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

# Risk Assessment and Risk Management Plan

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

## DIR 044/2003

## Title: Agronomic assessment and seed increase of GM cottons expressing insecticidal genes (*cry1Fa* and *cry1Ac*) from *Bacillus thuringiensis*)

Applicant: Dow AgroSciences

May 2004

## ABBREVIATIONS

| | |
|---|---|
| ANZFA | Australia New Zealand Food Authority (now FSANZ) |
| APVMA | Australian Pesticides and Veterinary Medicines Authority |
| *bar* | gene encoding a phosphinothricin acetyl transferase (PAT) protein |
| Bt | *Bacillus thuringiensis* |
| Bta | *Bacillus thuringiensis* variety *aizawai* |
| Btk | *Bacillus thuringiensis* variety *kurstaki* |
| CCI | Confidential Commercial Information |
| Cry | crystal insecticidal proteins of Bt |
| *cry1Ac* | gene encoding the Cry1Ac insecticidal protein |
| Cry1Ac | Cry1Ac insecticidal protein |
| *cry1Fa* | gene encoding the Cry1Fa insecticidal protein |
| Cry1Fa | Cry1Fa insecticidal protein |
| DIR | dealing involving intentional release |
| DNA | deoxyribonucleic acid |
| ELISA | enzyme linked immunosorbent assay |
| FAO | Food and Agriculture Organisation of the United Nations |
| FSANZ | Food Standards Australia New Zealand (formerly ANZFA) |
| g | gram |
| GM | genetically modified |
| GMO | genetically modified organism |
| GTTAC | Gene Technology Technical Advisory Committee |
| ha | hectare |
| IgE | Immunoglobulin E |
| m | metre |
| MRL | maximum residue limit |
| mRNA | messenger ribonucleic acid |
| nos | nopaline synthase gene |
| NRA | National Registration Authority for Agicultural and Veterinary Chemicals |
| OECD | Organisation for Economic Cooperation and Development |
| OGTR | Office of the Gene Technology Regulator |
| ppm | parts per million |
| *pat* | gene encoding the phosphinothricin acetyl transferase (PAT) protein |
| PAT | phosphinothricin acetyl transferase (PAT) protein |
| T-DNA | transfer deoxyribonucleic acid of Agrobacterium tumefaciens |
| TGA | Therapeutic Goods Administration |
| USDA | United States Department of Agriculture |
| US EPA | United States Environmental Protection Agency |
| US FDA | United States Food and Drug Administration |
| WHO | World Health Organisation |
| µg/g | microgram per gram |

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# EXECUTIVE SUMMARY

## INTRODUCTION

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 or the environment that can not be managed. As part of the evaluation process, 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. 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 Act is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication. The OGTR liaises closely with other regulators to ensure the identification, evaluation and management of risks that may be associated with development and use of gene technology.

The Regulator has made a decision to issue a licence in respect of application DIR 044/2003 from Dow AgroSciences Australia Pty Ltd (Dow AgroSciences).

## THE APPLICATION

Dow AgroSciences has applied for a licence (application DIR 044/2003) for the intentional release, under limited and controlled conditions, of three genetically modified (GM) insecticidal/herbicide tolerant cotton lines[1]. Dow AgroSciences initially proposed to conduct trials over two summer growing seasons and two winter growing seasons on up to 25 sites covering a total cumulative area of up to 10 hectares over 3 years (May 2004 to May 2006) in New South Wales, Queensland, Northern Territory and Western Australia. However, the company subsequently amended their application to slightly increase the number and size of sites (up to 30, covering a total cumulative area of up to 12.2 hectares over the same 3 years) and withdrew the request for trialing in Northern Territory.

In accordance with the provisions of section 185 of the Act, Dow AgroSciences sought and received approval for some specific documents, which contain details of the gene constructs, gene sequence information and molecular characterisation of the inserted genetic materials, as Confidential Commercial Information (CCI), in connection with a previous licence application DIR 040/2003.

[1] The term 'line' has been used, throughout this risk assessment, to denote cotton containing a specific genetic modification derived from specified individual transformation event/s (note that Widestrike™ cotton contains 2 events).

While the Regulator is satisfied that the public interest in the release as proposed did not outweigh the prejudice that disclosure would cause the applicant, the CCI was made available to the various prescribed expert groups that were consulted on the preparation of the RARMP for this application.

Two of the three GM cotton lines proposed for release contain a single insecticidal gene, either *cry1Fa* (line 281-24-236 or Cry1Fa cotton) or *cry1Ac* (line 3006-210-23 or Cry1Ac cotton). The third line (line 281-24-236/3006-210-23 or WideStrike™ cotton) contains both *cry1Fa* and *cry1Ac* genes, and was produced by conventional breeding of the two single-gene insecticidal cotton lines. The insecticidal genes were derived from the common soil bacterium *Bacillus thuringiensis* (Bt) and express insecticidal proteins (Bt toxins) that are toxic to specific lepidopteran caterpillar insects, including the major caterpillar pests of cotton.

The *cry1Fa* and *cry1Ac* genes in these GM cotton lines are chimeric genes, each containing parts of two other *cry1* genes of Bt. The chimeric genes were developed to improve the level of expression of the encoded Cry1Fa and Cry1Ac proteins in plants, and enhance their solubility in the insect gut. However, the encoded Bt toxins are very similar to the native Cry1Fa and Cry1Ac proteins. Within the functional (core) toxin, the amino acid sequences of the native and chimeric proteins are 99.3% and 99.6% identical, respectively. Therefore, the species specificity of the introduced Cry1Fa and Cry1Ac proteins to lepidopteran larvae is the same as native bacterial Cry1Fa and Cry1Ac proteins.

All three lines also contain a herbicide tolerance selectable marker gene, *pat*, that confers tolerance to the herbicide glufosinate ammonium (the active ingredient of Liberty® and Basta® herbicides). The marker gene was used in the laboratory during the development of the GMOs for identification and selection of plant tissues in which the insecticidal genes were also present. Dow AgroSciences does not intend to apply glufosinate ammonium herbicide to the GM cotton lines in the proposed release.

Dow AgroSciences proposes to conduct a small scale, multi-site trial in a range of Australian cotton growing regions. The aims of the proposed release are: to test the efficacy (effectiveness) of the two-gene (*cry1Fa*/*cry1Ac*) insecticidal cotton line, WideStrike™ cotton, against lepidopteran caterpillars pests of cotton as compared to its two parental lines (Cry1Fa cotton and Cry1Ac cotton); to evaluate their respective agronomic performance; and to collect data for developing an insect resistance management plan. Additionally, the applicant proposes to measure the expression levels of the insecticidal proteins in cotton tissues and residues of these proteins in soil, and to test the effect of GM cotton lines on non-target organisms. Seed would also be retained for subsequent seasons of the trial or for potential future releases (subject to further approvals).

Note that Dow AgroSciences' decision whether to progress the commercialisation of these GM cotton lines in Australia will depend on the outcomes of these trials. If these GM cottons do not prove to be suitable for the Australian cotton production system during the first two seasons of the trial, Dow AgroSciences intends to stop the trial at the end of second season (2004/05 summer season). The efficacy data generated during the initial stages of this trial will inform future research and potential commercialisation plans. The GM cotton lines proposed for release are derived from the original American cultivar that was used in the initial breeding program. If Dow AgroSciences wishes to conduct additional trials, including breeding the GM insecticidal traits into cultivars more suitable for Australian conditions, additional licence applications and approvals would be required.

