodstandards.gov.au

Section 2.3 International approvals

26. The GM insecticidal cottons proposed for release have not been commercially released in any other country. Since 2000, the US Department of Agriculture has approved a number of field trials of the same GM cotton events proposed for release in the application.

27. Glufosinate ammonium tolerant cotton (Liberty® cotton), including the LL25 event used as a parent for one of the GM cotton lines, has been field tested since 1997 in the US.

28. Other GM plants containing the bar gene have been field trialed in the US, Canada, Argentina and Japan, and include glufosinate ammonium tolerant maize, sugarbeet, canola, corn, soybean and rice. LibertyLink® crops that are tolerant to glufosinate ammonium such as corn, canola and soybeans have also been commercially released in these countries.

29. No adverse effects on human health or the environment from these releases have been reported.
CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN

30. The Act and the Regulations require that risks associated with dealing with GMOs are identified and assessed as to whether they can be managed to protect human health and safety and the environment (see Appendix 8).

SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

31. Comments received in response to the consultation with expert groups and authorities on the preparation of the risk assessment and risk management plan (RARMP) under by Section 50 of the Act, and with the same stakeholders and the public on the RARMP, under Section 52 of the Act (see Appendix 8), were very important in finalising the plan, which formed the basis of the Regulator’s final decision on the application.

32. Written submissions in relation to DIR 036/2003 received from the agencies and authorities raised the following issues relating to risks to human health and safety or the environment that have been addressed the RARMP:

- the possibility that the GM cotton may have toxic or allergenic properties (Appendix 2 refers);
- the emergence of insects resistant to the insecticidal protein in the GM cotton (Appendix 6 refers);
- the possibility that the GM cotton may be harmful to the environment because of inherent weediness or increased potential for weediness, particularly in favourable habitats such as adjacent to natural or artificial waterways (Appendix 4 refers);
- the extent of cross-pollination from GM cottons to other cotton crops (Appendix 5 refers);
- the possibility that the new genes introduced into the cotton can transfer to other organisms with adverse consequences (Appendix 5 refers);
- measures to limit the unintentional dispersal of GM cottonseed in the environment (Appendices 4, 5 and 7 refer);
- additional data requirements for the future development of the GMOs (Chapter 2 and Appendix 7 refer).

33. In total, the Regulator received three submissions from the public on this application. A summary of these written submissions is provided in Appendix 9. The key issues raised by the public that related to human health and safety or the environment were:

- the potential for increased weediness of the GM cottons (Appendix 4 refers); and
- the management of herbicide and insecticide resistance (Appendix 6 refers).

34. Public submissions also raised issues such as reduced pesticide use, State and Territory Government measures to delay the commercial release of GM crops until uncertainties regarding
market access and segregation issues are resolved, tracking and tracing of GM products, effect on markets, labelling of GM products, and communication of issues relating to GM products, which are outside the scope of evaluations conducted under the Act and therefore have not been considered as a part of the assessment process.

35. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to the protection of human health and safety and the environment in finalising the risk assessment and risk management plan. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

SECTION 2 FINALISATION OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN

36. The Regulator has conducted a risk assessment in relation to the proposed dealings, and prepared a risk management plan in accordance with the Act and the Regulations, using a Risk Analysis Framework as detailed in Appendix 8. The risk assessment process identified a number of hazards that may be posed by the proposed dealings. The risks posed by these hazards were assessed as being either ‘negligible’, ‘very low’, ‘low’, ‘moderate’, ‘high’ or ‘very high’, by considering:

- the likelihood (probability) of the hazard occurring;
- the likely consequences (impact) of the hazard, were it to be realised; and
- risk management options to mitigate any identified risks.

37. The following table (Table 1) lists each of the potential hazards that were considered during the risk assessment process in the Hazard Identification column and summarises the assessment of each hazard under the column headed Risk. A comprehensive assessment of each identified hazard is provided in Appendices 2-6, as cross-referenced in the column headed Summary of Risk Assessment.

38. Where it is considered, on the basis of a combination of possible adverse impacts and likelihood and occurrence, that risk management may be required to protect the health and safety of humans and/or the environment, the Risk Management column identifies the methods selected to limit the potential for risk exposure and the reasons they were chosen. The risk management plan for the proposed dealing has been given effect by specific conditions within the licence. These conditions are summarised in the final column, headed Licence Conditions, and detailed in Appendix 7.

39. As outlined in Section 4, the licence also imposes research conditions, some of which are intended to confirm the results of previous work. In addition, the evaluation process identified a range of research data required for the assessment of future applications.
Table 1  Summary of the risk assessment and the risk management plan (including licence conditions)

<table>
<thead>
<tr>
<th>Hazard Identification</th>
<th>Risk (combines 'likelihood' and 'impact')</th>
<th>Summary of Risk Assessment (refer to appendices for details)</th>
<th>Does risk require management?</th>
<th>Risk Management Method(s) and Reason(s) for selection</th>
<th>Is risk managed?</th>
<th>Licence conditions (See Appendix 7 for detailed licence conditions)</th>
</tr>
</thead>
</table>
| TOXICITY AND ALLERGENICITY FOR HUMANS: Food | Low | See Appendix 2  
• none of the GM material from the release will be used in human food or animal feed;  
• the toxicity of VIP3A protein is specific to lepidopteran caterpillars;  
• humans are commonly exposed to VIP3A, PAT and HPT, as these proteins are naturally widespread in the environment; and  
• FSANZ approval would be required before the GM materials could be used for human food. | Yes | Prevent seed from entering human food supply: prevents exposure through food.  
• Limit scale of release: decreases likelihood of exposure.  
• Destroy all seed not required for further trials: prevents unintended exposure.  
• Ensure secure transport and storage of retained seed: prevents unintended exposure. | Yes | 1. Prevent entry into human food supply: no cotton to enter human food supply.  
2. Limit scale: restrict area to 25 hectares in total at 15 sites over 3 growing seasons.  
3. Destroy seed: destroy all seed not required for future trials.  
4. Secure transport and storage: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within. |
### Summary of Risk Assessment

#### TOXICITY AND ALLERGENICITY FOR HUMANS:
- **Occupational exposure**
  - Low Risk
  - See Appendix 2
  - Cotton pollen is not wind-dispersed and therefore unlikely to be an air-borne allergen;
  - Exposure to the introduced proteins through working with cotton plants is very low;
  - Humans are commonly exposed to VIP3A, PAT and HPT, as these proteins are naturally widespread in the environment;
  - VIP3A, PAT and HPT proteins are not toxic or allergenic to humans; and
  - Although dust and lint from cotton can be created at processing facilities, respiratory irritation can be addressed by the use of protective equipment, and fibre characteristics of the GM cottons are likely to be the same as for non-GM cotton.

#### TOXICITY AND ALLERGENICITY FOR HUMANS:
- **Wearing & using household items containing cotton products**
  - Very Low Risk
  - See Appendix 2
  - Cotton lint used in clothing and household items contains no proteins or DNA and cotton products from GM cottons can not be distinguished from non-GM products.

#### TOXICITY FOR OTHER ORGANISMS:
- **Mammals and wildlife including birds and fish**
  - Low Risk
  - See Appendix 3
  - The release is small in size and limited in duration in each location;
  - Exposure of livestock and wildlife to these GM cottons is low, and cotton seed will not be used as stock feed;
  - The introduced proteins are already widespread in the environment, through the presence of the bacteria from which they are derived;
  - Preliminary studies indicated that the toxicity of VIP3A protein is highly specific to lepidopteran insect larvae; and
  - Toxicity studies with purified VIP3A indicated that the GM cottons will be no more toxic to mammals, birds or fish.

### Does risk require management?
- Yes

### Risk Management Method(s) and Reason(s) for selection
- Limit scale of release: decreases likelihood of exposure.
- Destroy all seed not required for further trials: prevents unintended exposure.
- Secure transport and storage of retained seed: prevents unintended exposure.

### Is risk managed?
- Yes

### Licence conditions
- 1. Limit scale: restrict area to 25 hectares in total at 15 sites over 3 growing seasons.
- 2. Destroy seed: destroy all seed not required for future trials.
- 3. Secure transport and storage: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.
- 4. Report adverse impacts: any adverse impacts on human health and safety must be reported to the Regulator.
### Chapter 2 - Summary of the Risk Assessment and Risk Management Plan

<table>
<thead>
<tr>
<th>Hazard Identification</th>
<th>Risk (combines 'likelihood' and 'impact')</th>
<th>Summary of Risk Assessment (refer to appendices for details)</th>
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<th>Is risk managed?</th>
<th>Licence conditions (See Appendix 7 for detailed licence conditions)</th>
</tr>
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</table>
| TOXICITY FOR OTHER ORGANISMS: Non-target invertebrates, including soil insects | Low | See Appendix 3  
- the introduced proteins are already widespread in the environment, through the presence of the bacteria from which they are derived;  
- the toxicity of VIP3A protein highly specific to lepidopteran insect larvae;  
- preliminary studies indicate that the introduced proteins are not known to be toxic to any other invertebrates;  
- although the risk is low, further information is required on toxicity for Australian non-target organisms. | Yes | Further research determines potential toxicity to non-target organisms. | Yes | 1. Require further research on the potential toxicity for non-target insects under Australian field conditions. |
| TOXICITY FOR OTHER ORGANISMS: Microbial organisms | Low | See Appendix 3  
- the introduced proteins are already widespread in the environment, through the presence of the bacteria from which they are derived; and  
- although the VIP3A protein is unlikely to have adverse effects on soil microorganisms, more information is required on toxicity for soil organisms. | Yes | Further research determines potential toxicity. | Yes | 1. Require further research on the potential toxicity for soil organisms; and potential for accumulation of VIP3A protein in soil. |
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<tr>
<th>Hazard Identification</th>
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<tbody>
<tr>
<td>WEEDINESS:</td>
<td>Low (Low)</td>
<td>See Appendix 4</td>
<td>Yes</td>
<td>Limit scale of the release: decreases likelihood of escape</td>
<td>Yes</td>
<td>1. Limit scale: restrict area to 25 hectares in total at 15 sites over 3 growing seasons.</td>
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<td></td>
<td>Surround the GM cotton with a pollen trap: minimises spread of the introduced genes beyond the release sites via pollen flow</td>
<td></td>
<td>2. Surround the GM cotton with a pollen trap: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site.</td>
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<td></td>
<td>Ensure secure transport and storage of retained seed: prevents escape of GM plant material outside the release sites.</td>
<td></td>
<td>3. Secure transport and storage: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</td>
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<td>Prevent cottonseed being used as stockfeed: prevents dispersal of cottonseed.</td>
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<td>4. Prevent seed from being used as stockfeed: no cottonseed to be used as stockfeed.</td>
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<td></td>
<td>Cleaning of equipment used at the release sites: prevents escape of GM plant material into the environment outside the release sites.</td>
<td></td>
<td>5. Cleaning of equipment used at the release sites: equipment must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned.</td>
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<td>Destroy any volunteers: prevents persistence.</td>
<td></td>
<td>6. Destroy volunteers: the release site must be monitored after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering.</td>
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<td>Further research informs the ongoing review of the data on spread/persistence of the GMOs in the environment and validates the efficacy of containment measures.</td>
<td></td>
<td>7. Research condition: surround the trial site (in excess of 1 ha) by a 400 m research zone for the purpose of conducting research on spread and persistence of the GMOs.</td>
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<tr>
<td>GENE TRANSFER: Plants</td>
<td>Low</td>
<td>See Appendix 5</td>
<td>Yes</td>
<td>Limit scale of the release decreases potential transfer.</td>
<td>Yes</td>
<td>1. Limit scale: restrict area to 25 hectares in total at 15 sites over 3 growing seasons.</td>
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<td></td>
<td>• cotton is mostly self-pollinated; and</td>
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<td>Surround the GM cotton with a pollen trap: minimises spread of the introduced genes beyond the release sites via pollen flow.</td>
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<td>• although the likelihood of some gene transfer from the GM cotton to cultivated cotton is possible, the risk posed by such gene transfer is low because gene transfer would not pose any risks additional to the low risks posed by the GM cotton itself.</td>
<td></td>
<td>Ensure secure transport and storage of retained seed: prevents escape of GM plant material outside the release sites.</td>
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<td>3. Secure transport and storage: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</td>
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<td>4. Cleaning of equipment used at the release sites: equipment must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned.</td>
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<td>Destroy any volunteers: prevents persistence.</td>
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<td>1. Limit scale: restrict area to 25 hectares in total at 15 sites over 3 growing seasons.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• cotton is mostly self-pollinated; and</td>
<td></td>
<td>Surround the GM cotton with a pollen trap: minimises spread of the introduced genes beyond the release sites via pollen flow.</td>
<td></td>
<td>2. Surround the GM cotton with a pollen trap: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• gene transfer to naturalised (feral) cotton populations is thought to be unlikely because of their geographic isolation from proposed trial areas.</td>
<td></td>
<td>Ensure secure transport and storage of retained seed: prevents escape of GM plant material outside the release sites.</td>
<td></td>
<td>3. Secure transport and storage: the GMOs must not be transported unless contained within a primary, sealed container that is packed into a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cleaning of equipment used at the release sites; prevents escape of</td>
<td></td>
<td>4. Cleaning of equipment used at the release sites: equipment</td>
</tr>
<tr>
<td>Hazard Identification</td>
<td>Risk (combines 'likelihood' and 'impact')</td>
<td>Summary of Risk Assessment (refer to appendices for details)</td>
<td>Does risk require management?</td>
<td>Risk Management Method(s) and Reason(s) for selection</td>
<td>Is risk managed?</td>
<td>Licence conditions (See Appendix 7 for detailed licence conditions)</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>-------------------------------------------------</td>
<td>-----------------------------</td>
<td>----------------------------------------------------------</td>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>GENE TRANSFER: Plants</td>
<td>Negligible</td>
<td>See Appendix 5 • genetic incompatibility and geographical isolation from native populations prevent the production of fertile hybrids.</td>
<td>No</td>
<td>GM plant material into the environment outside the release • Destroy any volunteers: prevents persistence. • Further research informs the ongoing review of the data on gene transfer and validates the efficacy of containment measures.</td>
<td>N/A</td>
<td>must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned. Destroy volunteers: the release site must be monitored after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering. 5. Research condition: surround the trial site (in excess of 1 ha) by a 400 m research zone for the purpose of conducting research on gene transfer.</td>
</tr>
<tr>
<td>GENE TRANSFER: Plants</td>
<td>Negligible</td>
<td>See Appendix 5 • well-established genetic incompatibility prevents successful cross pollination with other plant genera.</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
<td>None required</td>
</tr>
<tr>
<td>GENE TRANSFER: Humans &amp; other animals</td>
<td>Negligible</td>
<td>See Appendix 5 • Products from the GM cottons are not intended for stockfeed or human food, thus the likelihood of gene transfer to animals or humans is negligible; • FSANZ approval would be required before the GM materials could be used for human food; • Limited probability of occurrence. The chance of interaction, uptake and integration of intact plant DNA by other organisms is extremely low, especially if it involves unrelated sequences (non-homologous recombination); and • Natural events of horizontal gene flow from plants to distantly related organisms are extremely rare.</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
<td>None required</td>
</tr>
<tr>
<td>GENE TRANSFER: Microorganisms</td>
<td>Negligible</td>
<td>See Appendix 5 • all of the introduced genes in these GM cottons are already widespread in the environment, and are readily available</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
<td>None required</td>
</tr>
</tbody>
</table>
### Summary of Risk Assessment

#### (bacteria)

- for transfer from these sources via demonstrated natural mechanisms; and
- gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.

<table>
<thead>
<tr>
<th>Hazard Identification</th>
<th>Risk (combines 'likelihood' and 'impact')</th>
<th>Summary of Risk Assessment (refer to appendices for details)</th>
<th>Does risk require management?</th>
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<th>Is risk managed?</th>
<th>Licence conditions (See Appendix 7 for detailed licence conditions)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RESISTANCE:</strong></td>
<td><strong>Insecticide</strong></td>
<td>NA</td>
<td>See Appendix 6</td>
<td>APVMA is responsible for assessing the risk.</td>
<td>APVMA would impose conditions</td>
<td>Not required If approved, licence would note the requirement to adhere to the APVMA conditions, including any insecticide resistance management strategy.</td>
</tr>
<tr>
<td></td>
<td><strong>Herbicide</strong></td>
<td>NA</td>
<td>See Appendix 6</td>
<td>APVMA is responsible for assessing the risk.</td>
<td>APVMA would impose conditions</td>
<td>Not required If approved, licence would note the requirement to adhere to the APVMA conditions, including any herbicide resistance management strategy.</td>
</tr>
</tbody>
</table>

**RESISTANCE:**

- **Insecticide**
  - APVMA is responsible for assessing the risk.
  - APVMA would impose conditions

- **Herbicide**
  - APVMA is responsible for assessing the risk.
  - APVMA would impose conditions

**Licence conditions:**

Not required

If approved, licence would note the requirement to adhere to the APVMA conditions, including any insecticide resistance management strategy.
SECTION 3 DECISION ON THE APPLICATION

40. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 8. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to protect human health and safety and the environment.

41. It is concluded that there are no significant risks to human health and safety or to the Australian environment arising from the proposed release of insecticidal or insecticidal/herbicide tolerant GM cottons that can not be adequately managed. Detailed risk analyses based on the available scientific information are provided in Appendices 2 – 6 in support of this conclusion.

42. Therefore the Regulator has issued licence number DIR 036/2003 in respect of this application.

SECTION 4 RESEARCH REQUIREMENTS

43. As part of the OGTR’s commitment to review of data, research conditions have been imposed for a number of cotton DIR licences. Some of this research is intended to confirm previous research on pollen and gene flow undertaken prior to the implementation of the Gene Technology Act 2000 and the commercial scale release of other insecticidal and herbicide tolerant GM cottons. For the proposed release, the following research is required:

- the potential toxicity of the introduced proteins on non-target organisms, including the persistence of VIP3A protein in the soil;
- the agronomic characteristics indicative of potential weediness of the GM cottons under Australian conditions;
- validation of previous research on the efficacy of the Pollen Trap; and
- validation of previous research on gene transfer from GM cotton to other cotton using the Research Zone.

44. Before any application for a larger scale or commercial release of these insecticidal or insecticidal/herbicide tolerant GM cottons could be evaluated, more detailed information would be required on:

- the levels of expression of the insecticidal and insecticidal/herbicide tolerance genes in different parts of the plant under Australian field conditions;
- genetic segregation, molecular characterisation and stability of the inserted genetic material;
- characterisation of molecular basis and details of specificity of the insecticidal VIP3A protein; and
- the potential for the introduced proteins to accumulate and persist in the soil and water.

45. In addition, the results of research required as condition of licence DIR 025/2002 would also be required by the Regulator.

46. It should be noted that provision of the additional data during the proposed release are not required to ensure the management of risks to human health and safety and the environment from the
proposed release. The risk management measures summarised in Table 1 and given effect by the licence conditions, will achieve this purpose.

47. The use of GM cottons in food would require approval from FSANZ.
APPENDIX 1 INFORMATION ABOUT THE GMO

48. In preparing the risk assessment and risk management plan, the Regulator is required under Section 49 (2) of the Act to consider the properties of the parent organism and the effects of the genetic modification.

49. This Appendix addresses these matters and provides detailed information about the GMOs proposed for release, the parent organism, the genetic modification process, the genes that have been introduced and the new proteins that are expressed in the genetically modified cottons.

50. In accordance with Section 185 of the Act, details of the gene constructs, including the plasmid maps and of some regulatory sequences, have been declared as CCI. However, all CCI was made available to the prescribed expert groups, which were consulted in the preparation of the risk assessment and risk management plan.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMOs

51. Three types of GM cottons, all containing a vegetative insecticidal protein gene (vip3A) derived from the common soil bacterium *Bacillus thuringiensis*, are proposed for release. This gene encodes a protein, VIP3A, that is toxic to certain insects, including lepidopteran caterpillar pests of cotton.

52. One insecticidal GM cotton (transformation event COT102, or COT102 cotton) contains thevip3A gene and an antibiotic resistance selectable marker gene, *hph*, derived from a bacterium *Escherichia coli*. The *hph* gene encodes an enzyme, hygromycin phosphotransferase (HPT), that confers resistance to the antibiotic hygromycin. This GM cotton has previously been trialed in Australia (refer to DIR 017/2002 and DIR 025/2002). COT102 cotton has shown consistent insecticidal protein expression and efficacy both in the laboratory and in the field.

53. It should be noted that this GM cotton was also considered in detail for a limited and controlled release by Syngenta Seeds under application DIR 034/2003, and that a licence for this field trial was issued on the 15 October 2003 (available at www.ogtr.gov.au).

54. The other type of GM cotton (COT200 series cotton lines containing transformation event COT202 or COT203) contains thevip3A gene under a control of a different plant gene promoter to that in COT102 cotton (see Section 3.1) and does not contain the antibiotic resistance *hph* gene.

55. A third GM cotton was produced by the conventional breeding of a COT102 cotton with a herbicide tolerant GM cotton (Liberty® cotton, transformation event LL25) which contains the *bar* gene. The *bar* gene is derived from the common soil bacterium *Streptomyces hygroscopicus*, and confers tolerance to the herbicide glufosinate ammonium (the active ingredient of Liberty® and Basta® herbicides).

56. GM Liberty® cotton has previously received approval for limited and controlled release in Australia and was considered in detail in the risk assessment and risk management plan for DIR 015/2002, available at www.ogtr.gov.au. A further application for the limited and controlled release of GM Liberty® cotton containing the *bar* gene is being considered separately under application DIR 038/2003.
57. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in the GM cottons. These are derived from a plant and from a common soil bacterium, Agrobacterium tumefaciens. Although A. tumefaciens is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not in themselves capable of causing disease.

58. Each of these GM events, generated in a cotton variety not suited to Australian conditions, were introduced into elite Australian cotton cultivars by conventional breeding.

SECTION 2 THE PARENT ORGANISM

59. A comprehensive review of the parent organism, Gossypium hirsutum L. (cultivated cotton), is provided in the document ‘The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au.

SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

Section 3.1 The insecticidal gene (vip3A)

60. The insecticidal vip3A gene is derived from a common soil the bacteria, Bacillus thuringiensis, variety kurstaki. The vip3A gene was modified for optimum expression in cotton cells, using protocols similar to those described by Perlak et al. (1991). Like the cry genes in other GM insecticidal cottons (INGARD® and Bollgard® II), the vip3A gene encodes a protein with specific toxicity to some lepidopteran insects, including Helicoverpa armigera and H. punctigera, pests that attack cotton in Australia. Preliminary bioassay data indicates that GM cotton expressing VIP3A protein has more potent insecticidal activity towards H. armigera than seen with INGARD® cotton (information provided by CSIRO).

61. In COT102 and COT200 series cottons, expression of the vip3A gene is controlled by different plant derived promoters. Under section 185 of the Act, the details of the origin and construction of the promoters in these GM cottons has been declared as CCI.

62. These promoters enable the bacterial gene to be expressed in the cotton plants and are expected to lead to expression of the VIP3A protein throughout the growing season and in most of the tissues of the GM cottons. Also required for gene expression in plants is a 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).

Section 3.2 The insecticidal protein (VIP3A)

63. B. thuringiensis produces a range of insecticidal proteins, each with specific toxicity to certain groups of insects. The biological role of the insecticidal proteins is unclear, but they clearly enhance the ability of these free-living bacteria to immobilise and colonise a ready source of nutrients in the insects they kill (information provided by CSIRO). A number of the crystal (Cry) insecticidal proteins are already expressed in genetically modified crop plants to protect them against agricultural pests.

64. The vip3A gene encodes a protein, VIP3A, that is secreted into the extracellular environment by B. thuringiensis during vegetative growth, and expression continues during the stationary (sporulation)
phase (Donovan et al. 2001). The detection of the VIP3A protein in growth stages before sporulation establishes a clear distinction from other described insecticidal proteins of *B. thuringiensis* that belong to the \( \delta \)-endotoxin family, such as the Cry proteins (Estruch et al. 1996).

65. The VIP3A protein shows no amino acid homology to the Cry1Ac and Cry2Ab proteins that are produced in INGARD\textsuperscript{®} and/or Bollgard\textsuperscript{®} II cotton. The VIP3A protein binds to specific receptors inside the insect gut (different to those bound by Cry1A proteins). Once bound, the protein inserts into the membrane and forms ion-specific pores, which result in disrupted digestion and subsequent death of the insect (EPA 2000). If an organism does not have the specific receptors that enable binding of the VIP3A protein, the organism (e.g., humans, other mammals, birds, fish and non-target insects) will not be susceptible to the toxic effects of the protein.

66. More detailed information on the mode of action of VIP3A insecticidal protein would be required if these GM cottons are proposed for future large scale or commercial releases (which would require further applications and assessments).

### Section 3.3 The herbicide tolerance gene (*bar*)

67. The *bar* gene in Liberty\textsuperscript{®} cotton, derived from the common aerobic soil bacterium (actinomycete) *Streptomyces hygroscopicus*, encodes the PAT enzyme (Thompson et al. 1987). This enzyme chemically modifies glufosinate ammonium (the active ingredient of Liberty\textsuperscript{®} and Basta\textsuperscript{®} herbicides) into an inactive form, thus conferring tolerance to glufosinate ammonium, both in tissue culture (used for selection of genetically modified cotton cells in the laboratory during development of the GM cotton) and when applied to plants in the field.

68. The promoter controlling expression of the *bar* gene in the GM cottons is the 35S promoter derived from cauliflower mosaic virus (Odell et al. 1985). The nature of this promoter suggests that the PAT protein will be expressed throughout the growing season and in most tissues of the GM cotton. The termination region is derived from the 3’end of the *nos* gene of *A. tumefaciens* (Depicker et al. 1982).

69. More information on the *bar* gene, including the properties and the mode of action of the PAT protein encoded by the gene, can be found in the risk assessment and risk management plan prepared previously for Liberty\textsuperscript{®} GM cotton (DIR 015/2002) and herbicide-tolerant canola (DIR 010/2002 and DIR 021/2002, available at [www.ogtr.gov.au](http://www.ogtr.gov.au)).

### Section 3.4 The antibiotic resistance marker gene (*hph*)

70. The COT102 insecticidal cotton also contains the *hph* antibiotic resistance marker gene from *Escherichia coli*. The *hph* gene, which encodes a hygromycin phosphotransferase (HPT) 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. It is in common use as a selectable marker in the production of GM plants. The only modification made to the bacterial *hph* gene was the addition of plant regulatory sequences (promoter and termination regions) to allow expression in plant cells.

71. Like the *vip3A* gene, expression of the *hph* gene is controlled by a different plant derived promoter. Under section 185 of the Act, the details of the origin and identity of the promoter has been
declared as CCI. The termination region is derived from the 3’end of the nos gene of A. tumefaciens (Depicker et al. 1982).

**SECTION 4  METHOD OF GENE TRANSFER**

72. The cotton transformation events COT102, COT202, COT203 and LL25 were each generated by Agrobacterium-mediated transformation using protocols similar to those described by Murray et al (1999).

73. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Agrobacteria are the only prokaryotic organisms known to be capable of transferring DNA to eukaryotic cells (Bundock & Hooykaas 1998). *Agrobacterium*’s gene transfer ability evolved from bacterial conjugal transfer systems, which mobilise plasmids for transfer between bacterial cells. Normally when using Agrobacterium vectors, only the T-DNA is transferred and integrated into the plant genome (de la Riva et al. 1998), although flanking vector sequences can also be transferred. The transfer of the T-DNA (located between specific border sequences on a plasmid) from *A. tumefaciens* occurs through the mediation of the genes from the *vir* (virulence) 7 region of the Ti (tumour inducing) plasmids. It is generally accepted that T-DNA transfer into plant cells by *Agrobacterium* is irreversible (Huttner et al. 1992) and cannot be re-mobilised to transfer elsewhere within the genome or to other organisms.

74. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes (*iaaM, iaaH* and *ipt*) responsible for the overproduction of auxin and cytokinin, which are required for tumour induction (Klee & Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* region to act in either *cis* or *trans* configurations to the T-DNA. This has allowed the development of two types of transformation systems:

- co-integration vectors that join the T-DNA that is to be inserted into the plant and the *vir* region in a single plasmid (Stachel & Nester 1986); and
- binary vectors that have the T-DNA and *vir* regions segregated on two plasmids (Bevan 1984).

75. Both provide functionally equivalent transformation systems. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing any biosafety problems.

76. In the COT102 event, the conventional, disarmed binary vector PCOT1 was used to introduce the insecticidal gene, *vip3A*, and antibiotic resistant marker gene, *hph*, using standard *Agrobacterium* transformation protocols. Details of the plasmid map have been declared as CCI. GM cotton cells were selected by their ability to grow in the presence of the antibiotic hygromycin sulphate, and GM cotton plants were regenerated from these cells.

77. In generation of the COT200 series GM lines, the insecticidal and antibiotic resistance genes were contained within the same vector, pNOV103, but in separate T-DNAs, such that the genes inserted independently of each other at different sites within the cotton genome. As above, GM cotton cells were selected in the presence of hygromycin and GM plants regenerated from these cells. Subsequent plant breeding lead to segregation (separate inheritance) of the two introduced genes.
Insecticidal plants without antibiotic resistance (ie. without the \textit{hph} gene) were chosen for further work.

78. Liberty\textsuperscript{\textregistered} cotton (GM event LL25) was also generated by \textit{Agrobacterium}-mediated transformation, using the plasmid vector pGSV71 which carried the bar gene within the T-DNA. GM cotton cells were selected using glufosinate ammonium. Liberty\textsuperscript{\textregistered} cotton itself is not proposed for release in this application. This GM cotton was conventionally bred with COT102 cotton to combine the insecticidal and herbicide tolerance traits. The resulting insecticidal/herbicide tolerant (COT102/LL25) cotton is proposed for release.

\textbf{SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION}

79. A single copy of each gene, \textit{vip3A} and \textit{hph}, are present in the cotton genome in the COT102 GM event, as determined by Southern blot analysis. The applicant states that detailed molecular characterisation of the inserted genetic material will be completed if this GMO exhibits commercial potential. The \textit{vip3A} and \textit{hph} genes have been inherited as tightly linked dominant Mendelian traits over five generations. The CSIRO reports that expression of the inserted \textit{vip3A} gene in the GM cotton has remained stable.

80. In each of the COT200 series GM cotton lines, Southern blot analyses has demonstrated a single copy of \textit{vip3A} gene within the cotton genome and shown stable inheritance over three generations. The CSIRO proposes to gather more data on segregation during the field trials. The detailed molecular characterisation of the inserted genetic material will be completed if any of the COT200 series lines exhibit commercial potential.

81. Southern blot analysis of GM Liberty\textsuperscript{\textregistered} cotton, event LL25, demonstrates the presence of a single copy of the bar gene within an intact inserted T-DNA. Preliminary field assessment of Australian cotton cultivars containing LL25, sprayed with a discriminating dose of glufosinate ammonium herbicide, has shown that the \textit{bar} gene is functional and has been stably inherited as a dominant Mendelian trait for 5 generations of crossing and back-crossing through the breeding program.

82. Southern blotting and highly sensitive polymerase chain reaction (PCR) techniques have been used to determine that only the T-DNA of the \textit{Agrobacterium} plasmid vector is present in these GM cottons.

83. Further characterisation of the inserted genetic materials will be required to confirm this if these GM cottons are proposed for future large scale or commercial releases (which would require further applications and assessments).

\textbf{SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS}

84. It is expected that the introduced proteins will be expressed in most tissues throughout the life of the plants in the COT102 cotton and COT200 series cotton lines, because of the nature of the promoters that control expression of the introduced genes (see Section 3.1 above).
85. Quantitative analysis of the VIP3A insecticidal protein in the COT102 cotton indicates relatively low expression levels, with 309 ng/mg soluble protein in leaf (0.03%), 105 ng/mg in flower buds, 99 ng/mg in the cotton boll, 83 ng/mg in immature fibre (lint) and 69 ng/mg in seed.

86. In a field survey conducted as part of DIR 017/2002, the VIP3A insecticidal protein in COT102 plants showed high and consistent efficacy against *Helicoverpa spp.* with no deleterious effects on non-target insect numbers.

87. Furthermore, expression of the HPT protein was either not detectable in most COT102 plants or the levels were too low to quantify (EPA 2001).

88. The applicant states that the evaluation of VIP3A protein expression in the COT200 series lines is currently being carried out and the preliminary data indicates expression to be twice as high as in COT102 cotton.

89. Preliminary data for Liberty® cotton indicates that the PAT protein is expressed at about 1% of total extractable protein in cotton leaves. The combination of the Liberty® LL25 and COT102 cotton GM events is not expected to influence PAT or VIP3A protein expression.

90. More detailed information on the levels of expression of the introduced proteins, VIP3A, HPT and PAT, under Australian conditions, as well as the potential for interaction of introduced genes in the GM insecticidal/herbicide tolerant cotton, would be required if these GM cottons were proposed for future large scale or commercial releases (which would require further applications and assessments).

SECTION 7 PLEIOTROPIC EFFECTS OF THE GENETIC MODIFICATION

91. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. A single gene inserted into a plant by genetic modification can also be pleiotropic and it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in agronomic characteristics. Preliminary data indicate that no pleiotropic effects have occurred in these GM cottons.