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. Following harvest, the applicant proposes that plant material remaining at the site will be slashed and incorporated into the soil by cultivation. Seed from pollen trap plants will also be destroyed. Any regrowth will be controlled by herbicide and/or cultivation. Cottonseed will be securely transported to prevent dissemination.

WideStrike™ cotton is currently being trialed by Dow AgroSciences under limited and controlled conditions on two sites covering a total area of 0.04 ha in New South Wales (DIR 040/2003). There have been no previous releases of either of the two single-gene insecticidal GM cotton lines (*cry1Fa* or *cry1Ac*) in Australia.

All three GM cotton lines proposed for release in the current application have been approved for field trials in the United States since 2001. These cotton lines are also approved for field trial in Argentina in 2004.

Previously, other GM insecticidal cottons containing *cry* insecticidal gene(s) derived from the same bacterium (*B. thuringiensis*) 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 022/2002 (INGARD® cotton) and DIR 012/2001 (Bollgard® II cotton). However, the chimeric *cry1Ac* gene in two of the GM cotton lines proposed for release in the current application differs slightly from the chimeric *cry1Ac* gene present in both INGARD® and Bollgard® II cottons.

Other GM plants incorporating the *pat* gene and expressing PAT protein have also been trialed in Australia (eg. DIR 010/2001, DIR 015/2002 and DIR 038/2003).

There have been no reports of adverse effects on human health or the environment resulting from these releases.

## THE EVALUATION PROCESS

A RARMP has been prepared in relation to licence application DIR 044/2003 from Dow AgroSciences in accordance with the Act, the Regulations, and the Risk Analysis Framework. This framework was developed as part of the establishment of the regulatory arrangements in consultation with the public, State, Territory and Australian Government agencies, key 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 she must consider in preparing a RARMP, are set out in Appendix 8 of the RARMP. The complete RARMP can be obtained from the OGTR by contacting the Office on 1800 181 030 or from its web site: *www.ogtr.gov.au*.

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

Through this process, potential hazards to human health and safety or the environment that may be posed by the proposed release of the three GM insecticidal/herbicide tolerant cotton lines were

identified. These have been evaluated to determine whether risks might arise, based on the likelihood of each hazard occurring and the likely impact of each hazard, were it to be realised.

The identified potential hazards relate to:

- **toxicity and allergenicity to humans:** could these GM cottons be more toxic or allergenic than non-GM cotton, as a result of the novel gene products or because of unintended effects?
- **toxicity to non-target organisms:** could these GM cottons 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 these GM cottons 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
- **insecticide resistance:** could target insects develop resistance to the insecticidal proteins produced by the introduced insecticidal genes in these GM cotton?

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has a complementary regulatory role in respect of this application due to its responsibility for agricultural chemical use in Australia. Insecticidal GM plants fall under the *Agricultural and Veterinary Chemicals Code Act 1994* definition of an agricultural chemical product. Further information about the APVMA's assessment and approval process is contained in Chapter 1 and Appendix 6 of the RARMP.

For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits for experimental work that allow restricted use of an agricultural chemical, for example, for a limited period of time or for a limited area. The APVMA can impose conditions of use on both registrations and permits, and must be satisfied that the proposed use would not present an undue risk to human health or the environment, and the insecticide is efficacious. The hazard of development of insecticide resistance in pests is also part of the APVMA assessment of insecticide use.

Dow AgroSciences has submitted an application to the APVMA for a research permit for the use of the insecticidal genes in the GM cotton lines for the proposed trial. The APVMA and the OGTR have worked closely to ensure the thorough, coordinated assessment of these parallel proposals, and that the decisions by both agencies coincide.

## CONCLUSIONS OF THE RISK ASSESSMENT

It is concluded the proposed release of three GM cotton lines does not pose significant risks to human health and safety and the environment as a result of the genetic modification. The Regulator has imposed licence conditions that will minimise potential exposure of humans and other organisms to the GM cottons, and limit the spread and persistence of the GMOs or the introduced genes while more data is gathered on the behaviour and interactions of the GMOs in the environment. The assessment of each potential hazard identified above is summarised under a separate heading below.

### Toxicity or allergenicity to humans

The GM insecticidal/herbicide tolerant cotton lines are unlikely to prove more toxic or allergenic to humans via occupational exposure than conventional cotton. Humans are commonly exposed to the native bacterial forms of the proteins produced from the introduced genes, as the organisms from which they are derived are naturally widespread in the environment. None of the proteins have any known human toxicity or allergenicity. There have been no reports of toxic or allergenic effects from previous releases of GM cotton lines containing insecticidal genes derived from the same bacterium. The proposed release is limited in scale and conditions are imposed to limit the spread of the GMOs and the introduced genes.

The applicant does not intend to use any cottonseed produced in the proposed release in human food or animal feed, or to sell lint or linters for processing, thus limiting potential exposure. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment, and FSANZ approval would be needed before products from these GM cottons could be used in human food.

It should be noted that FSANZ is currently evaluating an application for approval for food use of oil and linters derived from one of the GM cotton lines, WideStrike™ cotton, proposed for release.

### Toxicity to non-target organisms

The GM insecticidal/herbicide tolerant cottons are unlikely to prove more toxic to non-target organisms than conventional cotton. As discussed above, although the introduced genes are chimeric, the encoded toxin proteins are very similar to the native toxins. Therefore the specificity of the expressed toxin proteins to lepidopteran caterpillars is the same as native bacterial Cry1Fa and Cry1Ac proteins. These proteins are naturally widespread in the environment and have no known toxicity to mammals, birds or fish. The proposed release is limited in scale and conditions are imposed to limit the movement of the GMOs and the introduced genes, and the applicant does not intend to use any material from the release in animal feed.

Results from laboratory and field studies in the USA indicate that the GM insecticidal/herbicide tolerant cotton lines proposed for release are not toxic to non-target invertebrates in that environment. However, the applicant will be required to provide information on the effect of the chimeric Cry1Fa and Cry1Ac proteins on non-target organisms under Australian field conditions before any application for a larger scale release of these GM cottons is evaluated.

### Weediness

The risk of the GM insecticidal/herbicide tolerant cotton lines establishing as problematic weeds, as a result of the proposed release in the areas of southern Australia is low and not likely to be greater than that of non-GM cotton. This is because the germination and persistence of both GM and non-GM cottons in southern Australia are limited by the availability of adequate soil moisture, nutrients, herbivory by non-lepidopteran species (vertebrate and invertebrate), fire, plant competition and/or frosts.

Limited experimental data suggests that insecticidal GM cottons may have the potential to be weedier than non-GM cotton in northern Australia in certain habitats where there is adequate soil moisture and nutrients, limited plant competition and protection from fire and no frosts. It is highly unlikely that the genetic modifications will affect the response of the GM cottons to these variables

and, thereby, alter the weediness of the GM cottons. Results from field studies in the USA indicate that the agronomic characteristics of the GM cottons which may relate to weediness potential have not been altered. However, licence conditions have been imposed to minimise the spread and persistence of these GM cottons in the environment.