92. COT102 cotton has been field tested in the US between 2000 and 2002, displaying agronomic characteristics within the normal range for the parental cotton variety. Similarly, trials of the COT102 cotton in Australia (PR151 and DIR 017/2002) have not indicated any secondary, pleiotropic effects.

93. The applicant also states that preliminary field trials of COT200 series cotton lines in the US indicate that their performance is within the normal range for the parental cotton variety.

94. Glass house and field trials of the LL25 event herbicide tolerant cotton, both in Australia (eg. PR124X(2) and DIR 015/2002) and in the US, have shown no alteration in characteristics such as morphology, seed set, seedling vigour or fibre yield compared to the parental variety.

95. The applicant proposes to conduct further agronomic characterisation of the COT102 cotton, COT200 series cotton lines and COT102/LL25 cotton under Australian field conditions as part of the proposed trials.
APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS

96. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. This Appendix considers potential hazards that may be posed to human health and safety as a consequence of any toxicity or allergenicity of the GMOs or their novel proteins.

97. It should be noted that one of the GM cottons proposed for release (GM insecticidal cotton event COT102) has been trialed under Australian field conditions since 2001 (DIR 017 and DIR 025) with no reported adverse effects to humans as a result of occupational exposure to these GM cottons. The same GMO was evaluated for a limited and controlled release under DIR 034/2003 (refer to RARMP available at www.ogtr.gov.au). In addition, one of the parent GMOs, Liberty® cotton is also being evaluated separately under application DIR 038/2003.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

98. Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000). 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 1986; FAO & WHO 2000). Allergy has a well-defined etiology (i.e biochemical causes) that is quite different from toxicity.

99. The GM cottons differ from conventional cotton in the expression of up to three additional proteins. The COT102 GM event cotton expresses the VIP3A insecticidal toxin as well as the antibiotic resistance HPT enzyme. The COT200 series GM cottons express the VIP3A toxin. The third GM cotton expresses the VIP3A insecticidal toxin, the HPT enzyme and the PAT protein, which confers tolerance to the herbicide glufosinate ammonium (see Appendix 1 for details of protein expression in the GMOs). The potential for these GM cottons to be toxic or allergenic to humans has been considered in detail in this Appendix. This could occur if the GM cottons were toxic or allergenic because of the novel gene products expressed in the plants or because of unintended effects of the genetic modification.

100. The APVMA has responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the Agricultural and Veterinary Chemicals (Code) Act 1994 (Refer to Chapter 1 for details). As part of their assessment of chemical use, the APVMA considers any potential human health effects, for example risks arising through occupational exposure or residues in food. Thus risks associated with the use of the insecticide or herbicide are not considered in the risk assessment of these GM cotton lines.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

101. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM insecticidal and insecticidal/herbicide tolerant cottons on human health and safety, the following factors were considered:
the inherent toxicity and allergenicity of conventionally bred cotton;

- the potential routes of exposure to these GM cottons, to their products and to the new proteins which are expressed in the cottons, the VIP3A, PAT and HPT proteins;

- the potential exposure to the VIP3A, PAT and HPT proteins from other sources in the environment;

- the potential toxicity and allergenicity of the new proteins expressed in the GM cottons; and

- the potential toxicity and allergenicity of the GM cottons.

Section 2.1 Toxicity and allergenicity of conventionally bred cotton

102. Cotton is a well-established field crop with a long history of safe use. A comprehensive review of conventional cotton, including information on its toxicity and allergenicity, is provided in the document ‘The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at www.ogtr.gov.au. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cottons being considered in this risk assessment.

103. Tissue from conventional cotton, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids (eg. dihydrosterculic, sterculic and malvalic acids).

104. Processed cotton fibre contains 99.8% cellulose and is widely used in pharmaceutical and medical applications because of its very low allergenicity. Cottonseed oil has been in common use since the middle of the nineteenth century and achieved GRAS status (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958 (ANZFA 2002).

105. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), and 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.

Section 2.2 Exposure of people to GM cottons

106. The applicant proposes to destroy all cottonseed produced in the release not required for use in further breeding and field trials. Since it is not intended that any cottonseed produced in the proposed release would be used in human food and animal feed, there will be no opportunity for human exposure to these GM cottons through food. The applicant proposes to sell the lint from the GM cottons and the surrounding non-GM pollen-trap plants from the release for use in fabric, upholstery and other non-food products. Cotton linters will be destroyed along with cottonseed after ginning. As a result of the scale and the scope of the release there is limited exposure of people to the GM cottons. In general, the potential routes of exposure of humans to these GM cottons are through:

- working with GM cottons (on cotton farms, in cotton processing facilities);
living in or near the areas where GM cottons are grown (general environmental exposure, eg. people breathing cotton pollen); and
- wearing cotton clothing or using household items made from cotton lint.

107. As there will be no opportunity for humans to consume food products from this proposed release of GM cottons, hazards to humans through food do not warrant detailed discussion here. It should be noted that FSANZ is evaluating an application for approval for food use of oil and linters derived from VIP insecticidal cotton containing the COT102 event.

2.2.1 Exposure to GM cottons through working with cotton and living near cotton plantations

108. Humans 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. Exposure to proteins (including the new proteins expressed in the GM cottons) or to other cellular components of the cotton plants will only occur if plant cells are ruptured. Even if the cells rupture, exposure to the new proteins expressed in GM cottons will be very low, as these proteins are only present at low levels in the GM cotton tissues (see Appendix 1 for details).

109. Three limited and controlled releases of the same GM insecticidal cotton (PR-151, DIR 017/2002 and DIR 025/2002) and six limited and controlled releases of Liberty® cotton (PR-82, PR-82X, PR-124, PR-124X, PR-124X(2) and DIR15/2002) have previously been undertaken in Australia with no adverse impacts on human health and safety reported.

110. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore limiting possible exposure to cotton pollen as a potential airborne allergen. The introduced proteins are expressed at low levels in the GM cotton tissues (see Appendix 1 for details).

111. The primary processing of cotton at cotton gins, and the bulk handling of cottonseed 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. Therefore, GM cotton lint is no more likely to induce adverse responses in workers than is lint from non-GM cotton.

2.2.2 Exposure to cotton products through wearing clothing and using household products made from cotton lint

112. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from the cotton lint (long fibres) which surrounds the cottonseed. Household products that may contain cotton linters include 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 and variety of plastics (Gregory et al. 1999). Cotton fibre is widely used in pharmaceutical and medical applications because of its very low allergenicity.

113. Processed cotton lint contains no detectable DNA or protein (Leffler & Tubertini 1976; Sims & Berberich 1996). Therefore the safety of wearing cotton clothing or using other products made from cotton is not likely to be affected by the genetic make-up of the cotton plants from which lint has been derived, that is, whether from GM cottons or from non-GM cotton.
Section 2.3 Exposure to the introduced proteins from other sources in the environment

114. The introduced proteins VIP3A, PAT and HPT proteins are widespread in the environment. The genes for these proteins have been derived from common soil and gut bacteria, and hence the VIP3A, PAT and HPT proteins are already a natural component of the soil.

115. The VIP3A protein is derived from the common soil bacterium *Bacillus thuringiensis* var *kurstaki* (Btk). It is produced during vegetative growth, and its expression continues into sporulation (Rice 1999; Donovan et al. 2001). Commercial Btk microbial formulations (produced by the fermentation of the same strain of bacterium from which the gene was derived) also contain this protein, and have been used over the past 30 years to protect food crops, including certified organic crops from insect attack. Bt proteins including VIP3A are found widely in both agricultural and natural environments, including soils, on plant leaves, in grain stores, in dead insects (Meadows 1993) and in a variety of fresh foods (such as lettuce and tomato) with no reported toxic or allergenic responses (ANZFA 1999).

116. The HPT protein is found commonly in bacteria isolated from animal digestive systems and soil. It is therefore already present in the environment and in food chains. The protein is not known to be toxic to vertebrate animals.

117. *Streptomyces hygroscopicus*, the source of the bacterial *bar* gene, which produces the PAT protein. It is a common soil bacterium, and therefore the PAT protein is already present in the environment. CSIRO states that the amount of PAT protein added to the soil from the GM cotton is likely to very small compared to the amount already present in the soil derived from microorganisms.

Section 2.4 Toxicity and allergenicity of the introduced proteins

118. Proteins when toxic, are known to act via acute mechanisms and at a very low dose levels (Sjoblad et al. 1992). Acute oral toxicity studies in animals can provide evidence about the toxicity of compounds.

119. Allergens usually share a number of characteristics:
- they are proteins ranging between 15-70kD in molecular weight;
- they are typically glycosylated and stable in the mammalian digestive system;
- they are stable at high temperatures (involved in cooking or processing) and are present as a major protein component in specific food (Taylor 1995; Astwood & Fuchs 1996; Astwood et al. 1996; Metcalfe et al. 1996; ANZFA 2001b).

120. There will be no opportunity for people to consume food products (oil and linters) from the GM cottons proposed for release, nor will cottonseed be fed to cattle.

121. One of the introduced proteins, PAT, in the GM cotton line (transformation event COT102 bred with LL25) is the same as that present in the glufosinate ammonium tolerant InVigor® hybrid canola, approved for commercial release (DIR 021/2002). Additionally, the herbicide tolerant GM cotton containing only the LL25 GM event is being evaluated separately under application DIR 038/2003. The potential toxicity and allergenicity of the VIP3A protein is also addressed in previous RARMPs for DIR017/2002, DIR025/2002 and DIR 034/2003. These documents are available on the OGTR website at [www.ogtr.gov.au](http://www.ogtr.gov.au) and are only presented here in summary. Similarly the
toxicity and allergenicity of the PAT protein is discussed in detail in the RARMPs for DIR010/2001 and DIR 021/2002.

### 2.4.1 The VIP3A protein

122. The *vip3A* gene from the bacterium *Bacillus thuringiensis*, codes for the VIP3A protein that is toxic to certain lepidopteran pests of cotton. Extensive testing has established that VIP3A is specific in its activity, and has demonstrated toxicity only to the larvae of certain lepidopteran species, including key pests of cotton. The protein must be ingested to be active and it binds to specific receptors inside the insect gut. This forms ion-specific pores which result in disrupted digestion and subsequent insect death (Lee M.K. et al. 2003).

123. Neither the GMO or any of its products from this trial will be used in human food or animal feed. However, a series of tests designed to evaluate the VIP3A protein for characteristics associated with food allergens and toxins have been done. Extensive animal testing has shown that the VIP3A protein is non-toxic to humans and animals. Detailed consideration of results of these studies are available in the risk assessment and risk management plan that was prepared for GM VIP insecticidal cotton, DIR 034/2003, available at [www.ogtr.gov.au](http://www.ogtr.gov.au).

124. In conclusion it is considered that the VIP3A protein is not toxic or allergenic to humans.

### 2.4.2 The PAT protein

125. The *bar* gene from the bacterium *Streptomyces hygroscopicus*, codes for the enzyme phosphinothricin acetyl transferase (PAT), which confers tolerance to the herbicide phosphinothricin (glufosinate ammonium). *Streptomyces hygroscopicus* is a common soil bacterium that may also exist in water and does not possess any pathogenic properties to humans and animals.

126. Neither the GMOs nor any of their products from this trial will be used in human foods or animal stockfeed.

127. Toxicity and allergenicity studies using the purified form of the introduced PAT protein have been conducted. Detailed descriptions of the results of these studies are presented in the risk assessment and risk management plan for DIR 021/2002, glufosinate ammonium tolerant GM canola, available at [www.ogtr.gov.au](http://www.ogtr.gov.au). The key results are summarised here.

128. The PAT protein is not a known allergen and it is not derived from a known source of allergens. Its molecular weight is 22-23kD, within the range of molecular weights usually shown by allergens. However the PAT protein lacks glycosylation sites which are common to plant food allergens (Bremmer & Leist 1996; EPA 1997b). It shares no similarities with known toxins or allergens present in the public sequence or protein databases.

129. A series of tests designed to evaluate the PAT protein for characteristics associated with food allergens and toxins have been done. Extensive animal testing has shown that the PAT protein is non-toxic to humans and animals. The enzymatic properties are within the range of biological function and are highly substrate specific for the herbicide glufosinate ammonium. The same gene has been assessed in other GM crops, including InVigor® canola and is considered to pose no risks to human health and safety (ANZFA 2001a)).
130. Wehrmann et al (Wehrmann et al. 1996) reported that when the PAT protein was subjected to simulated gastric conditions, the protein was degraded within seconds. The PAT enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions (European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b). Other studies have determined that the PAT enzyme is heat labile and completely inactivated by temperatures above 75°C (EPA 1997a).

131. In conclusion it is considered the PAT protein is not toxic or allergenic to humans.

2.4.3 The HPT protein

132. The antibiotic resistance HPT protein is produced naturally by strains of the bacterium *Escherichia coli*, which are widespread in the environment and in human and animal digestive systems. The HPT protein shows no amino acid sequence homology to known allergens. The HPT protein is not derived from a source known to produce allergens nor is it targeted to a cellular pathway for glycosylation in the plant. Furthermore, it is rapidly degraded upon exposure to simulated gastric fluid.

133. Currently, hygromycin is not in clinical use and therefore the likelihood of inactivation of an oral dose of hygromycin in humans, by consuming plant material containing the HPT protein is negligible. As noted previously, the materials from the proposed release are not intended for human or animal consumption.

134. In conclusion it is considered that the HPT protein is not toxic or allergenic to humans.

Section 2.5 Toxicity and allergenicity assessment of GM insecticidal and insecticidal/herbicide tolerant cottons

135. The *vip3A* gene expressed in the GM insecticidal cotton is derived from a common soil bacterium *Bacillus thuringiensis*. The *vip3A* protein is present at low levels in the tissues of all GM insecticidal cotton and represents only around 1% of the total extractable protein in the leaves. Expression data on VIP3A protein showed that the stems and leaves of GM insecticidal cotton expressed the highest amounts of the novel protein when compared to the roots and pollen.

136. The *bar* gene expressed in the Liberty® cotton is derived from a common aerobic soil actinomycete, *Streptomyces hygroscopicus*, which is a soil microorganism not implicated in disease. The PAT protein is present at low levels in Liberty® cotton tissues and represents only around 1% of the total extractable protein in the leaves. The USEPA concludes that toxicity data support the prediction that the PAT protein would be non-toxic to humans. Other Governmental regulatory authorities in Canada, Japan and European Union have also made decisions that the presence of the PAT protein in plants does not render them unsafe.

137. The HPT protein is produced naturally by strains of the bacterium *Escherichia coli*, which are widespread in the environment and in human and animal digestive systems. The *hph* gene encodes a protein that confers resistance to the aminoglycoside antibiotic hygromycin. It is in common use as a selectable marker in the production of GM plants. The only modification made to the *hph* gene was the addition of plant regulatory sequences to allow expression in plant cells.
138. As detailed in Section 2.4, examination of the nature of the VIP3A, PAT and HPT proteins raise no safety concerns to date. The enzymatic properties are within the range of biological function and are highly specific. A battery of tests designed to evaluate the introduced proteins for characteristics associated with food allergens and toxins raises no concern. The introduced proteins share no sequence homology with known allergens and toxins and are not stable in digestive environments.

139. The combination of insecticidal/herbicide tolerant traits are not likely to increase the risks of toxicity or allergenicity of the GM cottons because the herbicide tolerance gene operates through independent and unrelated biochemical mechanisms. However further data would be required to evaluate future applications for a larger scale or commercial releases of the same GM cottons.

SECTION 3 CONCLUSIONS REGARDING TOXICITY AND ALLERGENICITY

140. It is considered that the risk of the GM cottons being toxic or allergenic to humans from the proposed small scale release is low because:

- the VIP3A, PAT and HPT proteins are not toxic or allergenic to humans;
- humans are commonly exposed to PAT protein as these proteins are naturally ubiquitous in the environment;
- none of the GM cotton material from the release will be used for human or animal food;
- like non-GM cotton, the GM cotton pollen is not wind-dispersed and is unlikely to be an air-borne allergen;
- dust and lint from cotton can be created at processing facilities, the use of personal protective equipment prevents respiratory irritation; and
- processed cotton lint used in clothing and household items contains no proteins or DNA.

141. As a condition of the licence, the licence holder must report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the cotton) or to the environment.
APPENDIX 3 TOXICITY TO NON-TARGET ORGANISMS

142. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and risk management plan. This Appendix considers potential hazards that may be posed through any potential toxicity of the GMO or its novel proteins to non-target organisms.