**Transfer of introduced genes to other organisms**

Some gene transfer from the GM insecticidal/herbicide tolerant cotton lines to other cultivated cottons would be likely under uncontrolled conditions, although 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 GM cottons themselves.

Herbarium records suggest that naturalised/feral cotton populations may occur, or might have occurred in the past, in central and south eastern Queensland, in the northern Northern Territory and in northern Western Australia. The remnants of these populations, a few of which may be within pollinating distance of commercial cotton crops, has not yet been confirmed. As part of the licence conditions for DIR 022/2003, a survey of naturalised cotton populations in Queensland, in locations suggested by herbarium records, is being conducted. The risk of transferring the introduced genes to native cotton is negligible, because of geographic separation and genetic incompatibility.

Licence conditions have been imposed to limit cross-pollination to compatible cotton plants outside the release sites.

The likelihood of transfer of the introduced genes to other organisms is negligible because of well established genetic incompatibility. Even if such transfer occurred it would be unlikely to pose any hazard to human health and safety or 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 data from research on gene flow undertaken prior to the implementation of the Act and prior to the commercial release of GM cottons, and in so doing validate the containment measures for field trials of GM cottons. In order to facilitate this review and data collection, the Regulator has imposed a licence condition to establish 400 metre research zones around field trial sites in excess of one hectare. The associated research program would be developed in consultation with the OGTR.

**Insecticide resistance**

Development of insects resistant to the insecticidal proteins in these three GM cotton lines might occur if they were grown on a larger scale without using any management strategies. However, given the limited scope of the release in scale and time, the likelihood of this risk resulting from the release is negligible.

In addition, as these GM cottons fall under the *Agricultural and Veterinary Chemicals Code Act 1994* definition of an agricultural chemical product due to their production of insecticidal substances, they are subject to regulation by the Australian Pesticides and Veterinary Medicines Authority (APVMA). The hazard of development of insecticide resistance in pests was also considered by the APVMA in considering Dow AgroSciences' permit application for the use of the insecticidal genes in the GM cottons. The APVMA has the regulatory responsibility for management of this hazard.

## 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 has been given effect by the licence conditions imposed. The key licence conditions are outlined below.

### Toxicity or allergenicity to humans

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release;
- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- destroy all GM materials 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:

- limit the scale of the release;
- prevent GM cottonseed being used as stockfeed;
- destroy all GM materials 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;
- prevent cottonseed being used as stockfeed;
- either surround the GM cottons by a 20 m pollen trap of non-GM cotton or ensure that there are no other cotton crops or naturalised cotton populations within 450 m of the GM cotton crops;
- securely transport and store the GM cottons;
- clean the release sites and 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 proposed which require the applicant to:

- limit the scale of the release;

- either surround the GM cottons by a 20 m pollen trap of non-GM cotton or ensure that there are no other cotton crops or naturalised cotton populations within 450 m of the GM cotton crops;
- provision for a 400 m wide research zone around trial sites in excess of one hectare for the purpose of conducting research on gene flow, and for development of an agreed research program to validate previous research on gene transfer containment measures for ongoing review of data;
- securely transport and store the GM cottons;
- clean the release sites and equipment used at release sites; and
- monitor release sites after harvest and destroy volunteers.

**Insecticide resistance**

No conditions have been imposed in relation to insecticide resistance management. The APVMA also has the regulatory responsibility for this issue. The applicant's obligation to comply with any conditions imposed by the APVMA is noted in the licence.

**General conditions**

Any 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;
- requirement that the applicant allow access to the release sites by the Regulator, or persons authorised by the Regulator, for the purpose 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 and safety or to the environment.

Chapter 2 of the risk assessment and 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.

**Research requirements**

Research conditions have been imposed in the licence to collect data on:

- levels of expression of the insecticidal and herbicide tolerance genes in the GM cotton tissues under Australian field conditions;
- effect of the GM cottons on non-target organisms under Australian field conditions;
- potential for the introduced proteins to accumulate in the soil under Australian field conditions;
- efficacy of gene flow containment measures for any release sites in excess of one hectare, within the associated Research Zone, and for any release site for which a pollen trap is not required, due to having no other cotton crops or populations within 450 m.

### Additional data

The proposed limited and controlled release is a small scale, multi-site trial over four cotton growing seasons. If the applicant makes any future applications for larger scale releases of GM insecticidal/herbicide tolerant cotton lines, more data are required to be provided on:

- agronomic characteristics of the GM cottons in relation to potential weediness under Australian field conditions;
- effect of the GM cottons on soil biota;
- unintended effects of the genetic modification.

### 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 unforseen problems.

# CHAPTER 1 BACKGROUND

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

## SECTION 1 THE APPLICATION

2. The OGTR has received an application (licence application number DIR 044/2003) from Dow AgroSciences Australia Pty Ltd (Dow AgroSciences) for the intentional release of three genetically modified (GM) insecticidal/herbicide tolerant cottons into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

**Project Title:** **Agronomic assessment and seed increase of GM cottons expressing insecticidal genes (*cry1Fa* and *cry1Ac*) from *Bacillus thuringiensis***

**Applicant:** Dow AgroSciences Australia Pty Ltd
Locked Bag 502
Frenchs Forest NSW 1640

**Common name of the parent organism:** Cotton

**Scientific name of the parent organism:** *Gossypium hirsutum* L.

**Modified trait(s):** Insecticidal and herbicide tolerance

**Identity of the gene(s) responsible for the modified trait(s):**

- chimeric *cry1Fa* gene from the bacterium *Bacillus thuringiensis* (insecticidal)
- chimeric *cry1Ac* gene from the bacterium *Bacillus thuringiensis* (insecticidal)
- *pat* gene from *Streptomyces viridochromogenes* (herbicide tolerance/selectable marker)

**Proposed Location(s)** Shires of Balonne, Banana, Bauhinia, Emerald, Millmerran, Murilla, Pittsworth, Waggamba, Wambo and Warroo in Queensland; Shires of Bourke, Carrathool, Gunnedah, Moree Plains, Narrabri, Walgett and Warren in New South Wales; Shires of Wyndham/East Kimberley and Derby/West Kimberley in Western Australia.

**Proposed Release Size:** A maximum of 30 sites covering up to a total of 12.20 hectares

**Proposed Time of Release** May 2004 – May 2006

| Season | No of Sites | Total Area (ha) |
|---|---|---|
| Winter 2004 | 3 | 0.20 |
| Summer 2004/5 | 11 | 1.00 |
| Winter 2005 | 3 | 5.00 |
| Summer 2005/6 | 13 | 6.00 |
| Total | 30 | 12.20 |

3. In the original application, Dow AgroSciences proposed to conduct the trial over four cotton growing seasons on up to 25 sites covering a total cumulative area of 10 hectares. However, Dow AgroSciences subsequently amended its application to slightly increase the number and size of sites (up to 30, covering a total cumulative area of up to 12.2 hectares over the same 3 years) and withdrew the request for trialing in Northern Territory.

4. In accordance with the provisions of section 185 of the *Gene Technology Act 2000* (the Act), Dow AgroSciences sought and received approval for details of the gene constructs, gene sequence information and molecular characteristics of the inserted genetic materials as Confidential Commercial Information (CCI) in connection with a previous licence application (DIR 040/2003). While the Regulator is satisfied that the public interest in the release did not outweigh the prejudice that disclosure would cause the applicant, the CCI was made available to the various prescribed expert groups that were consulted on the preparation of the risk assessment and risk management plan for this application.

## Section 1.1 The proposed dealings

5. Dow AgroSciences proposes to conduct a small scale, multi-site trial in a range of cotton growing regions. The aims of the proposed release are to test the efficacy (effectiveness) of a GM cotton line, WideStrike™ cotton, containing two insecticidal genes (*cry1Fa* and *cry1Ac*) against lepidopteran caterpillars pests of cotton, as compared to its two parental lines (line 3006-210-23 or Cry1Ac cotton and line 281-24-236 or Cry1Fa cotton); to evaluate their respective agronomic performance; and to collect data for development of an insecticide resistance management plan. In addition, the applicant proposes to measure the expression levels of the insecticidal proteins in cotton tissues and residues of these proteins in soil, and to test the effect of the GM cotton lines on non-target organisms. Seed would also be retained for subsequent trial seasons for the proposed release or for potential future releases (subject to further approvals). None of the cotton plants from the release, or their by-products, would be used for human food and animal feed, nor would lint or linters be sold for processing.

6. Note that Dow AgroSciences has not yet decided whether to commercialise these GM cotton lines in Australia. If these GM cottons do not prove to be suitable for the Australian cotton production system during the first two seasons of the trial, Dow AgroSciences intends to stop the trial at the end of second season (2004/05 summer season). The efficacy data generated during the initial stages of this trial will inform future research and potential commercialisation plans. The GM cotton lines proposed for release are derived from the original American cultivar that was used in the initial breeding program. If Dow AgroSciences wishes to conduct additional trials, including breeding the GM insecticidal traits into cultivars more suitable for Australian conditions, additional licence applications and approvals would be required.

## Section 1.2 Parent organism

7. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in New South Wales and Queensland and on a trial basis in Western Australia and the Northern Territory. 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

8. Dow AgroSciences proposes to release three GM cotton lines, two of which contain a single insecticidal gene, *cry1Fa* (line 281-24-236 or Cry1Fa cotton) or *cry1Ac* (line 3006-210-23 or Cry1Ac cotton). The third line, WideStrike™ cotton, was produced by conventional breeding of these two single-gene insecticidal lines. Therefore WideStrike™ cotton contains both *cry1Fa* and *cry1Ac* genes.

9. The insecticidal genes in these GM cotton lines are derived from the common soil bacterium *Bacillus thuringiensis* (Bt). However, the *cry1Fa* and *cry1Ac* genes are chimeric genes, ie. they contain parts of two other *cry1* genes of Bt, but the proteins they express retain the species specific toxicity of native Cry1Fa and Cry1Ac proteins for lepidopteran caterpillar insects, including the major caterpillar pests of cotton (see Appendix 1 Section 3 for details). The chimeric genes were developed to achieve improved expression in plants and solubility of the Bt toxins in the insect gut.

10. In addition to the chimeric *cry1Fa* and/or *cry1Ac* genes, these cotton lines contain a selectable marker gene (*pat*) from the common soil bacterium S*treptomyces viridochromogenes*. The *pat* gene encodes the PAT (phosphinothricin acetyl transferase) protein, which confers tolerance to the herbicide glufosinate ammonium (the active ingredient of Liberty® and Basta® herbicides). The marker gene was used in the laboratory during the development of the GMOs for identification and selection of plant tissues in which the insecticidal genes were also present. Dow AgroSciences does not intend to apply the herbicide glufosinate ammonium to the GM cotton lines during the proposed release.

11. 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, *Zea mays* (corn), and from a common 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.

12. Additional information on the chimeric *cry1Fa*, *cry1Ac* genes, the *pat* gene, and molecular characterisation of the inserted genetic materials and the new proteins expressed by the GM cottons is provided in Appendix 1.

## Section 1.4 Method of gene transfer

13. The two *cry1* genes were each introduced separately into the American cotton variety GC510, in combination with one copy of the *pat* gene, by *Agrobacterium*-mediated DNA transformation (Zambryski 1992). The genes were introduced into the genome of cotton from plasmid vectors carried by *A. tumefaciens*. These vectors were 'disarmed', lacking the genes that encode the tumour-inducing functions of *A. tumefaciens* (see Appendix 1, Section 4 for details).

14. The GM cotton plants containing the single insecticidal gene (*cry1Fa* or *cry1Ac*), or their progeny from self-pollination, were then crossed and repeatedly backcrossed, to another elite American commercial cotton variety, PSC355 (the 'recurrent parent' in the breeding program) to generate the two 'parental' GM cotton lines (referred to as Cry1Fa cotton and Cry1Ac cotton). The two insecticidal traits were then combined by conventional cross breeding between these two GM cotton lines to generate the third GM cotton line, referred to as WideStrike™ cotton. Thus the

GM WideStrike™ cotton line contains two insecticidal genes and two copies of the herbicide tolerance *pat* gene.

## SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

### Section 2.1 Previous releases in Australia

15. One of the GM cotton lines, WideStrike™ cotton, proposed for release is currently being trialed under limited and controlled conditions on two sites covering a total area of 0.04 hectares in New South Wales (DIR 040/2003). There have been no previous releases of either of the two single-gene insecticidal GM cotton lines (Cry1Fa and Cry1Ac cottons) in Australia.

16. Previously, other GM cottons containing *cry* insecticidal genes derived from the same bacterium (*Bacillus thuringiensis*) have been trialed extensively in Australia (eg. DIR s 005/2201, 006/2001, 008/2001 and 009/2001), as well as commercially released under licences DIR 022/2002 (INGARD® cotton) and DIR 012/2001 (Bollgard® II cotton). The chimeric *cry1Ac* gene in two of the GM cotton lines proposed for release in the current application differs slightly from the chimeric *cry1Ac* gene present in INGARD® and Bollgard® II cottons.

17. Other GM plants expressing PAT proteins have also been trialed in Australia (eg. DIR 010/2001, DIR 015/2002 and DIR 038/2003).

### Section 2.2 Approvals by Other Australian Government Agencies

18. The OGTR is responsible for assessing the risks to human health and the environment associated with development and use of gene technology. Other government regulatory requirements may be relevant in respect of the release of GMOs, and the use of products of the GMOs. In this instance the responsibilities of the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standards Australia New Zealand (FSANZ) are relevant.

#### 2.2.1 Food Standards Australia New Zealand

19. FSANZ is responsible for human food safety assessment and labelling, including GM food. FSANZ's approval would need to be obtained before any materials from the GM cotton lines proposed for release under this application could be used for human food.

20. Currently, FSANZ is evaluating an application for approval for food use of oil and linters derived from WideStrike™ cotton. However, it should be noted that none of the products from this release will be used for human food.