143. It should be noted that one of the GM cottons proposed for release (GM insecticidal cotton event COT102) has been trialed under Australian field conditions since 2001 (DIR 017 and DIR 025) with no reported adverse effects on non-target organisms. The same GMO was evaluated for a limited and controlled release under DIR 034/2003 (refer to RARMP available at [http://www.ogtr.gov.au](http://www.ogtr.gov.au)). In addition, one of the parent GMOs, Liberty® cotton is also being evaluated separately under application DIR 038/2003.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

144. The GM cottons differ from conventional cotton in the expression of up to three additional proteins. The COT102 GM event cotton expresses the VIP3A insecticidal toxin as well as the antibiotic resistance HPT enzyme. The COT200 series GM event cotton expresses the VIP3A toxin. The third cotton line expresses the VIP3A insecticidal toxin, the HPT enzyme and the PAT protein, which confers tolerance to the herbicide glufosinate ammonium (see Appendix 1 for details of protein expression in the GMOs). The potential for these cottons to be toxic to organisms, other than the target pests of cotton (lepidopteran caterpillars) is considered in detail in this Appendix. This could occur if the GM cottons were toxic because of the novel gene products expressed in the plants or because of unintended effects of the genetic modifications.

145. If the GM cottons are toxic to non-target organisms, the potential hazards could include adverse impacts on:

- wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (pollinators, parasitoids or predators of insect pests); and
- microbial organisms, particularly soil microorganisms.

146. Toxicity for the lepidopteran target organisms may also present indirect hazards with potential to harm the natural environment (for example, adverse impacts on native biodiversity), through:

- secondary effects on populations of specialist parasitoids and predators that feed on lepidopteran insects; and
- secondary effects on populations of organisms that are preyed on by non-target lepidopteran insects.

147. As discussed in Chapter 1, the APVMA has responsibility for agricultural chemical use, including insecticides and herbicides in Australia under the *Agricultural and Veterinary Chemicals (Code) Act* 1994. As part of their assessment of chemical use, the APVMA considers any potential environmental effects, such as toxicity to non-target organisms and herbicide residues in the environment. Thus risks associated with the use of the insecticide or herbicide are not considered in the risk assessment of these GM cotton lines.
SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

148. In assessing the likelihood of adverse impacts on non-target organisms due to toxicity of the GM insecticidal and insecticidal/herbicide tolerant cotton, a number of factors were considered, including:

- the inherent toxicity of conventionally bred cotton (OGTR 2002);
- the potential exposure to the introduced proteins from other sources in the environment;
- information about the likely routes of exposure to GM cottons and to the introduced proteins, through direct contact with the crop or through contact with soil in which the crop is grown; and
- the potential toxicity of the new proteins expressed in the cotton for particular species, including mammals, fish and birds, non-target invertebrates and soil microorganisms.

149. The potential toxicity of the VIP3A protein is also addressed in previous RARMPs for DIR017/2002, DIR025/2002 and DIR 034/2003. These documents are available on the OGTR website at www.ogtr.gov.au and are only presented here in summary. Similarly the toxicity and allergenicity of the PAT protein is discussed in detail in the RARMPs for DIR010/2001 and DIR 021/2002.

Section 2.1 Toxicity of conventionally bred cotton

150. Cotton is a well established field crop with a long history of safe use. A comprehensive review of conventional cotton, including information on its toxicity and allergenicity, is provided in the document ‘The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at http://www.ogtr.gov.au/. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cottons being considered in this risk assessment.

151. Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids (eg. dihydrostercolic, stercolic and malvalic acids).

152. Mammals avoid feeding on cotton plants due to both the gossypol content and the morphology of the plant. The presence of gossypol and cyclopropenoid fatty acids in cottonseed limits the use of whole cottonseed 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 catfish, poultry and swine. The meal and hulls of cottonseed 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.

153. Best Management Practices for the Australian cotton industry prohibits the use of cotton trash and stubble as a feed for animals, due to residues of other pesticides that could be found in the cotton trash and stubble.
Section 2.2  Other sources of the introduced proteins in the environment

154. As discussed in Appendix 2, the VIP3A, PAT and HPT proteins are widespread in the environment. The genes for these proteins have been derived from common soil and gut bacteria, and hence the VIP3A, PAT and HPT proteins are already a natural component of the soil.

155. The VIP3A protein is derived from the common soil bacterium *Bacillus thuringiensis* var *kurstaki* (Btk). It is produced during vegetative growth, and its expression continues into sporulation (Rice 1999; Donovan et al. 2001). Commercial Btk microbial formulations (produced by the fermentation of the same strain of bacterium from which the gene was derived) also contain this protein, and have been used over the past 30 years to protect food crops, including certified organic crops from insect attack. Bt proteins including VIP3A are found widely in both agricultural and natural environments, including soils, on plant leaves, in grain stores, in dead insects (Meadows 1993) and in a variety of fresh foods (such as lettuce and tomato) (ANZFA 1999).

156. The HPT protein is found commonly in bacteria isolated from animal digestive systems and soil. It is therefore already present in the environment and in food chains. The protein is not known to be toxic to vertebrate animals.

157. *Streptomyces hygroscopicus*, the source of the bacterial *bar* gene, which produces the PAT protein. It is a common soil bacterium, and therefore the PAT protein is already present in the environment. CSIRO states that the amount of PAT protein added to the soil from the GM cotton is likely to very small compared to the amount already present in the soil derived from microorganisms.

Section 2.3  Potential toxicity hazard for livestock and wildlife, including mammals, birds and fish

2.3.1 Exposure of livestock and wildlife to cotton

158. None of the cotton plants from the release or their by-products will be used as stockfeed. As discussed in Section 2.1, most mammals avoid feeding on cotton plant or cotton trash due to the presence of gossypol and other components of cotton tissues and the morphology of the plant. Use of cotton products as stock feed is also limited for this reason (OGTR 2002).

159. In the field, seed cotton is present as large lint-covered seeds that are unattractive to avian species (OGTR 2002), so birds are not likely to be exposed to the introduced proteins in the GM cottons. Some exposure of wildlife could occur following planting. However, the introduced proteins are present at low levels (refer to Section 6, Appendix 1). The cottonseed from the proposed release will not be used for stockfeed.

160. Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation practices used by cotton growers in Australia retain irrigation water run-off, as well as the first 15mm of storm water run-off, on farm to minimise the entrance of pesticide residues into natural waterways. In New South Wales this is a legislative requirement, while in Queensland this is part of Good Management Practice of the cotton industry. The licence includes a requirement to separate GM cotton trials from natural waterways by at least 50 metres.
Section 2.3.2  Toxicity of GM cottons to livestock and wildlife

161. Acute oral toxicity studies have been carried out with mice and bobwhite quail (data supplied by applicant). No apparent toxicity was observed in mice or quail at doses of 2700 mg or 400 mg VIP3A protein/kg body weight, respectively, over 14 days of examination.

162. A two-year chronic rat feeding study was undertaken with Bt microbial products at doses of up to 8400 mg/kg of body weight/day. A decrease in weight gain was observed at the highest dose, but in the absence of any other adverse findings this was not considered to be related to Bt protein toxicity (McClintock et al. 1995).

163. The antibiotic resistance protein, HPT is found in bacteria isolated from animals and humans. It is therefore already present in the environment and in food chains. The HPT protein is not known to be toxic to vertebrate animals. Although the antibiotic is used in veterinary medicine, the restricted scale of the release would limit any exposure of animals to the GM cotton.

164. The PAT protein is present at a low level in Liberty® cotton, representing around 1% of the total extractable protein in the leaves. The level of PAT protein in the pollen and nectar is expected to be negligible. Consequently the level of exposure to the novel protein in the genetically modified crop is not likely to be significant, and may be further limited depending on possible routes of exposure.

165. No data has been provided on the biodegradability of the PAT protein. However, data relating to the stability of the PAT protein to digestion in mammalian digestive systems does not indicate any unusual degree of stability. The PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH (DIR 010/2002 and DIR 021/2002) Risk Assessment and Risk Management Plan, available on the OGTR website.

166. As noted above, none of the cotton plants from the release, or their by-products, are intended for use as stockfeed. Currently there is insufficient information available to assess the effect of the GM cottons to a range of non-target organisms. Accordingly, licence conditions have been imposed to ensure that the seed produced from this release is not used for stockfeed.

Section 2.4  Potential toxicity hazard for invertebrates

2.4.1 Exposure of invertebrates to GM cottons

167. Non-target invertebrates may be exposed directly, through feeding on the GM plants, or indirectly through eating other organisms, including the lepidopteran target organisms, that feed on the plants. However, the introduced proteins are produced at very low levels (VIP3A equals 0.03% of total soluble protein, refer to Appendix 1 section 6 for details) in all three types of GM cotton. Relative exposure will be greatest for other herbivorous species feeding on the cotton plants. Pollinator species and various adult insects that feed on pollen will also have exposure to the proteins. Species feeding on lepidopteran larvae may be exposed to the VIP3A protein. Sap feeders, such as aphids, will have minimal exposure, as the sap is composed primarily of sugars and mineral salts dissolved in water, rather than protein.

168. Non-target lepidopteran species may also be exposed to the GM cottons and may be affected by the VIP3A protein. However, cotton is not the preferred food source for non-target Lepidopteran species, and their populations would be sustained on other types of plants found around the release
location. In addition, a field survey conducted as a part of DIR 017/2002 demonstrated that the numbers of non-target insects were not affected by the insecticidal protein VIP3A.

169. The PAT protein is not toxic (to humans or animals) and is ubiquitously present in the soil. Therefore the contribution that the transgenic plants would make to this existing protein pool would be negligible. Levels of PAT protein entering the environment are likely to be small (see Appendix 1, section 6). In nature, invertebrates and insects are already exposed to this protein and therefore are not considered as a new route of exposure to the PAT protein.

170. More information on the PAT protein in relation to effects on invertebrates and insects can be found in the risk assessment and risk management plans for a herbicide tolerant canola, which expresses the bar gene (DIR 010/2002 and DIR 021/2002 available at the OGTR website).

171. The HPT protein is also widespread in the environment in naturally occurring hygromycin-resistant microorganisms found in animal digestive systems and soil.

2.4.2 Toxicity of GM cottons for invertebrates

172. Preliminary data supplied by the applicant indicates that the VIP3A protein is not toxic to any of the non-target insects listed in the Table below.

173. Table: Non-target species insensitive to the VIP3A protein

<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidoptera</td>
<td>Ostrinia nubilalis</td>
<td>European corn borer</td>
</tr>
<tr>
<td></td>
<td>Plodia interpunctella</td>
<td>Indian meal moth</td>
</tr>
<tr>
<td></td>
<td>Hyphantria cunea</td>
<td>Fall webworm</td>
</tr>
<tr>
<td></td>
<td>Plutella xylostella</td>
<td>Diamondback moth</td>
</tr>
<tr>
<td></td>
<td>Danaus plexippus</td>
<td>Monarch butterfly</td>
</tr>
<tr>
<td>Diptera</td>
<td>Musca domestica</td>
<td>House fly</td>
</tr>
<tr>
<td></td>
<td>Drosophila melanogaster</td>
<td>Fruit fly</td>
</tr>
<tr>
<td></td>
<td>Culex pipiens</td>
<td>Northern house mosquito</td>
</tr>
<tr>
<td>Homoptera</td>
<td>Myzus persicae</td>
<td>Green peach aphid</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>Apis mellifera</td>
<td>Honey bee</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Diabrotica virgifera</td>
<td>Western corn rootworm</td>
</tr>
<tr>
<td></td>
<td>Diabrotica longicornis</td>
<td>Northern corn rootworm</td>
</tr>
<tr>
<td></td>
<td>Papillia japonica</td>
<td>Japanese beetle</td>
</tr>
<tr>
<td></td>
<td>Leptinotarsa decemlineata</td>
<td>Colorado potato beetle</td>
</tr>
<tr>
<td></td>
<td>Tenebrio molitor</td>
<td>Yellow meal worm</td>
</tr>
<tr>
<td></td>
<td>Diabrotica undecimpunctata</td>
<td>Southern corn rootworm</td>
</tr>
<tr>
<td></td>
<td>Coleomegilla maculata</td>
<td>Pink spotted ladybeetle</td>
</tr>
<tr>
<td>Isotomidae</td>
<td>Folsomia candida</td>
<td>Springtails</td>
</tr>
<tr>
<td>Neuroptera</td>
<td>Chrysoperla carnea</td>
<td>Green lacewings</td>
</tr>
<tr>
<td>Thysanoptera</td>
<td>Frankliniella occidentalis</td>
<td>Western flower thrips</td>
</tr>
</tbody>
</table>

174. A number of studies on the effect of the PAT protein on invertebrates and insects have shown no detrimental effects, as discussed in detail in the DIR 010/2002 and DIR 021/2002 risk assessment and risk management plans, available at the OGTR website.

175. Currently there is no information available on the potential toxicity of the GM cottons to invertebrates and insects under Australian field conditions. The licence conditions require the applicant
to conduct research to study the effect of insecticidal proteins on non-target organisms including Australian invertebrates and insects during 3 years of field trials (refer to Chapter 2 and Appendix 7 for details). Similar research conditions have been imposed under DIR 034/2003 licence.

Section 2.5 Potential toxicity hazard for microorganisms

2.5.1 Exposure of microorganisms to GM cottons

176. The VIP3A protein is naturally produced in a common soil bacterium, *Bacillus thuringiensis* (Bt). Thus the VIP3A protein produced during both vegetative growth and sporulation of Bt, is already a natural component of soil. Microbial formulations of Bt that contain VIP3A (and other Bt toxins) are regularly applied as biological pesticides to numerous Australian crops, increasing the level of VIP3A in the environment.

177. The HPT protein is also widespread in the environment in naturally occurring hygromycin-resistant microorganisms found in animal digestive systems and soil.

178. Microorganisms may be exposed to GM cotton plants during growth or decomposition of the plant material. After harvest of lint and seed, the remaining cotton plant residues are typically tilled into the soil, so that soil microorganisms are likely to be exposed to the introduced proteins as the GM cotton residues are broken down. Exposure of microorganisms in soil to VIP3A residues may also occur as a result of root exudations, as has been observed in Bt corn expressing Cry1Ab (Saxena et al. 1999; Stotzky 2000). Preliminary work by (Gupta et al. 2002) has shown that roots of INGARD® cotton, expressing the Cry1Ac insecticidal protein, release this protein into soil during growth. Root breakage also increases the release of protein into the soil. It is expected that the roots the GM cottons in this release will express the VIP3A and HPT proteins, and that some of these will enter the soil during growth.

179. Microbial resistance to glufosinate ammonium is already widespread and the contribution that the transgenic plants would make to this existing pool would be negligible. The PAT protein is naturally expressed in a common soil bacterium, *Streptomyces hygroscopicus*. Thus the PAT protein is already a natural component of soil.

180. The risk associated with the PAT protein being released into the environment is considered negligible because of its non-toxic and biodegradable nature. Biologically produced molecules typically have very short half-lives in the environment due to breakdown by soil microbes and as a result these substances do not accumulate in the soil or contaminate groundwater (Shatters 1999).

2.5.2 Toxicity of GM cottons for microorganisms

181. There are no reported studies on the effect of the VIP3A protein on microorganisms and therefore more data is required.

182. A number of studies on the effect of the PAT protein on soil microorganisms have shown no detrimental effects, as discussed in detail in the DIR 021/2002 risk assessment and risk management plan, available on the OGTR website.

183. As part of the licence conditions, there is a requirement to conduct research on the potential effect on soil biota to the new proteins expressed in GM insecticidal cottons, including information on
the biodegradability of the introduced proteins during the three years of the field trial (Refer to Chapter 2 and Appendix 7 for details).

SECTION 3 CONCLUSIONS REGARDING TOXICITY TO NON-TARGET ORGANISMS

184. It is considered that the risk of GM cotton being toxic to non-target organisms (those other than Lepidoptera) is low because:

- the release is small in size;
- the introduced proteins are already widespread in the environment, through the presence of the bacteria from which they are derived;
- exposure of livestock and wildlife to these GM cottons is low;
- the toxicity of the VIP3A protein is specific to lepidopteran insect larvae;
- the introduced proteins PAT and HPT are not known to be toxic to any organisms;
- laboratory and field studies suggest that populations of key non-target invertebrates are unlikely to be affected by VIP 3A protein;
- the introduced proteins are unlikely to have adverse effects on soil microorganisms.

185. As part of the licence conditions, the licence holder is required to report any adverse effects on the environment (for example, any indication of toxicity of the GM cottons for non-target organisms) and undertake research to examine the effects of VIP3A protein on non-target organisms and confirm the risk management strategies imposed for the trial.
APPENDIX 4 WEEDINESS

186. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this Appendix, risks posed by the proposed dealing to the environment are considered in relation to the potential for the GMOs to become a problematic weed.

187. There are numerous definitions of weeds (Richardson et al. 2000) including ‘a plant growing where it should not be’. Richardson et al. (2000) recommend terminology pertaining to weeds as plants (not necessarily alien) that grow in sites where they are not wanted and which usually have detectable economic or environmental effects (synonyms: plant pests, harmful species; problem plants). According to Richardson et al. (2000), ‘environmental weeds’ are alien plant taxa that invade natural vegetation, usually adversely affecting native biodiversity and/or ecosystem functioning. From analysis of global data sets, (Daehler 1998) found that ‘agricultural weeds’ tend to be herbaceous, rapidly reproducing, abiotically dispersed species, whereas plants that are most likely to become invaders of native ecosystems tend to be primarily aquatic or semi-aquatic, grasses, nitrogen-fixers, climbers, and clonal trees.

188. Weeds become a problem to the community when their brief presence or abundance interferes with the intended use of the land they occupy. Weeds may also represent a source of food to various organisms hence the introduction of weeds to an environment may also bring about ecological change by altering the structure of food webs. Weeds are thought to share a number of life history characters that enable them to rapidly colonise and persist in ecosystems, particularly those that are regularly disturbed. These characteristics include:

- ability to germinate, survive, and reproduce under a wide range of environmental conditions;
- long-lived seed with extended dormancy periods;
- rapid seedling growth;
- rapid growth to reproductive stage;
- long continuous seed production;
- ability to self pollinate but are not exclusively autogamous;
- use of unspecialised pollinators or wind when outcrossing;
- high seed output under favourable conditions;
- special adaptations for long distance and short distance dispersal; and
- being good competitors (Baker 1965).

SECTION 1 NATURE OF THE WEEDINESS HAZARD

189. The GM insecticidal and insecticidal/herbicide tolerant cottons differ from conventional cotton in the expression of up to three additional proteins. These are VIP3A protein (in all three types of GM cottons), HPT protein (in two types of GM cottons) and PAT protein (in one type of GM cotton) (See Appendix 1 for details).
190. The possibility was considered that the GM insecticidal and insecticidal/herbicide tolerant
cottons might have the potential to be harmful to the environment, because of inherent weediness or
increased potential for weediness, either due to expression of the novel gene products or as a result of
unintended effects of the genetic modification.

191. This could occur if the GM cottons displayed altered characteristics such as increased fitness or
increased fecundity. If the GM cottons were to spread in the environment as weeds, this could result
in impacts such as loss of native biodiversity.

192. The APVMA has responsibility for agricultural chemical use, including insecticides and
herbicides in Australia under the Agricultural and Veterinary Chemicals (Code) Act 1994 (Refer to
Chapter 1 for details). As part of their assessment of chemical use, the APVMA considers any
potential environmental effects such as development of glufosinate resistant weeds, levels of glufosinate
ammonium in the environment and drift from spray applications. Thus risks associated with the use of
the insecticide or herbicide are not considered in the risk assessment of these GM cotton lines.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

193. In assessing the likelihood of adverse impacts due to weediness of GM cottons, a number of
factors were considered, including:

- the inherent weediness of conventionally bred cotton;
- the means of dispersal of cottonseed into the environment;
- the potential weediness of GM insecticidal and insecticidal/herbicide tolerant cottons;
- the potential selective advantage conferred by the introduced VIP3A, PAT and HPT
proteins; and
- data gathered from the environmental monitoring program for currently commercially
released Roundup Ready® and Roundup Ready®/INGARD® cotton.

Section 2.1 Inherent weediness of conventional cotton

194. 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) that was
produced in order to inform the risk assessment processes for licence applications involving GM
cotton. This document can be accessed at www.ogtr.gov.au. In summary, the document concludes
that non-GM cotton is not a problematic weed in Australia, because factors including soil moisture,
nutrient limitation, and roadside management practices limit the establishment and/or persistence of
cotton seedlings. Further information on the weediness of non-GM cotton is included here to establish
a baseline for comparison with the GM cottons being considered.

195. Cotton is not considered to possess the characteristics commonly associated with successful
weeds, such as seed dormancy, long persistence in the soil, germination under a broad range of
environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed
dispersal and long-distance seed dispersal (Keeler 1985; Keeler 1989).

196. Another important element in prediction of weediness is taxonomic relationship, considering
weediness within a taxon, including history of weediness in any part of the world (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 pest. Cotton is not considered to be a problematic weed in Australia (Groves et al. 2000; Groves et al. 2002). There are about 50 species of *Gossypium* (Fryxell 1992; Craven et al. 1994) of which only one (*G. tomentosum*) is listed as a weed in the USA (Holm et al. 1997).

**Section 2.2 Potential weediness of the GM insecticidal and insecticidal/herbicide tolerant cottons**

197. Many of the characteristics associated with weediness are also important agronomic characteristics. Consequently these are assessed as part of the agronomic evaluations during the development of new cotton varieties, including GM varieties.

198. INGARD® cotton (an insecticidal cotton that acts on the same targeted insects as the GM cotton in this release but contains a *cry* gene instead) has been in commercial release since 1996 and Roundup Ready® and Roundup Ready®/INGARD® cotton since 2000, both in the cotton growing regions of NSW and Qld. Since their commercial release, cottonseed from these GM cottons has been used as stockfeed. Over this period there has been no evidence that GM cotton has become weedier than non-GM cotton or is more invasive than non-GM cotton. Surveys of volunteer cotton in the southern cotton growing areas of Australia and experimental research on the weedy potential of GM cotton consistently suggest that major factors limiting cotton establishment and survival include water and nutrient availability, herbivory by non-lepidopteran species (vertebrate and invertebrate), plant competition, frost and fire (Eastick 2002; Farrell & Roberts 2002).

199. The results of this research strongly indicate that the likelihood of GM cotton establishing and persisting as a weed in southern Australia at higher levels than the very low rate of establishment of non-GM cotton is negligible. This is because the environmental variables are the key limitations on cotton populations and these are not affected by the genetic modifications. Experiments conducted by Eastick (2000) indicate that cotton has poor survival attributes away from agricultural land.

200. Preliminary data from US and Australian glass-house and field trials indicate that the agronomic characteristics of the homozygous GM COT102 cotton plants are within the range of current commercial non-GM cotton varieties (information provided by the applicant). The evaluation of agronomic characters in backcrosses with Australian elite varieties is ongoing, however the only known difference between the GM cotton and non-GM cotton is the presence and expression of the *vip3A*, *bar* and *hph* genes. From the trials conducted until now, it appears that the GM cotton is likely to behave similarly to non-GM cotton except for the modified traits.

201. In the licence, access to the release sites is restricted to authorised personnel. Hence, dispersal of the GMO from the release sites is unlikely to occur. The release sites would also be required to be at least 50m away from natural waterways. Hence the dispersal of the GM cottons from the release sites is likely to be minimal.

202. As proposed by the applicant, the licence imposes conditions to limit outcrossing to non-GM cotton by physical separation of several kilometres from all other cotton crops (information provided by the applicant). Since pollen dispersal studies have shown that outcrossing is localised around the pollen source and decreases significantly with distance (Umbeck et al. 1991; Llewellyn & Fitt 1996), the applicant is required to plant a 20 m pollen trap around the GMO cotton lines to contain localised
pollen dispersal by insects. (Pollen flow is also discussed in Appendix 5, Section 1 in relation to gene transfer.)

203. Following harvest of the seed cotton from the release sites, the remaining plant material will be slashed and incorporated into the soil. During harvesting, some seed may fall to the ground and get incorporated into the soil. Such a seed represents an opportunity for the GMO cottons to persist in the environment. However, cotton has little dormancy, meaning that seed germinates with the arrival of favourable soil moisture and temperature conditions following harvest.

204. Post harvest inspection and destruction of volunteer cotton is required as a condition of the licence, to ensure that the GM cotton lines do not persist in the environment at the release site.

2.2.1 Key findings of the environmental monitoring program on GM herbicide tolerant (Roundup Ready®) and herbicide tolerant/insecticidal (Roundup Ready®/INGARD®) cotton

205. The environmental monitoring program for Roundup Ready® and Roundup Ready®/INGARD® cotton demonstrated that, for regions of Australia south of latitude 22º South, the establishment and persistence of these GM cottons occurs infrequently and does not occur at levels disproportionate to the amount of cottonseed entering the environment. That is, for southern Australia, there is no indication that either Roundup Ready® or Roundup Ready®/INGARD® cotton is weedier than the negligibly weedy non-GM cotton. Moreover, where volunteers had persisted, they were managed easily either mechanically or with herbicides other than the glyphosate.

Section 2.3 Potential selective advantage conferred by the introduced proteins

206. In order for the GM insecticidal and insecticidal/herbicide tolerant cottons to spread and persist in the environment more than non-GM cotton, following the release, the expression of any one or all of the introduced proteins would need to confer a selective advantage.

2.3.1 VIP3A

207. The VIP protein could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton.

208. A detailed discussion of the potential of the Cry1Ac protein (an insecticidal protein derived from the same bacterium and with the same target organisms as the VIP3A protein) to influence weediness is provided in the risk assessment document for DIR 022/2002, available at www.ogtr.gov.au. A detailed assessment of the potential of both the Cry1Ac and Cry2Ab insecticidal proteins together to influence weediness is provided in the risk assessment for DIR 012/2002. These risk assessments conclude that GM cottons expressing the insecticidal and insecticidal/herbicide tolerant genes do not enhance the weediness potential of these GM cottons as compared to non-GM cottons in the southern cotton growing areas of Australia.

209. Since the commercial release of insecticidal (INGARD®) cotton in 1996 (DIR 022/2002) and herbicide tolerant/insecticidal (Roundup Ready/INGARD®) cotton in 2000 (DIR 023/2002), there has been no reports of enhanced weediness of these GM cottons in southern Australia.
210. There may be some limited potential for the *vip3A* gene to confer a selective advantage on the GM cotton, if insect herbivory regulated populations. An investigation into the potential weediness of GM insecticidal cottons (INGARD® and Bollgard® II) in northern Australia concluded that in some situations, where moisture and nutrients are high due to human activity (eg stock yards, drains), there was an increase in fecundity of INGARD® cotton compared to non-GM cotton. However, there was no evidence that the addition of the Bt genes conferred additional fitness to cotton plants in natural habitats, and lepidopteran insect herbivory was not a limiting factor on cotton (Eastick 2002). Nonetheless, it should be noted that the current limited and controlled release of GM cottons will occur in southern Australia only where insects do not regulate cotton populations.

### 2.3.2 PAT protein

211. The herbicide tolerant PAT protein in GM cotton is produced in the leaves in low amounts (refer Appendix 1 for more details) and is not know to have any other biochemical pathway other than the acetylation of glufosinate ammonium and its derivatives. The PAT protein present in the GM cotton is specific in mode of action and only confers resistance to the herbicides Liberty® and Basta® and does not confer any selective advantage.

212. The PAT protein is derived from a common soil bacterium *Streptomyces hygroscopicus* which is also naturally present in water.

213. Non-GM cotton displays very few characteristics considered important for a plant to be an effective weed (Lee 1984; Keeler 1989) and this potential for weediness is not likely to change in GM plants expressing the herbicide tolerant PAT protein (LaSota 1992).

### 2.3.3 HPT protein

214. The antibiotic resistance *hph* gene will not confer a selective advantage on the GM cotton, since antibiotics are not applied to cotton crops and are not likely to be present in any environment where cotton grows. In addition, there is no evidence, nor any reason to expect that expression of the protein would alter any of the characteristic attributes of cotton that would be important for weediness.

### Section 2.4 Dispersal of GM cottonseed beyond the release sites

215. The proposed dealing includes cultivation of GM insecticidal and insecticidal/herbicide tolerant cottons and retention of all cottonseed for storage or planting in the subsequent two season field trials authorised by the same licence. Cottonseed produced in the proposed field trials will not be used for human food or stockfeed.

216. Surveys (Eastick 2002; Farrell & Roberts 2002) have found that seed cotton (ie. cottonseed in its natural form with lint attached) is the most likely form of seed to be dispersed in the environment, as opposed to fuzzy (ginned) or black (delinted) seed. Seed cotton, as well as being the natural form of the seed produced on the plant, can be dispersed by falling from cotton modules during transport to cotton gins after harvest. Other forms of seed are present only as a result of human activity, for example, transport of seed to storage facilities or new planting sites. Seed cotton generally does not germinate until the season after its production, when climatic conditions become favourable.
217. There is only limited potential for movement of seed cotton in waterways (Eastick 2002). Seed cotton has poor seed dormancy. After seed production, seed germinates as soon as climatic conditions become favourable.

218. A survey of the transport routes between Emerald (in the Queensland cotton growing region) and the Atherton Tablelands (north of 22° South) indicated that cotton plants had established in roadside environment only infrequently, despite 12 years of use of these routes for transporting fuzzy seed for stockfeed (Farrell & Roberts 2002).

219. Specific licence conditions have been imposed to require harvested material to be securely wrapped before transporting away from the release sites so as to prevent the dispersal and escape of seed cotton into the environment. In addition, access to the release site is limited to authorised personnel, to prevent dispersal of the seed by humans.

220. Licence conditions have been imposed to establish a 400 m wide area of land (called a Research Zone) around trial sites larger than one hectare for the purpose of conducting research to inform the ongoing review of data on spread and persistence of the GMOs in the environment and validate the efficacy of containment measures.

Section 2.5 Persistence of the GM cotton at the release sites

221. Following harvest of the seed cotton from the release sites, the remaining plant material will be slashed and incorporated into the soil. Some seed may fall to the ground during harvesting and also be incorporated into the soil. A soil seed bank represents an opportunity for the GMOs to persist in the environment. Seed cotton has little dormancy, meaning seed will germinate with the arrival of favourable soil moisture and temperature conditions. GM cotton volunteers are expected to germinate in the wet season following harvest.

222. Licence conditions have been imposed to require post harvest monitoring, and destruction of cotton volunteers to ensure that the GM cottons do not persist in the environment at the release sites.

Section 3 Conclusions regarding weediness

223. It is considered that the risk of the GM insecticidal cotton establishing as a weed as a result of the proposed limited and controlled release is low because:

- the scale of the release is small;
- cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment;
- the genetic modifications have not enhanced the weediness of GM cottons as compared to non-GM cottons;
- the presence of the insecticidal gene in the GM cotton may confer a selective advantage if cotton is limited by lepidopteran insects, however, evidence suggests that this is not the case;
- major constraints on weediness of GM and non-GM cotton in southern Australia are the availability of water and nutrients, plant competition, herbivory by non-lepidopteran species, frost and fire;
the presence of the herbicide tolerance gene in the GM cotton may confer a selective advantage only in the presence of specific herbicides (Liberty® or Basta®); and

surveys carried out since the commercial release of herbicide tolerant (Roundup Ready®) and herbicide tolerant/insecticidal (Roundup Ready®/INGARD®) cottons indicate that these GM cottons have not become problematic weeds in the southern cotton growing areas of Australia.

224. It is further considered that the risks of GM cotton establishing as a weed can be managed to an acceptable level by requiring various strategies to minimise the spread and persistence of the GM cottons from the release site. Refer to Chapter 2 and Appendix 7 for risk management conditions.

225. Further information on the impact of the genetic modifications on agronomic characteristics of GM cottons in Australian field conditions that may be indicative of potential weediness (eg. dormancy of seeds, competitive ability, susceptibility to natural enemies and spread within the environment) is needed. Hence, a licence condition has been imposed to require research of the potential weediness of the GM cottons proposed to be released in this field trial.
APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

226. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. This Appendix considers potential hazards that may be posed through the transfer of the introduced genes from the GM insecticidal cottons to other organisms.

227. Gene transfer is the movement of genes between individuals. Within a species genes are routinely exchanged between individuals of successive generations through sexual reproduction. Hybrids can be produced between closely related species through sexual reproduction. For example, in plants cross pollination of wheat and rye produces triticale, in animals fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning hybridisation may or may not lead to the introgression of a gene or genes into a population. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except among bacteria. However gene transfer between sexually incompatible organisms can occur. Detailed examination of DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. In general there seems to have been only very limited transfer of genes from plants to other types of organisms.

228. The likelihood of hazards arising from gene transfer is dependent on a number of factors that form a necessary chain, including:

- **opportunity** for gene transfer to occur such that the recipient organism is exposed to the genetic material in the form of pollen, plant cells or DNA; and
- **incorporation** of the genetic material into the genome of the recipient organism at a site and in a configuration that allows the gene to be functional; and
- **persistence** of the transferred genetic material such that the newly modified organism is able to survive, reproduce and maintain the genetic modification; and
- **significance** of the transferred genetic material such that its presence and/or expression in the recipient organism will result in an adverse impact on human health and safety, or the environment.

229. For ease of reference, the assessment of gene transfer to other organisms is presented in three sections:

- Section 1 details the nature and likelihood of genes introduced to GM cotton lines transferring to other plants, including other cotton crops;
- Section 2 details the nature and likelihood of genes introduced to GM cotton lines transferring to microorganisms; and
- Section 3 details the nature and likelihood of genes introduced to GM cotton lines transferring to animals, including humans.