21. GM corn containing Cry1Fa core toxin has been approved for food use in Australia since 2003.

22. Further information is 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*

### 2.2.2 Australian Pesticides and Veterinary Medicines Authority

23. The APVMA has a complementary regulatory role in respect of this application due to its responsibility for agricultural chemical use in Australia. Insecticidal GM plants fall under the *Agricultural and Veterinary Chemicals Code Act 1994* definition of an agricultural chemical product due to their production of insecticidal substances encoded by the introduced genes. Any use of herbicide on the GM cotton is also subject to APVMA regulation. For commercial products, the normal form of approval is through registration, but the APVMA may also issue permits allowing restricted use of an agricultural chemical, for example for a limited period of time or on a limited area.

24. Dow AgroSciences has submitted an application to the APVMA for a research permit for the use of the insecticidal genes in the GM cottons for the proposed trial. Dow AgroSciences does not intend to apply glufosinate ammonium herbicide to the GM cottons in the proposed release. The APVMA and the OGTR have worked closely to ensure the thorough, coordinated assessment of these parallel proposals, and that the decisions by both agencies coincide.

25. In considering applications for registrations or permits, the APVMA also considers and, if necessary, imposes conditions in relation to a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of the product and resistance development.

26. Further information 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*

## Section 2.4 International approvals

27. All three GM insecticidal/herbicide tolerant cotton lines proposed for release under the current application have been released for field trials in the United States of America since 2001. A current Experimental Use Permit has been issued by the United States Environmental Protection Agency (US EPA). The US EPA has also issued tolerance exemptions for residues of the Cry1Fa and Cry1Ac insecticidal proteins and their genes in all raw agricultural commodities when applied/used as plant-incorporated protectants (US EPA 1997; US EPA 2003). Dow AgroSciences has also been conducting a field trial of the GM cottons in Argentina since 2004 (*http://www.sagpya.mecon.gov.ar/12/ingles/release.htm*).

# CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN

28. The Act and the Regulations require that risks associated with dealings 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

29. 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 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.

30. Written submissions in relation to DIR 044/2003 received from the agencies and authorities suggested the following issues relating to risks to human health and safety or the environment, which have been addressed in the RARMP:

- the potential toxicity and allergenicity of GM cottons (Appendices 2 and 3 refer);
- the possibility that the GM cottons 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, section 2 refers);
- the possibility that the new genes introduced into the cottons can transfer to other organisms with adverse consequences (Appendix 5 refers);
- the emergence of insects resistant to the insecticidal proteins in the GM cottons (Appendix 6 refers);
- measures to limit the unintended dispersal in the environment of GM cottonseed and GM pollen (Chapter 2 and Appendix 7 refer); and
- additional data requirements for the future development of the GMOs (Chapter 2 refers).

31. The Regulator received two 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:

- effect of the GM cottons on human and animal health and safety; and
- effect of the GM cottons on soil organisms and the environment.

32. One of the public submissions also raised benefits of insecticidal GM cottons, particularly reduced pesticide use, which are outside the scope of evaluation conducted under the Act and therefore have not been considered as part of the assessment process.

33. In accordance with section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to human health and safety and to 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 RISK MANAGEMENT PLAN

34. 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. The risk assessment process used a Risk Analysis Framework developed in consultation with the public and key stakeholders (available from the OGTR website *www.ogtr.gov.au*). A number of hazards were identified 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 of the hazards occurring; and
- the likely consequences (impact) of the hazards, were they to be realised.

35. 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 risk assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed *Summary of Risk Assessment*.

36. Where it is considered that risk management may be necessary to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the selected methods and the reasons they were chosen. A risk management plan for the proposed dealing is given effect by specific conditions imposed in the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 7.

## SECTION 3 RESEARCH REQUIREMENTS

37. The licence conditions include the requirement that the applicant collect and provide to the Regulator further information regarding:

- levels of expression of the insecticidal and herbicide tolerance genes in the GM cottons tissues under Australian field conditions;
- effect of the GM cottons on non-target organisms under Australian field conditions;
- potential for the introduced proteins to accumulate in the soil under Australian field conditions; and
- efficacy of gene flow containment measures for any release sites in excess of one hectare, within the associated Research Zone, and for any release site for which a pollen trap is not required, due to having no other cotton crops or populations within 450 m.

## SECTION 4 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES

38. The proposed limited and controlled release is a small scale, multi-site trial over four cotton growing seasons. If the applicant makes any applications for future larger scale releases of GM insecticidal/herbicide tolerant cotton lines, more data would be required to be provided on:

- agronomic characteristics of the GM cottons in relation to potential weediness under Australian field conditions;
- effect of the GM cottons on soil biota; and
- unintended effects of the genetic modification.

39. It should be noted that collection of the above data during the proposed release is not required to ensure the management of risks to human health and safety and the environment. The risk management measures summarised in Table 1, and given effect by the licence conditions, will effectively manage any such risks.

40. It should also be noted that the use of products of the GM cottons in food would require approval from FSANZ.

## SECTION 5 DECISION ON THE APPLICATION

41. 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 risk posed by the dealings are able to be managed so as to protect human health and safety and the environment.

42. It is concluded that the proposed release of the GM cotton lines does not pose significant risks to human health and safety and the environment as a result of the genetic modification. The Regulator has imposed licence conditions to minimise potential exposure of humans and other organisms to the GM cottons, and to limit the spread and persistence of the GMOs or the introduced genetic materials while additional data is collected on the behaviour and interactions of the GMOs in the Australian environment. Detailed risk analyses based on the available scientific information are provided in Appendices 2-6 in support of this conclusion.

43. Therefore, the Regulator has issued licence DIR 044/2003 in respect of this application.

## Table 1 Summary of risk assessment and risk management plan (including imposed licence conditions)

GM cottons: the genetically modified cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cotton) proposed for release.
Lepidoptera: the caterpillar insect pests targeted by the GM cottons belong to this order of insects.
Bt toxins: the Cry1Fa and Cry1Ac proteins may also be referred to as a Bt toxins, because they are some of the many protein toxins produced by the bacteria *Bacillus thuringiensis* (Bt) in nature.
PAT: the protein encoded by the introduced *pat* gene, which provides tolerance to the herbicide glufosinate ammonium.
N/A: not applicable.

| Hazard Identification | Risk (combines 'likelihood' & 'impact') | Summary of Risk Assessment (refer to appendices for details) | Does Risk Require Management? | Risk Management *Method(s) and Reason(s) for selection* | Is Risk Managed? | Licence conditions (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| **TOXICITY AND ALLERGENICITY FOR HUMANS:** Food | **Low** | **See Appendix 2**<br>▪ products of the GM cotton lines will not be used for human food or animal feed, or processed for lint or oil production;<br>▪ the introduced core proteins are very similar to the native bacterial proteins which are already widespread in the environment and present in human food;<br>▪ the introduced proteins are of very low oral toxicity;<br>▪ the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; and<br>▪ compositional analyses have not indicated any differences between the GM and non-GM cottonseed, other than the presence of the introduced proteins. | Yes | ▪ **Prevent seed from entering human food supply:** prevents exposure through food;<br>▪ **Destroy all seed not required for possible future trials:** prevents unintended exposure; and<br>▪ **Ensure secure transport and storage of retained seed:** prevents unintended exposure. | Yes | ▪ **Prohibit entry into human food supply:** no materials from the GMOs to be used in human food or stock feed;<br>▪ **Destroy seed:** destroy all seed not required for possible future trials;<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed must be transported within a primary, sealed container that is packed in a secondary unbreakable container; store in sealed container within a locked facility that is signed to indicate GM cottonseed is stored within. |
| **TOXICITY AND ALLERGENICITY FOR HUMANS:** Occupational exposure | **Low** | **See Appendix 2**<br>▪ exposure to the introduced proteins through working with cotton plants is very low;<br>▪ cotton pollen is not wind dispersed and therefore not likely to be an airborne allergen;<br>▪ processing of the GM seed cotton will only occur on a small scale (for preparation of seed for subsequent season trial or possible future trials) and GM cotton lint is no more likely to induce adverse responses in workers than is lint from non-GM cotton;<br>▪ the introduced core proteins are very similar to the native bacterial proteins which are already widespread in the environment and present in human food;<br>▪ the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; | Yes | ▪ **Limit scale of release:** decreases likelihood of exposure;<br>▪ **Destroy all seed not required for possible future trials:** prevents unintended exposure;<br>▪ **Ensure secure transport and storage GM material:** prevents unintended exposure; and<br>▪ **Report any adverse impacts on human health and safety:** ensures identification of unexpected adverse impacts. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Destroy seed:** destroy all seed not required for possible future trials;<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed must be transported within a primary, sealed container that is packed in a secondary unbreakable container; store in sealed container within a locked facility that is signed to indicate GM cottonseed is stored within; and<br>▪ **Report adverse impacts:** any adverse impacts on human health and safety must be reported to the Regulator. |

| Hazard Identification | Risk (combines 'likelihood' & 'impact') | Summary of Risk Assessment (refer to appendices for details) | Does Risk Require Management? | Risk Management *Method(s) and Reason(s) for selection* | Is Risk Managed? | Licence conditions (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| | | ▪ the introduced proteins are of very low oral toxicity; and<br>▪ compositional analyses have not indicated any differences between the GM and non-GM cottonseed, other than the presence of the introduced proteins and the intended plant traits. | | | | |
| **TOXICITY FOR OTHER ORGANISMS:** Mammals and wildlife, including birds and fish | **Low** | **See Appendix 3**<br>▪ the introduced core proteins are very similar to the native bacterial proteins which are already widespread in the environment, including in soil, on plants and on fresh produce, in the microorganisms from which the genes were derived;<br>▪ the release is small in size and limited in duration;<br>▪ the toxicity of Cry1Fa and Cry1Ac proteins is specific to lepidopteran caterpillar larvae;<br>▪ the PAT protein is not known to be toxic to any organism;<br>▪ exposure of livestock and wildlife to the GM cotton lines would be low, and no materials from the release are proposed to be used in stockfeed; and<br>▪ toxicity studies with purified proteins and Bt microbial preparations indicate that the Cry1Fa and Cry1Ac proteins are not toxic to mammals, birds or fish. | Yes | ▪ **Limit scale of release:** decreases likelihood of exposure;<br>▪ **Prevent seed from being used as stockfeed:** prevents exposure of animals;<br>▪ **Destroy all seed not required for further trials:** prevents unintended exposure; and<br>▪ **Ensure secure transport and storage GM material:** prevents unintended exposure. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Prevent seed from being used as stockfeed:** no material from the GMOs to be used in stock feed.<br>▪ **Destroy seed:** destroy all seed not required for possible future trials; and<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed must be transported within a primary, sealed container that is packed in a secondary unbreakable container; store in sealed container within a locked facility that is signed to indicate GM cottonseed is stored within. |
| **TOXICITY FOR OTHER ORGANISMS:** Non-target invertebrates, including soil dwelling organisms | **Low** | **See Appendix 3**<br>▪ the introduced core proteins are very similar to the native bacterial proteins which are already widespread in the environment, including in soil, on plants and on fresh produce, in the microorganisms from which the genes were derived;<br>▪ the release is small in size and limited in duration;<br>▪ the toxicity of the Cry1Fa and Cry1Ac proteins is specific to lepidopteran caterpillar larvae;<br>▪ the PAT protein is not known to be toxic to any organism;<br>▪ laboratory and field studies carried out in the USA indicate that populations of non-target invertebrates are unlikely to be affected by Cry1Fa, Cry1Ac and PAT proteins; and<br>▪ although the risk is low, further information is required on toxicity for Australian non-target organisms. | Yes | ▪ **Limit scale of release:** decrease the likelihood of exposure.<br>▪ **Further Research:** investigate potential toxicity to non-target organisms under Australian field conditions. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Require research:** investigate potential toxicity to non-target organisms under Australian field conditions. |

| Hazard Identification | Risk (combines 'likelihood' & 'impact') | Summary of Risk Assessment (refer to appendices for details) | Does Risk Require Management? | Risk Management *Method(s) and Reason(s) for selection* | Is Risk Managed? | Licence conditions (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| **TOXICITY FOR OTHER ORGANISMS:** Micro-organisms | **Low** | **See Appendix 3**<br>▪ the introduced core proteins are very similar to the native bacterial proteins which are already widespread in the environment, including in soil, on plants and on fresh produce, in the microorganisms from which the genes were derived;<br>▪ the release is small in size and limited in duration; and<br>▪ although the introduced proteins are unlikely to have adverse effect on soil microorganisms, more information is required on toxicity for soil microorganisms under Australian field conditions. | Yes | ▪ **Limit scale of release:** decrease the likelihood of exposure.<br>▪ **Further research:** investigate persistence of the insecticidal proteins in soil under Australian conditions. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Require research:** investigate the potential for the introduced proteins to accumulate in soil under Australian conditions. |
| **WEEDINESS:** | **Low** | **See Appendix 4**<br>▪ cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment;<br>▪ the genetic modifications in the GM cotton lines are unlikely to affect these characteristics;<br>▪ other GM insecticidal cottons (targeted to lepidopteran caterpillar pests) grown commercially in Australia have not become problematic weeds;<br>▪ major constraints on weediness of both GM and non-GM cottons are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire;<br>▪ the presence of the insecticidal gene(s) in the GM cottons may confer a selective advantage if the cotton is limited by lepidopteran insects, however, the risk is low; and<br>▪ although the two traits (insecticidal and herbicide tolerance) may have an additive effect on competitiveness, neither of these traits individually has been found to be significant for weediness. | Yes | ▪ **Limit scale of the release:** decreases likelihood of escape.<br>▪ **Either surround the GM cottons with a pollen trap or isolate from other cotton crops or naturalised cotton populations:** minimises spread of the introduced genes beyond the release sites via pollen flow.<br>▪ **Ensure secure transport and storage GM material:** prevents spread of GM plant material outside the release sites.<br>▪ **Clean equipment used with the GMOs:** prevents spread of GM plant material outside the release sites.<br>▪ **Prevent cottonseed being used as stockfeed:** prevent dispersal of cotton seed.<br>▪ **Destroy any volunteers:** prevents persistence. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Pollen trap or isolation:** each release site must be surrounded by a 20 m pollen trap of non-GM cotton; or be at least 450 m away from any other cotton crops and naturalised cotton populations.<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed must be transported within a primary, sealed container that is packed in a secondary unbreakable container; store in sealed container within a locked facility that is signed to indicate GM cottonseed is stored within.<br>▪ **Clean equipment:** equipment must be cleaned before it is used for any other purpose. If the GM cottons are grinned, the gin must be cleaned immediately following its use, before any other cotton is ginned.<br>▪ **Prevent seed from being used as stockfeed:** no cottonseed to be used as stockfeed.<br>▪ **Destroy volunteers:** the release sites must be inspected after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering. |