230. Section 4 draws together the conclusions from these sections.
SECTION 1  GENE TRANSFER FROM GM INSECTICIDAL AND INSECTICIDAL/HERBICIDE TOLERANT COTTON LINES TO OTHER PLANTS

Section 1.1  Nature of the gene transfer hazard

231. Transfer of the introduced genes (vip3A, bar and hph) or regulatory sequences (promoter and terminator regions) from GM cotton to cultivated non-GM cotton plants or to naturalised (feral) cotton would present the same hazards, and have the same potential impacts, as the presence of the genes in GM cotton. These risks are considered in Appendices 2 - 4. However, if such a transfer occurred, it would increase the possibility that these genes could further spread in the environment.

232. If gene transfer to other plant species were to occur, the hazards to the environment associated with any such transfers could be highly varied, broadly depending upon the nature of the genes and of the species to which transfer occurred. Transfer of the introduced genes or regulatory sequences into other plant species, in particular to native flora, may have adverse effects on biodiversity if the recipient plants gained a selective advantage, such as enhanced survival or reproductive capacity.

1.1.1  Potential hazards from the introduced genes

THE vip3A (INSECTICIDAL) GENE

233. Plants expressing this gene would be toxic to lepidopteran insects. This could confer a selective advantage on the plants or adversely affect survival of lepidopteran insects and consequently other organisms linked to lepidopteran insects through food webs.

THE bar (HERBICIDE TOLERANCE) GENE

234. Plants expressing this gene could become tolerant to the herbicide glufosinate ammonium, conferring a selective advantage on the plant in the presence of glufosinate ammonium use (Liberty® and Basta® herbicides).

THE hph (ANTIBIOTIC RESISTANCE) GENE

235. Plants expressing this gene could become resistant to the antibiotic hygromycin. This would only have an impact on plant survival if the antibiotic was used on the plants, or otherwise present in the environment of the plant, and were limiting its growth. Antibiotics are not generally applied to crops and would not limit their growth except at very high concentrations not found in the natural or agricultural environment. Expression of the hph gene enabled selection of plant cells containing the genetic modification in the laboratory.

PROMOTERS AND OTHER REGULATORY SEQUENCES

236. If these sequences were to be transferred to other plants without the associated genes of the GM cottons, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.
237. Some of the introduced regulatory sequences are derived from a plant pathogen  
(Agrobacterium tumefaciens). However these sequences are not pathogenic in themselves nor do  
they cause any disease symptoms in GM plants.

238. All of the introduced regulatory sequences operate in the same manner as do endogenous plant  
regulatory elements. The transfer of endogenous regulatory elements to a new genetic context occurs  
naturally in all plant genomes and could also result in unpredictable effects. Thus the potential hazard  
from the introduced sequences is no different to that posed by sequence transfer from non-GM plants  
or sequence transfer occurring within the genome of a plant species.

Section 1.2  Likelihood of a hazard arising through gene transfer

239. The likelihood of gene transfer creating a hazard for human health and safety or the environment  
depends on the characteristics of introduced gene sequences, as well as on the likelihood of transfer  
itself.

1.2.1 Transfer to cultivated cotton

240. Cotton is primarily self pollinating, however in a cropping situation a low level of pollen transfer,  
by insect pollinators, to other nearby vegetation would be likely. Cotton pollen dispersal studies  
consistently show that outcrossing is localised around the pollen source and decreases significantly with  
distance. Studies by (Llewellyn & Fitt 1996) demonstrated that outcrossing was very rare (less than  
0.01%) or was not detected at a distance of 10 m from GM cotton plants and no outcrossing was  
detected at 20 m. For a detailed consideration of the likelihood of this occurring, including an overview  
of the pollination biology of cotton, see the document 'The Biology and Ecology of Cotton  
(Gossypium hirsutum) in Australia', available at www.ogtr.gov.au, that was produced in order to  
inform the risk assessment processes for licence applications involving GM cotton.

241. Gossypium barbedense (pima cotton) is also used for commercial cotton production, but only  
to a very minor extent in Australia (Lake Tandou and Bourke, NSW). G. hirsutum and  
G. barbedense are closely related and hybridisation between the two species can occur, yielding  
fertile progeny. Hybrid progeny exhibit characteristics intermediate to the parents but typically with  
lower capacity to produce fruit. After several generations, progeny of the hybrids revert to the  
characteristics of one or other of the parents. G. barbedense and hybrids are not more weedy or  
difficult to control than is G. hirsutum (personal communication, Warwick Stiller & Greg Constable,  
CSIRO).

242. Transfer of the introduced genes or regulatory sequences to other cotton plants growing in  
cultivation would present the same hazards as the presence of the genes in GM cottons proposed for  
release (see Appendices 2-6).

243. For this proposed release, the likelihood of the hazard would be further minimised because  
licence conditions include a requirement that GM cotton be isolated from other cotton by a 20 m  
pollen trap to limit cross-pollination to plants outside the release sites (see Appendix 7 for details).

244. As part of the OGTR’s ongoing commitment to the review of data, specific research conditions  
have been imposed in the licence. The research is intended to confirm previous research on gene flow  
undertaken prior to the implementation of the Act and the commercial release of GM cottons and  
validate the containment measures imposed during the field trials of new GM cottons. In order to
facilitate this review and data collection, the Regulator has imposed conditions to establish 400 m research zones surrounding the proposed GM cotton trials comprising plantings in excess of one hectare. This research program will be developed in consultation with the OGTR.

1.2.2 Transfer to volunteer and naturalised cotton

245. Off farm, cotton volunteers may establish along roadsides in cotton growing areas, primarily due to transport of harvested seed cotton (see Appendix 4). The majority of these volunteers are in pollinating distance of cotton crops.

246. Transfer of the introduced genes to naturalised cotton may increase the likelihood that the genes could spread and/or persist in the environment (away from cotton farming systems). Gene transfer to naturalised cotton populations is thought to be unlikely because of the geographic distances between these naturalised populations and the cotton growing regions of NSW and QLD. However herbarium records of *G. hirsutum* and *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in central and south eastern Queensland. The remnants of these populations, which may be within pollinating distance of cotton crops, has not been confirmed. As part of the licence conditions for DIR 022/2002 (INGARD® cotton), Monsanto will be conducting a survey of naturalised cotton populations in Queensland, in location suggested by herbarium records.

247. Licence conditions have been imposed to limit cross-pollination to plants outside the release sites (see Chapter 2 and Appendix 7 for details).

248. Licence conditions have been imposed to establish a 400 m wide area of land (called a Research Zone) around trial sites greater than one hectare in size for the purpose of conducting research to inform the ongoing review of data on gene transfer and validate the efficacy of containment measures.

1.2.3 Transfer to native cottons and other plant species

249. Australian flora contains 17 native *Gossypium* species. All of the Australian *Gossypium* species are diploids (C, G or K genomes), while the cultivated cottons are tetraploids (AD-genomes). The native species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids have been produced without application of plant hormones, when plants were planted in close proximity of each other. However these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations.

250. 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 in NSW and QLD. Thus gene transfer from GM insecticidal cottons to native cottons is prevented not only by genetic incompatibility but also by geographic constraints to cross-pollination (OGTR 2002).

251. The failure of cross-pollination due to well established genetic incompatibility also prevents gene transfer from GM insecticidal cottons to other plant species.
SECTION 2  GENE TRANSFER FROM GM INSECTICIDAL OR INSECTICIDAL/HERBICIDE TOLERANT COTTONS TO MICROORGANISMS

Section 2.1  Nature of the gene transfer hazard

252. The transfer of genes from plants to microorganisms cannot occur through cross-pollination. Horizontal gene transfer is defined as the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Conner et al. 2003). There is growing evidence that horizontal gene transfer has been a principal force in the evolution of bacteria (Ochman et al. 2000; Nielsen 1998; Smalla et al. 2000; Stanhope et al. 2001).

253. The potential hazards associated with the introduced genes of GM insecticidal and insecticidal/herbicide tolerant cottons transferring to microorganisms could be highly varied, broadly depending upon the phenotype of the recipient and any changes to its survival or reproductive capacity. The impact of any hazard arising through gene transfer would also depend on other sources of the introduced genes in the environment.

2.2.1 Potential hazards from the introduced genes

THE VIP3A (INSECTICIDAL) GENE

254. Microorganisms expressing this gene would be toxic to lepidopteran insects. This could impact on survival of lepidopteran insects if the recipient microorganisms were ingested at high levels. Microorganism populations could also be affected if toxicity to lepidopteran insects gave the recipient a survival or reproductive advantage.

THE BAR (HERBICIDE TOLERANCE) GENE

255. Microorganisms expressing this gene would gain resistance to glufosinate ammonium and to the tripeptide antibiotic phosphinothricyl-L-alanyl-L-alanine (bialaphos) naturally produced by the bacteria *S. hygroscopicus* and *S. viridochromogenes* (Organisation for Economic Co-operation and Development (OECD) 1999) from which the *bar* and related *pat* genes, respectively, are derived. *Streptomyces spp.* are saprophytic, soil-borne bacteria and are not considered a pathogen of plants, humans, or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). Thus transfer of this gene is unlikely to present a hazard in relation to pathogenesis. Bialaphos is not used in human or veterinary therapy.

THE HPH (ANTIBIOTIC RESISTANCE) GENE

256. Microorganisms expressing this gene would be resistant to the antibiotic hygromycin. The consequences of this for human health and safety and the environment would depend on other characteristics of the microorganism (for example pathogenicity), the use and significance of the antibiotic(s) in clinical and/or veterinary practice and whether these antibiotics limit growth or survival of the microorganism in other circumstances.

257. Hygromycin is not currently in clinical use in Australia, and has only limited veterinary use (JETACAR 1999). The *hp* gene, and other genes conferring resistance to hygromycin, are already
widespread in microbial communities in the environment, including in human and animal digestive systems.

258. Some microorganisms may be limited by antibiotics, either due to the use of antibiotic medicines or in some limited environmental situations where competing microorganisms produce antibiotics. Viruses are not limited by antibiotics.

PROMOTERS AND OTHER REGULATORY SEQUENCES

259. If these sequences were to be transferred to microorganisms without the associated genes of GM cottons, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

260. Some of the introduced regulatory sequences are derived from plant pathogens (Agrobacterium tumefaciens). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

261. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.

Section 2.2.2 Other sources of the introduced genes in the environment, and their potential for horizontal transfer

262. Information on other sources of the introduced genes in the environment is discussed here to provide baseline information on the prevalence and transfer of these genes that would happen naturally, irrespective of the GM cottons.

263. All of the introduced genes in GM insecticidal and insecticidal/herbicide tolerant cottons are already widespread in the environment, being derived from common soil bacteria. The regulatory sequences are derived from common bacteria or plants.

THE vip3A (INSECTICIDAL) GENE

264. The vip3A insecticidal gene expressed in the GM insecticidal and insecticidal/herbicide tolerant cottons occur naturally in the common soil bacteria Bacillus thuringiensis (Bt). Bt has been isolated from a wide range of sources such as forest, soil, grain dust, bat dung, sea water and dead insects (Martin & Travers 1989).

265. Many Bt toxin genes are not carried in chromosomal DNA, but are encoded on extra-chromosomal DNA, known as plasmids. Plasmids are known to be exchanged between bacterial species in nature by conjugation and transformation. The native cry1Ac gene has been identified on a plasmid of Bt kurstaki strain HD-73 (Lereclus et al. 1993). It has been demonstrated in the laboratory that Bt strains can interchange toxin-encoding plasmids with other Bt strains and with other bacterial species (Glare & O'Callaghan 2000). Horizontal gene transfer may also occur by transduction mediated by bacteriophages (Glare & O'Callaghan 2000).
THE BAR (HERBICIDE TOLERANCE) GENE

266. The herbicide-tolerance bar gene was originally isolated from the common soil bacteria *Streptomyces hygroscopicus*, which is not considered pathogenic to plants, humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). This gene is already present naturally in the environment. Transfer of this gene would not present a hazard to human health or the environment.

THE HPH (ANTIBIOTIC RESISTANCE) GENE

267. Microorganisms expressing the hph gene could become resistant to the antibiotic hygromycin. Hygromycin is not currently in clinical use in Australia, and has only limited veterinary use (JETACAR 1999). The hph gene, and other genes conferring resistance to hygromycin, are already widespread in microbial communities in the environment, including in human and animal digestive systems.

PROMOTERS AND OTHER REGULATORY SEQUENCES

268. If these sequences were to be transferred to other plants without the associated genes of the GM cottons, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

269. Some of the introduced regulatory sequences are derived from a plant pathogen (*Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

270. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context occurs naturally in all plant genomes and could also result in unpredictable effects. Thus the potential hazard from the introduced sequences is no different to that posed by sequence transfer from non-GM plants or sequence transfer occurring within the genome of a plant species.

Section 2.2.3 Likelihood of a hazard arising through gene transfer to microorganisms occurring

271. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the characteristics of introduced gene sequences, as well as on the likelihood of the transfer itself.

272. Most gene transfers have been identified through analyses of gene sequences (Ochman et al. 2000; Worobey & Holmes 1999). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence & Ochman 1998). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000).

273. In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses are exceedingly rare (Mayo & Jolly 1991; Nielsen et al. 1998; Nielsen et al. 2000; Harper et al. 1999; Schoelz & Wintermantel 1993; Greene & Allison 1994; Pittard 1997; Aoki & Syono 1999; Worobey & Holmes 1999). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Nielsen et al. 2000; Schoelz & Wintermantel 1993; Greene & Allison...
1994). However, in all cases this was achieved only under controlled conditions with the presence of related gene sequences (homologous recombination), and using powerful selection methods to detect extremely rare gene transfer events.

**BACTERIA**

274. Natural transformation is a mechanism by which transfer of DNA from plants to microorganisms could have occurred during evolution (Bertolla & Simonet 1999) and is the mechanism that is most likely to contribute to a horizontal gene transfer from transgenic plants to bacteria (Smalla et al. 2000). Natural transformation enables competent bacteria to generate genetic variability by taking up and integrating free DNA that is present in their surroundings. This uptake of DNA does not necessarily depend on DNA sequence, thus indicating the potential of gene transfer from divergent donor organisms (Nielsen 1998).

275. A number of steps and conditions would need to be fulfilled for functional natural transformation to occur (Bertolla & Simonet 1999), many of which are highly unlikely, making the overall likelihood of gene transfer, and of resulting hazard, extremely low:

- release of the DNA molecules from plant cells into the environment;
- persistence of the free DNA in the environment;
- presence of bacterial genotypes capable of developing competence for natural transformation;
- appropriate biotic and abiotic conditions for the development of the competent stage;
- uptake of DNA fragments;
- chromosomal integration via recombination or autonomous replication of the transforming DNA;
- expression of the genes by the recipient bacterium;
- selective advantage to fix the transformation into the gene pool.

276. Thus horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure (Gebhard & Smalla 1999; Mercer et al. 1999; Nielsen et al. 1998) and even then only, at a very low frequency.

277. Using antibiotic selection to detect extremely rare events, *Acinobacter sp.* cells containing a defective copy of the neomycin resistance (*nptII*) gene (with 10 bp or 317 bp of DNA deleted) were observed to incorporate DNA from GM plants (sugarbeet, tomato, potato or oilseed rape) carrying the intact *nptII* gene, leading to restoration of neomycin resistance. Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in *Acinobacter sp.* (Nielsen et al. 2000; De Vries et al. 2001) or in *Pseudomonas stutzeri* (De Vries, 2001).
Appendix 5 - Transfer of introduced genes to other organisms

RELEASE AND PERSISTENCE

278. Several studies have demonstrated the persistence of plant DNA in the soil (Gebhard & Smalla 1999; Paget & Simonet 1994; Widmer et al. 1996; Paget & Simonet 1997; Widmer et al. 1997). Bacteria residing on the plant surface can access nutrients leaking from the leaf or exuded from the root and they often aggregate in biofilms that can facilitate cell-to-cell contact and thereby possibly DNA transfer. Several studies have also demonstrated the persistence of plant DNA in the gastrointestinal tract of animals, in contact with the microorganisms that colonise the whole length of the gastrointestinal tract and aid in the digestive process. However, the proportion of DNA which may derive from the introduced genes of GM plants in the animal diet is extremely low (see Section 3.2.3).

BACTERIAL COMPETENCE AND DNA UPTAKE

279. The major limiting factor for natural transformation remains the presence of potentially competent bacterial species and the development of competence (Smalla et al. 2000). Competence in bacteria is not usually constitutively expressed: bacterial species that are transformable need to enter a physiologically regulated state of competence for the uptake of exogenous DNA (Lorenz & Wackernagel 1994). Few bacteria induced to express competence in the laboratory have subsequently been shown to be able to express competence under natural conditions (Nielsen 1998).

280. Electrical fields and current are also known to be capable of permeabilising bacterial cell membranes under laboratory conditions, facilitating experimental transformation. Given that the environment is subjected to regular thunderstorms and lightning discharges that induce enormous electrical perturbations, the possibility of natural electro-transformation of bacteria has been investigated. Bacteria added to soil have been transformed via simulated lightning in the laboratory (Demaneche et al. 2001), however there is no direct evidence that this is occurring in nature.

DNA INTEGRATION

281. Integration of genes into the genome of recipient bacteria is known to be dependent on sequence homology between the captured DNA and that of the recipient bacteria. It seems that heterology between these sequences is the main barrier to the stable introduction of diverged DNA in bacteria (Baron 1968; Rayssiguier 1989; Matic 1995; Vulic 1997). In enterobacteria there is an exponential relationship between recombination frequencies and sequence similarity of introduced DNA (Vulic et al. 1997). Although there is a higher probability of recombination when the sequences are more similar, the consequent risk of adverse effect is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties is low.

EXPRESSION AND SELECTION

282. Even if the barriers to uptake and integration are overcome, there are also barriers to expression of the exogenous genes. For example:

- many plant promoters will not be active in bacteria;
- processing of the intermediate RNA may be required for protein expression (eg. removal of introns to generate functional mRNA for translation), which will not occur in bacteria;
> coding sequences of plant genes may not be efficiently translated in bacteria due to differences in codon usage (note that the coding sequences of the bacterially derived \textit{vip3A}, \textit{bar} and \textit{hph} genes were modified to enhance expression in plants); and
> processing of an encoded ‘pro-protein’ may be required for production of a functional product.

283. Prokaryotes have efficient genomes and generally do not contain extraneous DNA sequences. If the genes are not useful to the organism then there will be no selective advantage in maintaining them in the genome, and they are not likely to persist. Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

\textbf{Viruses}

284. There is a theoretical possibility of recombination between sequences that have been introduced into the genome of GM plants and the genome of viruses that infect the plants (Ho et al. 2000; Hodgson 2000a; Hodgson 2000b). Recombination between viral genomes and plant DNA has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, eg regeneration of infectious virus by complementation of a defective virus by viral sequences introduced into a GM plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999). With homologous sequences the consequent risk of adverse effects arising from gene transfer is reduced because with highly similar sequences the likelihood of any recombinants expressing novel properties is low.

285. Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

\textbf{Fungi}

286. Fungi are known to be transformable, and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by \textit{Plasmodiophora brassicae} (Bryngelson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by \textit{Aspergillus niger} (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen et al. 1998)

287. Thus the risk of gene transfer leading to hazardous consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.2.2).

\textbf{SECTION 3  GENE TRANSFER FROM GM INSECTICIDAL AND INSECTICIDAL/HERBICIDE TOLERANT COTTONS TO ANIMALS}

\textbf{Section 3.1  Nature of the gene transfer hazard}

288. The potential hazards associated with the introduced genes in GM insecticidal and insecticidal/herbicide tolerant cottons transferring to animals, including humans, could be highly varied,
broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny.

Section 3.1.1 Potential hazards from the introduced genes

THE VIP3A (INSECTICIDAL) GENE

289. Animals could become toxic to lepidopteran insects. This is not likely to pose any consequences for lepidopteran insects, nor would such a transfer confer a selective advantage to the animal.

THE BAR (HERBICIDE TOLERANCE) GENE

290. The expression of this gene in animals would not be expected to lead to any adverse effects, since animals are not controlled by glufosinate ammonium. Furthermore, expression of the PAT enzyme in an animal would not be expected to produce any adverse metabolic effect, since PAT has extremely high substrate specificity for L-PPT, and cannot acetylate any other amino acid or any protein (Wehrmann et al. 1996) (Canadian Food Inspection Agency 1995).

THE HPH (ANTIBIOTIC RESISTANCE) GENE

291. Animals could gain the ability to degrade the antibiotic hygromycin. If the transfer occurred to humans or other animals, which may be treated with this antibiotic, treatment may be affected. However the gene product, the HPT enzyme, would only be active within the animal cells, where appropriate conditions and co-factors for its activity exist, therefore interference with any antibiotic treatment is unlikely. Animals are not controlled by antibiotics, so no selective advantage would result.

PROMOTERS AND OTHER REGULATORY SEQUENCES

292. If these sequences were to be transferred to animals without the associated genes of GM cottons, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on the resulting phenotypic change induced. However the same is true of any plant gene regulatory sequences, if transferred into a new genetic context. Thus the potential hazard is generally not increased relative to that of transfer from non-GM plants.

293. Some of the introduced regulatory sequences are derived from plant pathogens (Agrobacterium tumefaciens). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

294. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.
Section 3.2  Likelihood of hazard arising through transfer from GM insecticidal and insecticidal/herbicide tolerant cottons to animals (including humans)

295. The likelihood of gene transfer creating a hazard for human health and safety or the environment depends on the characteristics of introduced gene sequences, as well as on the likelihood of transfer itself, as discussed in following sub-sections.

3.2.1 Humans

296. The most significant route for entry of foreign DNA into humans is through food, as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract is exposed to foreign DNA released from food. Microorganisms colonise the whole length of the gastrointestinal tract, aiding the digestive process.

297. No products from the GM cottons in the proposed field trials will be used for human food. Thus, the likelihood of gene transfer to humans is negligible. It is worth noting that cottonseed oil and linters are the only fraction of cotton plants used in human food. Since these processed products are free of DNA, even if products of the GM cottons were approved by FSANZ for use in food, humans would not be exposed to GM cotton DNA via the digestive system, excluding the possibility of gene transfer to human cells in the gut.

3.2.2 Animals

298. GM cottonseed from the proposed field trials will not be fed to livestock. If GM cottonseed were fed to animals, the most significant route for entry of foreign DNA, as with humans, would be through food as it passes through the gastrointestinal tract. Thus, the likelihood of gene transfer to animals is negligible. The fate of DNA in the digestive tract of various animals has been studied and is discussed in the risk assessment for DIR 021/2002 and DIR 22/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment (Section 2.2.2).

SECTION 4  CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS

Section 4.1  Conclusions regarding gene transfer to other plants

299. It is considered that although some gene transfer from GM cottons to cultivated cotton (of both *G. hirsutum* and *G. barbedense*) at a low level is likely, the risks posed are low because:
   - gene transfer would not pose any risks additional to the low risks posed by GM insecticidal and insecticidal/herbicide tolerant cottons.

300. Licence conditions have been imposed to limit gene transfer to cultivated cotton (See Chapter 2, Appendix 7). Additional licence conditions have been imposed to establish a 400 m wide Research Zone for the purpose of conducting research to inform the ongoing review of the efficacy of containment measures.

301. Although transfer of the introduced genes from GM cottons to naturalised cotton (both *G. hirsutum* and *G. barbedense*) may increase the likelihood that the genes could spread and/or persist
in the environment, it is considered that the likelihood of a risk arising through gene transfer to volunteer or naturalised cotton is low, because:

- gene transfer would not pose any risks additional to the low risks posed by GM insecticidal and insecticidal/herbicide tolerant cottons; and
- gene transfer to naturalised cotton populations is thought to be unlikely because of the geographic isolation.

302. Licence conditions have been imposed to limit gene transfer to naturalised cotton (See Chapter 2, Appendix 7).

303. It is considered that the risk of gene transfer from GM cottons to native cotton species is negligible, because:

- genetic incompatibility and geographical isolation prevent the production of fertile hybrids.

304. It is considered that the risk of gene transfer from GM cottons to other plant genera is negligible, because:

- well established genetic incompatibility prevents successful cross pollination with other plant species.

Section 4.2 Conclusions regarding gene transfer to microorganisms

305. It is considered that the risk of a hazard arising through transfer of the introduced genes from GM cottons to microorganisms is negligible, because:

- all of the introduced genes in GM cottons are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and
- gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.

306. Therefore, no management conditions have been imposed in the licence.

Section 4.3 Conclusions regarding gene transfer to animals, including humans

307. Products from the GM cottons will not be allowed to be fed to animals or enter the human food supply. The most significant route of entry of foreign DNA into animals and humans is through food. Thus the likelihood of gene transfer to animals or humans is negligible.

308. It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely affected.

309. Therefore, no management conditions have been imposed in the licence.
APPENDIX 6 INSECTICIDE AND HERBICIDE RESISTANCE

310. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this Appendix, risks posed by the proposed dealing to the environment are considered in relation to the potential for development of herbicide resistance among weeds, and insecticide resistance among targeted pests.

311. Regulation of agricultural chemicals, including insecticides and herbicides, is principally the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the Agricultural and Veterinary Chemicals Code (1994). The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to a product that is already on the market must also be referred to the APVMA. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of a chemical product, for example for a limited period of time or for a limited area.

312. 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 efficacy of herbicides and insecticides, and herbicide and insect resistance management. The APVMA can impose conditions on the use of chemical products in registrations and permits. These conditions can include implementation of insect resistance management or herbicide resistance management plans, and ongoing reporting on compliance and effectiveness.

313. All submissions to the APVMA are treated on their merits and applicants are free to address any issue through the provision of data or through scientific argument. The APVMA can only grant a permit if it is satisfied that the use of a product as specified in the permit:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues;
- would not be likely to have an effect that is harmful to human beings;
- would not be likely to have an effect that is harmful to animals, plants or things or to the environment;
- would not unduly prejudice trade or commerce between Australia and places outside of Australia;
- would be effective according to criteria that the APVMA has proposed for the product;
- that any requirements prescribed by the regulations have been complied with;
- if the product is not registered, that there are reasonable grounds for an application not having been made or for issuing a permit pending determination of an application for registration; and
- that the applicant is a suitable person to hold the permit, according to criteria determined by the APVMA.

314. As part of its charter, the APVMA also manages a national compliance program in partnership with the States and Territories to ensure that products are used in accordance with their registration and their labels continue to meet the conditions of approval.
315. The APVMA and the OGTR work closely together to ensure thorough coordinated assessments are undertaken and, wherever possible, that timing of assessments and decisions by both agencies coincide. Further information about how the APVMA’s assessment and approval processes can be obtained from their website www.apvma.gov.au.

SECTION 1 INSECTICIDE RESISTANCE HAZARD

316. If the GM cotton were cultivated extensively, *Helicoverpa armigera*, *H. punctigera* and other susceptible lepidopteran insect species that feed on the GM cottons could be placed under selection pressure for resistance to the VIP3A insecticidal protein. If resistance were to occur in target pests, the insecticidal efficacy of these GM cottons would be adversely affected, potentially attenuating the benefits of these GM cottons.

317. CSIRO has submitted an application to the APVMA for obtaining a research permit for the use of the insecticidal gene on the types of GM cotton that are for this release.

SECTION 2 HERBICIDE RESISTANCE HAZARD

318. Selection for herbicide resistance has an increased probability of occurring in the long-term if the Liberty® cotton was grown on a large scale and without taking any steps to manage this risk.

319. CSIRO has submitted an application to the APVMA for obtaining a research permit for the use of the herbicide Liberty® (formulation of glufosinate ammonium) on the types of GM cotton that are for this release. Further trials or commercial release of Liberty® cotton will also require further applications and approvals by the Regulator. The applicant may need to consider providing additional information on the management of herbicide resistance if, or when, they seek approval for the registration of changed use of a herbicide.

SECTION 3 CONCLUSIONS REGARDING INSECTICIDE AND HERBICIDE RESISTANCE

320. This hazard will be assessed by the APVMA in considering CSIRO’s permit application for the use of the insecticidal gene as an insecticide in the insecticidal GM cotton lines and the use of Liberty® herbicide on the glufosinate ammonium tolerant cotton lines, respectively. Therefore, the Regulator has imposed no specific conditions in the licence in relation to management of insecticide or herbicide resistance, however the requirement to comply with any conditions imposed by the APVMA has been noted.
APPENDIX 7 LICENCE CONDITIONS

Note in relation to approval of genetically modified foods for human consumption

Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority), is responsible for human food safety assessment. Currently, CSIRO has not applied to FSANZ for evaluation of material from the GM cottons for use in human food. FSANZ approval would need to be obtained before any parts of the GM cottons such as oil and linters derived from GM cottonseed could be used as human food.

Note in relation to herbicide tolerance and insecticide resistance management

The Gene Technology (Consequential Amendments) Act (2000) requires the Australian Pesticides and Veterinary Medicines Authority (APVMA, formerly the National Registration Authority for Agricultural and Veterinary Chemicals, NRA) to consult the Gene Technology Regulator for the purposes of making certain decisions regarding registration or issuing a permit for a chemical product that is or contains a genetically modified product.

One of the genetically modified organisms (GMOs) referred to in this licence has been modified to be tolerant to a herbicide. The APVMA has responsibility for setting registration conditions for the use of herbicides in Australia, including implementation of herbicide resistance management programs. Conditions of this licence do not relate to use of herbicide, and do not replace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to the use of herbicides in connection with these GMOs.

The GMOs referred to in this licence also falls into the Agricultural and Veterinary Chemicals Code (1994) definition of an agricultural chemical product, due to the production of an insecticidal substance, and therefore is subject to regulation by the APVMA. Conditions of this licence do not relate to management of insecticide resistance, and do not replace any conditions set by the APVMA. The licence holder must comply with any conditions imposed by the APVMA in relation to dealings with this GMO.
SECTION 1 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 CSIRO.

Project Supervisor

3. 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

4. Persons covered by this licence must not deal with the GMO except as expressly permitted or contemplated by this licence.

GMOs covered by this licence

5. The GMOs covered by this licence are insecticidal (COT102 and COT200 series) and insecticidal/herbicide tolerant (COT102/LL25) Cottons.

Permitted dealings

6. 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.

Persons covered by this GMO licence

7. 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 the GMOs grown in a Location pursuant to this Licence.

Informing people of their obligations

8. The licence holder must inform each 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.

9. 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.
Licence holder to notify of circumstances that might affect suitability

10. 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 licence holder of this licence to meet the conditions in it.

Additional information to be given to the Regulator

11. The licence holder must 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.

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

12. 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

13. The licence holder must at all times remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.
SECTION 2 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:

- Words and phrases used in this licence have the same meanings as they do in the Act and the Regulations;
- Words importing a gender include any other gender;
- Words in the singular include the plural and words in the plural include the singular;
- Words importing persons include a partnership and a body whether corporate or otherwise;
- 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;
- Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word or phrase has a corresponding meaning;

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;
- 'Clean' (or 'Cleaned'), as the case requires, means:
  - (a) in relation to a Location or other area, the Destruction of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants 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, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants 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) root cutting; or
  - (d) burning; or
(e) treatment with herbicide; or
(f) hand weeding.

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 alone 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 genetically modified organism or organisms authorised for release by this licence;

'Location' means an area of land where the GMOs are planted and grown;

'Material from Pollen Trap plants' means seed, stubble, pollen or any material (including parts of a plant) that is derived from or produced by Cotton from a Pollen Trap;

'Material from the GMOs' means material (including part of a plant) that is derived from or produced by the GMOs;

'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;

'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 Cotton from a Pollen Trap;

'Regulator' means the Gene Technology Regulator;

'Research Zone' means an area of land, extending at least 400 metres in all directions from the outside edge of a Location;

'Volunteer plants' means progeny of the GMOs or Pollen Trap plants, or regrowth of GM or non-GM Cotton plants.
SECTION 3 SPECIFIC CONDITIONS

Locations and size of trial

1. The permitted dealings with the GMOs may be undertaken within the Shires set out below in Table 1.

Table 1. Shires in which the GMOs may be grown

<table>
<thead>
<tr>
<th>NSW</th>
<th>QLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bourke</td>
<td>Balonne</td>
</tr>
<tr>
<td>Carathool</td>
<td>Banana</td>
</tr>
<tr>
<td>Gunnedah</td>
<td>Bauhinia</td>
</tr>
<tr>
<td>Moree Plains</td>
<td>Emerald</td>
</tr>
<tr>
<td>Narrabri</td>
<td>Milmeran</td>
</tr>
<tr>
<td>Walgett</td>
<td>Murilla</td>
</tr>
<tr>
<td>Warren</td>
<td>Pittsworth</td>
</tr>
<tr>
<td></td>
<td>Waggamba</td>
</tr>
<tr>
<td></td>
<td>Wambo</td>
</tr>
<tr>
<td></td>
<td>Warroo</td>
</tr>
</tbody>
</table>

2. The maximum number of Locations where permitted dealings may be conducted are set out in Table 2 at Column 2. The maximum combined area of all Locations where permitted dealings may be occur is limited to those set out in Table 2 at Column 3. The GPS coordinates for the 2003/2004 plantings are given in Table 3.