| Hazard Identification | Risk (combines 'likelihood' & 'impact') | Summary of Risk Assessment (refer to appendices for details) | Does Risk Require Management? | Risk Management *Method(s) and Reason(s) for selection* | Is Risk Managed? | Licence conditions (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| **GENE TRANSFER:**<br>Plants:<br>Other cotton crops | **Low** | **See Appendix 5**<br>▪ cotton is mostly self-pollinated; and<br>▪ gene transfer to other cotton crops or volunteer cotton would not pose any risks additional to the low risks posed by the GM cottons themselves. | Yes | ▪ **Limit scale of the release:** decreases potential transfer.<br>▪ **Either surround the GM cottons with a pollen trap or isolate from other cotton crops or naturalised cotton populations:** minimises spread of the introduced genes beyond the release sites via pollen flow.<br>▪ **Ensure secure transport and storage of retained seed:** prevents spread of GM plant materials outside the release sites.<br>▪ **Clean equipment used with the GMOs:** prevents spread of GM plant materials into the environment outside the release sites.<br>▪ **Destroy any volunteers:** prevents persistence.<br>▪ **Further research:** in order to inform the ongoing review of the data on gene transfer and validate the efficacy of containment measures. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Pollen trap or isolation:** each release site must be surrounded by a 20 m pollen trap of non-GM cotton; or be at least 450 m away from any other cotton crops and naturalised cotton populations.<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed must be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; store in sealed container within a locked facility that is signed to indicate GM cottonseed is stored within.<br>▪ **Clean equipment:** equipment must be cleaned before it is used for any other purpose. If the GM cottons are grinned, the gin must be cleaned immediately following its use, before any other cotton is ginned.<br>▪ **Destroy volunteers:** the release sites must be inspected after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering.<br>▪ **Require research:** provide for a 400 m research zone surrounding release sites in excess of 1 ha, for the purpose of conducting research on gene flow and conduct research on efficacy of gene flow containment measures for sites with no pollen traps but 450 m away from other cotton populations. |

| **Hazard Identification** | **Risk** (combines 'likelihood' & 'impact') | **Summary of Risk Assessment** (refer to appendices for details) | **Does Risk Require Management?** | **Risk Management Method(s) and *Reason(s) for selection*** | **Is Risk Managed?** | **Licence conditions** (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| **GENE TRANSFER:**<br><br>Plants:<br>Feral (naturalised) cotton | **Low** | **See Appendix 5**<br>▪ cotton is mostly self-pollinated; and<br>▪ gene transfer to naturalised cotton would not pose any risks additional to the risks posed by the GM cottons themselves. | Yes | ▪ **Limit scale of the release:** decreases potential transfer.<br>▪ **Either surround the GM cottons with a pollen trap or isolate from other cotton crops or naturalised cotton populations:** minimises spread of the introduced genes beyond the release sites via pollen flow.<br>▪ **Ensure secure transport and storage of retained seed:** prevents spread plant material outside the release sites.<br>▪ **Clean equipment used with the GMOs:** prevents spread of GM plant material into the environment outside the release sites.<br>▪ **Destroy** any **volunteers:** prevents persistence. | Yes | ▪ **Limit scale:** restrict area to 12.2 ha over four cotton growing seasons;<br>▪ **Pollen trap or isolation:** each release site must be surrounded by a 20 m pollen trap of non-GM cotton; or be at least 450 m away from any other cotton crops and naturalised cotton populations.<br>▪ **Secure transport and storage:** harvested material to be securely wrapped for transport; GM cottonseed 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 cottonseed is stored within.<br>▪ **Cleaning equipment:** 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.<br>▪ **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. |
| **GENE TRANSFER:**<br>Plants<br>Native cottons | **Negligible** | **See Appendix 5**<br>▪ genetic incompatibility and geographical isolation from native populations prevent the production of fertile hybrids. | No | N/A | N/A | None required |
| **GENE TRANSFER:**<br>Plants<br>▪ Other genera | **Negligible** | **See Appendix 5**<br>▪ Well-established genetic incompatibility prevents successful cross-pollination with other plant species. | No | N/A | N/A | None required |

| **Hazard Identification** | **Risk** (combines 'likelihood' & 'impact') | **Summary of Risk Assessment** (refer to appendices for details) | **Does Risk Require Management?** | **Risk Management Method(s) and *Reason(s) for selection*** | **Is Risk Managed?** | **Licence conditions** (See Appendix 7 for detailed licence conditions) |
|---|---|---|---|---|---|---|
| **GENE TRANSFER:** Micro-organisms | **Negligible** | **See Appendix 5**<br>▪ the introduced genes in the GM cottons are derived from, and are similar to, native bacterial genes that are already widespread in the environment, and are readily available for transfer from these sources via demonstrated natural mechanisms; and<br>▪ gene transfer from plants to microorganisms 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. | No | N/A | N/A | None required |
| **GENE TRANSFER:** Animals, including humans | **Negligible** | **See Appendix 5**<br>▪ the introduced genes in the GM cottons are derived from, and are similar to, native bacterial genes that are already widespread in the environment;<br>▪ transfer or the introduced genes would be unlikely to pose a hazard to human health and safety or to the environment;<br>▪ products from the GM cottons are not intended for stockfeed or human food;<br>▪ most animals avoid feeding on GM or non-GM cotton plants; and<br>▪ even if the GM cottons were approved by FSANZ for use in food, the cotton by-products used in food do not contain DNA; | No | N/A | N/A | None required |
| **RESISTANCE:** Insecticide | **N/A** | **See Appendix 6**<br>▪ APVMA is responsible for assessing and managing this risk | Managed by the APVMA | APVMA would impose appropriate conditions | | None required<br>Licence notes the requirement to adhere to any APVMA conditions, including any insecticide resistance management strategy. |

# APPENDIX 1 INFORMATION ABOUT THE GMOS

44. 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 genetic modification.

45. 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 GM cotton lines.

46. It should be noted that some specific Dow AgroSciences documents, which contain details of the plasmid vectors, gene sequence information and molecular characterisation of the inserted genetic materials, have previously been declared as Confidential Commercial Information (CCI) under section 185 of the Act, in connection with a previous Dow AgroSciences' licence application, DIR 040/2003. However, the 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

47. Dow AgroSciences proposes to release three GM cotton lines, two of which contain a single insecticidal gene, chimeric *cry1Fa* (line 281-24-236 or Cry1Fa cotton) or chimeric *cry1Ac* (line 3006-210-23 or Cry1Ac cotton). The third line, WideStrike™ cotton, was produced by conventional breeding of these two single-gene insecticidal lines. Therefore WideStrike™ cotton contains both *cry1Fa* and *cry1Ac* genes. The insecticidal genes are derived from the common soil bacterium *Bacillus thuringiensis* (Bt) and express insecticidal proteins (Bt toxins) that are toxic to specific lepidopteran caterpillar insects, including the major caterpillar pests of cotton.