Table 2. Maximum numbers of Locations and combined areas

<table>
<thead>
<tr>
<th>Growing season</th>
<th>Maximum number of Locations</th>
<th>Maximum combined area of all Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer 2003/2004</td>
<td>4</td>
<td>1.1 ha</td>
</tr>
<tr>
<td>Summer 2004/2005</td>
<td>5</td>
<td>3 ha</td>
</tr>
<tr>
<td>Summer 2005/2006</td>
<td>6</td>
<td>18 ha</td>
</tr>
</tbody>
</table>

Table 3. GPS coordinates for 2003/2004 plantings

<table>
<thead>
<tr>
<th>Trait</th>
<th>Field (Narrabri)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>COT102</td>
<td>S</td>
<td>30°12.717</td>
<td>30°12.723</td>
<td>30°12.727</td>
<td>30°12.721</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>149°35.811</td>
<td>149°35.837</td>
<td>149°35.835</td>
<td>149°35.811</td>
</tr>
<tr>
<td>COT102/200</td>
<td>S</td>
<td>30°12.360</td>
<td>30°12.360</td>
<td>30°12.360</td>
<td>30°12.360</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>149°35.340</td>
<td>149°35.340</td>
<td>149°35.340</td>
<td>149°35.340</td>
</tr>
<tr>
<td>COT102</td>
<td>A2</td>
<td>30°11.846</td>
<td>30°11.852</td>
<td>30°11.897</td>
<td>30°11.891</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>149°36.404</td>
<td>149°36.441</td>
<td>149°36.432</td>
<td>149°36.395</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>30°11.894</td>
<td>30°11.900</td>
<td>30°11.943</td>
<td>30°11.938</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>149°36.344</td>
<td>149°36.380</td>
<td>149°36.372</td>
<td>149°36.335</td>
</tr>
<tr>
<td>COT200</td>
<td>L1</td>
<td>30°10.751</td>
<td>30°10.758</td>
<td>30°10.761</td>
<td>30°10.753</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>149°35.653</td>
<td>149°35.670</td>
<td>149°35.669</td>
<td>149°35.652</td>
</tr>
</tbody>
</table>

3. Planting the GMOs must not commence after 31 December 2005.

4. 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.
5. At least 7 days prior to commencing to grow the GMOs at a Location, the Location’s GPS coordinates and either a street address, or other directions to the Location, must be provided to the Regulator by notice in writing.

Notification of planting of the GMOs

6. The licence holder must provide notices in writing to the Regulator of the actual date or dates of commencement of planting of the GMOs at each Location (and Pollen Trap in respect of each Location) (the actual planting date notice). This notice must be provided within 7 days of commencement of planting of the GMOs at the Location.

Notification of commencement of flowering of the GMO

7. The licence holder must provide notices in writing to the Regulator in respect of each of the following:

   (a) the short term forecasted date or dates of commencement of flowering of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the short term forecast flowering date notice’). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of flowering set out in the notice; and

   (b) the actual date or dates of commencement of flowering of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the actual flowering date notice’). This notice must be provided within 7 days of commencement of flowering of the GMOs at a Location.

Notification of commencement of seed set of GMO

8. The licence holder must provide notices in writing to the Regulator in respect of each of the following:

   (a) the short term forecasted date or dates of commencement of seed set of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the short term forecast seed set date notice’). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of seed set, as set out in the notice; and

   (b) the actual date or dates of commencement of seed set of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the actual seed set date notice’). This notice must be provided within 7 days of commencement of seed set of the GMOs at a Location.

Notification of commencement of harvest of GMOs

9. The licence holder must provide notices in writing to the Regulator in respect of each of the following:

   (a) the short term forecasted date or dates of commencement of harvesting of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the short term forecast harvest date notice’). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of harvesting set out in the notice; and

   (b) the actual date or dates of commencement of harvesting of the GMOs at each Location (and Pollen Trap in respect of each Location) (‘the actual harvest date notice’). This notice
Pollen Traps

10. A Pollen Trap must surround every Location.

11. Each Pollen Trap must contain non-GM Cotton that is grown in such a way as to reasonably promote a dense and vigorous growth and flowering of the non-GM Cotton at the same time as the GMOs.

12. 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.

13. 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 Material from Pollen Trap plants must be handled and controlled as if it is Material from the GMOs (ie subject to other applicable conditions elsewhere in this licence).

14. 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.

Research Zones

15. Each Location in excess of one hectare in size that is planted to the GMOs must be surrounded by a Research Zone unless the licence holder has a notice in writing from the Regulator that a Research Zone in connection with the Location is not required.

16. Each Research Zone must be able to be accessed and controlled by the licence holder to the extent necessary to enable the licence holder to meet its obligations under this licence to conduct research in the Research Zone.

Harvest and post-harvest procedures

17. If the GMOs or Pollen Trap plants are harvested, they must be harvested separately from any other Cotton.

18. If seed Cotton harvested from the GMOs or from Pollen Trap plants is ginned, it must be ginned separately from any other Cotton.

19. The licence holder must ensure that the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants are not consumed by humans or used for stockfeed.

20. 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 cottonseed is stored within the facility;
   (b) exported; or
   (c) Destroyed by burning.

21. Any GM 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).

22. Cotton lint obtained from ginning of seed Cotton harvested from the GMOs or Pollen Trap plants may be sold.
Cleaning - post harvest and generally

23. Equipment, a Location or other area used pursuant to this licence in respect of GMOs, Material from GMOs, Pollen Trap plants or Material from Pollen Trap plants, must be Cleaned.

24. For each Location, either within 14 days of harvest of the GMOs or by 30 June 2006, whichever occurs first, the Location must be Cleaned.

25. 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.)

26. Cleaning must occur immediately or as soon as practicable after the 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 Cotton is ginned).

27. 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

28. Following Cleaning of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants at a Location or other area, the following places must be monitored 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; and
   (d) any areas used to Clean Equipment used in connection with the GMOs or to Destroy the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.

29. Inspection must be performed by a person who is able to recognise Volunteer plants.

30. All the places required to be monitored must be monitored either at least once every 2 months for a period of at least 12 months that commences on the last day of Cleaning of the Location, or until the GMOs are once again grown at the Location in a new growing season, pursuant to this Licence.

31. The results of inspection activities must be recorded in a logbook. The logbook must be available on request for examination or photocopying by the OGTR. The findings of the inspections as recorded in the logbook must be included in the licence holder's annual report to the Regulator. The logbook 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 number of Volunteer plants observed, if any;
   (e) details of the development stages reached by the Volunteer plants, if any; and
   (f) details of methods used to Destroy Volunteer plants, if any.
32. Any Volunteer plant identified must be Destroyed prior to the plant flowering.

**General conditions on use of Locations post-harvest**

33. If the GMOs are grown at a Location, no other Cotton plant of any kind may be grown at the Location, or Pollen Trap in respect of the Location, after harvest of the GMOs or Pollen Trap plants, except in accordance with this licence, until inspection obligations are completed.

34. If the GMOs are grown at a Location, no plants may be planted at the Location, or Pollen Trap in respect of the Location, except in accordance with this licence, until inspection obligations are completed unless:

   (a) the plants are grasses (grass pastures), cereals (cereal crop); or
   (b) the plants are plants agreed to in writing by the Regulator; and
   (c) the Regulator is satisfied that inspection and Destruction of Volunteer plants prior to flowering will not be adversely affected by the planting.

**Transportation of the GMOs, Material from GMOs, Pollen Trap plants and Material from Pollen Trap plants**

35. Subject to the conditions immediately below in respect of transportation, the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants must be transported in accordance with the OGTR Guidelines for the Transport of GMOs (June 2001) issued by the Regulator.

36. Harvested GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants 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'); or
   (b) completely enclosed within a layer of tarpaulin inside a layer of shade cloth ('double wrapped in tarpaulin and shade cloth');
   (c) contained within an enclosed chain-bed truck specifically designed for the purpose of transporting Cotton modules.

*Explanatory note: double wrapping is intended to prevent dissemination of the enclosed material during transportation.*

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

38. Every container used to transport the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants must be labelled:

   (a) to indicate that it contains GM 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.

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

40. Within 30 days of the date of the commencement 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, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants outside an area that must be inspected.

41. 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, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants; and
   (c) inspect and Destroy any Volunteer plants that may exist as a result of the event.

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

Compliance Management Plan

43. 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

44. The licence holder must provide the Regulator with a written report within 90 days of each anniversary of this licence, in accordance with any Guidelines issued by the Regulator in relation to annual reporting. This report must include information on any adverse impacts on human health and safety or the environment, caused as a result of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.

Research requirements

45. The licence holder must, in consultation with the OGTR, develop an agreed research program to collect information regarding:
   (a) the potential toxicity of the introduced proteins on non-target organisms, including the persistence of VIP3A protein in the soil under Australian conditions;
   (b) the agronomic characteristics indicative of potential weediness of the GM Cottons under Australian conditions;
   (c) validation of previous research on the efficacy of the Pollen Trap; and
   (d) validation of previous research on gene transfer from GM Cotton to other Cotton using the Research Zone.

46. In accordance with any Guidelines issued by the Regulator in relation to annual reporting, the Licence holder must provide the Regulator with a written report of the progress and results of the research program. This report must accompany the annual report to be sent to the Regulator.
Testing methodology

47. 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 in a recipient organism. The instrument must be provided within 12 months of the issuing of this licence.
APPENDIX 8 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

310. The Gene Technology Act 2000 (the Act) took effect on 21 June 2001. The Act, supported by the Gene Technology Regulations 2001, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia’s nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

311. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

312. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Commonwealth regulatory agency located within the Health and Ageing portfolio.

313. The Act prohibits persons from dealing with GMOs unless the dealing is exempt, a Notifiable Low Risk Dealing, on the Register of GMOs, or licensed by the Regulator (see Section 31 of the Act).

314. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans (RARMPs) are discussed in detail in Division 4, Part 5 of the Act and summarised below.

315. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website (www.ogtr.gov.au).

SECTION 2 THE LICENCE APPLICATION

316. Licence applications for dealings involving the intentional release (DIR) of a genetically modified organism into the environment must be submitted in accordance with the requirements of Section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:

- the parent organism;
- the GMOs;
- the proposed dealing with the GMOs;
- interaction between the GMOs and the environment;
- risks the GMOs may pose to the health and safety of people;
- risk management;
- previous assessments of approvals; and
- the suitability of the applicant.
317. The application must also contain:

- additional information required for a GMO that is:
  - a plant;
  - a micro-organism (not living in or on animals and not a live vaccine);
  - a micro-organism that lives in or on animals;
  - a live vaccine for use in animals;
  - a vertebrate animal;
  - an aquatic organism;
  - an invertebrate animal;
  - to be used for biological control;
  - to be used for bioremediation; and
  - intended to be used as food for human or vertebrate animal consumption;

- supporting information from the Institutional Biosafety Committee.

318. A preliminary screening of an application is undertaken by OGTR staff to determine whether it complies with the Act and the Regulations, by containing the required information. If this information is provided in the application, the Regulator may then accept the application for formal consideration. Section 43 of the Act provides that the Regulator is not required to consider an application if the application does not contain the required information.

319. After accepting an application for consideration, the Regulator must decide to issue, or refuse to issue, a licence. The decision must be taken following an extensive consultation and evaluation process, as detailed in Sections 3-6 of this Appendix. Regulation 8 of the Regulations prescribes a period of 170 working days within which this decision must be taken. This period does not include weekends or public holidays in the Australian Capital Territory. Also, this period does not include any days in which the Regulator is unable to progress the application because information sought from the applicant in relation to the application has not been received.

SECTION 3 THE INITIAL CONSULTATION PROCESSES

320. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a RARMP from prescribed agencies:

- State and Territory Governments;
- the Gene Technology Technical Advisory Committee (GTTAC);
- prescribed Australian Government agencies (Regulation 9 of the Gene Technology Regulations 2001 refers);
- the Environment Minister; and
- relevant local council(s) where the release is proposed.

321. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people
or to the environment, the Regulator must publish a notice (in national and regional newspapers, in the Gazette and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

322. As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application’s receipt on the OGTR website and directly advising those on the OGTR mailing list. Copies of applications are available on request from the OGTR.

SECTION 4 THE EVALUATION PROCESSES

323. The risk assessment process is carried out in accordance with the Act and Regulations, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories, Commonwealth government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

324. In undertaking a risk assessment, the following are considered and analysed:

- the data presented in the proponent’s application;
- data provided previously to GMAC, the interim OGTR or the OGTR in respect of previous releases of relevant GMOs;
- submissions or advice from States and Territories, Commonwealth agencies and the Environment Minister and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

325. In considering this information and preparing the RARMP, the following specific matters are taken into account, as set out in Section 49 and required by Section 51 of the Act:

- the risks posed to human health and safety or risks to the environment;
- the properties of the organism to which the dealings relate before it became a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred on the properties of the organism;
- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings;
- any likely impacts of the proposed dealings on the health and safety of people.
326. In accordance with Regulation 10 of the Regulations, the following are also taken into account:

- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
- the potential of the GMO concerned to:
  - be harmful to other organisms;
  - adversely affect any ecosystems;
  - transfer genetic material to another organism;
  - spread, or persist, in the environment;
  - have, in comparison to related organisms, a selective advantage in the environment; and
  - be toxic, allergenic or pathogenic to other organisms.
- the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

327. Having prepared a risk assessment and a risk management plan, the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.

328. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. Section 56 of the Act requires that these be taken into account in making a decision on whether or not to issue a licence for the proposed release.

329. Comments received in written submissions on this RARMP are very important in shaping the final RARMP and in informing the Regulator’s decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the final RARMP.

330. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to protect human health and safety and the environment. Matters in submissions that do not address these issues and/or concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

SECTION 6 DECISION ON LICENCE

331. Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (Section 55 of the Act). The Regulator must not issue the licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.
332. The Regulator must also have regard to any policy guidelines issued by the Ministerial Council that relate to risks to human health and safety and the environment, or the management of such risks. At this time no policy guidelines have been issued.

333. The Regulator must also not issue a licence if this would be inconsistent with a policy principle issued by the Ministerial Council. The Gene Technology Ministerial Council recently issued a policy principle “Gene Technology (Recognition of Designated Areas) Principle 2003” (the Principle), which allows for recognition of GM or non-GM designated areas for marketing purposes. The Principle is designed to ensure the valid operation of State and Territory laws declaring areas to be GM, non-GM or both for marketing purposes.

334. The Regulator must also be satisfied, under section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence eg.:
   - any relevant convictions;
   - any relevant revocations or suspensions of a licences or permits; and
   - the capacity of the person or company to meet the conditions of the licence.

335. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

336. The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.

337. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). For example, conditions may be imposed to:
   - limit the scope of the dealings;
   - require documentation and record-keeping;
   - require a level of containment;
   - specify waste disposal methods;
   - manage risks posed to the health and safety of people, or to the environment;
   - require data collection, including studies to be conducted;
   - limit the geographic area in which the dealings may occur;
   - require contingency planning in respect of unintended effects of the dealings; and
   - limit the dissemination or persistence of the GMO or its genetic material in the environment.

338. It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (Section 63 of the Act). Access to the site of a dealing must also be provided to persons authorised by the Regulator for the purpose of
auditing and monitoring the dealing and compliance with other licence conditions (Section 64 of the Act). It is a condition of any licence that the licence holder inform the Regulator of:

- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
- any contraventions of the licence by a person covered by the licence; and
- any unintended effects of the dealings authorised by the licence.

339. It should be noted that, as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to enforce compliance with licence conditions, and indeed, to direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors trial sites to determine whether the licence holder is complying with the licence conditions, or whether there are any unintended effects.
APPENDIX 9  SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

Submission from: A: agricultural organisation; I: individual; F: food interest organisation.


<table>
<thead>
<tr>
<th>Sub. No.</th>
<th>Type</th>
<th>Summary of issues raised</th>
<th>Issue</th>
<th>Consideration of issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Maintenance of food integrity and consumer confidence and the effects on markets</td>
<td>MA</td>
<td>OSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GM tracking and tracing, communication and labelling</td>
<td>FSANZ</td>
<td>OSA</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>Govern any release of GMOs using the precautionary principle until more research completed.</td>
<td>RA, D</td>
<td>App 2-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maintain State and Territory Government measures to delay the commercial release of GM food crops.</td>
<td>SG</td>
<td>OSA</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Benefits of insecticidal and herbicide tolerant transgenic cotton on industry and environment.</td>
<td>EN</td>
<td>OSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction in pesticide use due to GM cottons</td>
<td>PU, EN</td>
<td>OSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The GM cottons will allow successful control of weeds in cotton.</td>
<td>PU</td>
<td>OSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced risk of weeds developing resistance.</td>
<td>HR, RM</td>
<td>App 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lack of cotton plants establishing themselves as weeds</td>
<td>W</td>
<td>App 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Likelihood of the GM cottons becoming weeds is low</td>
<td>W</td>
<td>App 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Supports the proposed field trials</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 10 REFERENCES


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