48. All three lines also contain a herbicide tolerance selectable marker gene, *pat*, that confers tolerance to the herbicide glufosinate ammonium (the active ingredient of Liberty® and Basta® herbicides). The marker gene was used in the laboratory during the development of the GMOs for identification and selection of plant tissues in which the insecticidal genes were also present.

49. Short regulatory sequences (promoters and terminators) that control expression of the introduced genes are also present in these GM cottons. These sequences are derived from a plant, *Zea mays* (corn), and a common 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.

50. The characteristics of the introduced genes and their products, and methods used to introduce the genes into cottons are discussed in Sections 3 & 4 of this Appendix.

## SECTION 2 THE PARENT ORGANISM

51. 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 cottons. This document can be accessed at *www.ogtr.gov.au*.

# SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

## Section 3.1 The *cry1Fa* and *cry1Ac* genes

52. The *cry1Fa* and *cry1Ac* genes in the GM insecticidal/herbicide tolerant cotton lines are chimeric genes, each combining parts of three different *cry* genes isolated from *Bacillus thuringiensis* (Bt). The part of the chimeric *cry1Fa* gene which corresponds to the active core toxin is derived from the native *cry1Fa* gene of Bt variety *aizawai* (Bta). The part of the chimeric *cry1Ac* gene which corresponds to the active core (functional) toxin is derived from the native *cry1Ac* gene of Bt variety *kurstaki* (Btk) strain HD73. The remainder of each of these genes, encoding the carboxy-terminal portion of the proteins which is cleaved off in the insect gut, is derived from parts of the *cry1Ca3* and *cry1Ab1* genes.

53. The carboxyl-terminal potion of the Cry1 proteins is not essential for toxicity. Its function appears to be in the maintenance of the unusual solubility of the Cry1 proteins (Luthy & Ebersold 1981). However it is a highly conserved region, a property which allows co-assembly of different Cry1 proteins into the same crystal (Hofte & Whiteley 1989). The chimeric genes were developed to improve the level of expression in plants and the solubility of the encoded Bt toxins in the insect gut. The coding sequence of the chimeric genes has been further modified to achieve optimal expression in plants, without affecting the encoded protein sequences.

54. The chimeric *cry1Fa* and *cry1Ac* genes each encode a protein toxin (Cry protein or Bt toxin), Cry1Fa and Cry1Ac, respectively which are very similar to native Cry1Fa and Cry1Ac proteins. Within the core toxin, the amino acid sequences of the native and chimeric proteins are 99.3% and 99.6% identical, respectively, and retain the species specificity of toxicity to larvae of lepidopteran insects (moths and butterflies) characteristic of native Cry1Fa and Cry1Ac proteins.

55. The chimeric *cry1Ac* gene in the GM cotton lines differs from the *cry1Ac* gene present in both INGARD® and Bollgard® II cottons, which is a chimeric gene derived from *cry1Ac* and *cry1Ab* genes of Bt. INGARD® and Bollgard® II are approved for commercial release in Australia under licences DIR 022/2002 and DIR 012/2002, respectively (see *www.ogtr.gov.au*).

56. Expression of the chimeric *cry1Fa* gene is controlled by the (4OCS)Δmas 2' promoter, a synthetic promoter derived from the mannose synthase gene (*mas*) promoter and octopine synthase gene (*ocs*) enhancer of *A. tumefaciens* (Ni et al. 1995). The mRNA termination region is provided by the bidirectional polyadenylation signal of *A. tumefaciens* open reading frame 25 (Barker et al. 1983).

57. Expression of the chimeric *cry1Ac* gene in these GM cottons is controlled by the Ubiquitin promoter of *Zea mays* (corn) (Christensen et al. 1992). The mRNA termination region is also provided by the bidirectional polyadenylation signal of *Agrobacterium tumefaciens* open reading frame 25 (Barker et al. 1983).

## Section 3.2 Origin and use of insecticidal *Bacillus thuringiensis*

58. Information on the origin and use of *B. thuringiensis* (Bt) is included here to provide background information on Bt and Bt products. Bt is a gram positive, spore-forming soil bacterium that is ubiquitous in the environment. It has been found in dead insects, insect breeding

environments, stored grain, soil and leaf surfaces (Dulmage 1982). Products consisting of bacterial spores and Cry proteins have been applied to crops for over 40 years in the USA (Walker et al. 2003). Discovered in the early 20th century in Japan by S. Ishiwata, the bacterium was first isolated from diseased flour moth larvae in Thuringia, Germany by E. Berliner (see Walker et al. 2003). Later, E. Kurstak of France and H. Dulmage of the US Department of Agriculture isolated and accumulated the first significant collection of strains. These strains that were active against lepidopteran insects were grouped together and given the subspecies name *kurstaki* (Dulmage 1982).

59. Bt first became available as a commercial insecticide in France in 1938 (Neppl 2000) and was used commercially in the USA in 1958. The first US Environment Protection Agency registration of Bt occurred in 1961 (Starnes & Liu 1993). The discovery of other Bt subspecies, such as *B. thuringiensis israelensis* that is active against mosquitoes and blackflies (Goldberg & Margalit 1977) and *B. thuringiensis tenebrionis* with activity against some coleopteran insects (eg. beetles) (Krieg et al. 1983) showed the broad insecticidal potential of Bt subspecies. Each subspecies demonstrated a certain level of insecticidal specificity, suggesting that Bt was ideal for integrated pest management (Glare and O'Callaghan, 2000). Thus Bt preparations have become the most widely used microbial insecticides (accounting for over 90% of all commercial sales) (Glare and O'Callaghan, 2000).

60. Bt was introduced to Australia in late 1970s (Teakle 1991). The first registration of Bt was in South Australia in 1985 for use in that State (*www.apvma.gov.au*).

### Section 3.3 Insecticidal Cry proteins (Bt toxins) and their mode of action

61. The Cry proteins, also referred to as δ-endotoxins, insecticidal crystal proteins or protoxins are one class of toxins produced by Bt. During sporulation, Bt produces a parasporal crystal composed of one or more Cry proteins. The formation of the parasporal crystal distinguishes *B. thuringiensis* from other *Bacillus* species. The Cry proteins of each *B. thuringiensis* subspecies are often toxic to specific insect genera.

62. The Cry proteins are classified according to their degree of amino acid homology (Hofte & Whiteley 1989; Crickmore et al. 2002), which also determines their target specificity. For example the Cry1 family of toxins, including the Cry1Fa and Cry1Ac toxins (from the native *cry1Fa* and *cry1Ac* genes), are highly specific against lepidopteran insects (moths and butterflies) (Macintosh et al. 1990; Chambers et al. 1991; Federici 2003). The Cry1Fa and Cry1Ac toxins have a different but overlapping spectrum of toxicity to lepidopteran insect species.

63. The species specificity of the Cry protein results from a series of steps which must occur before the toxicity is realised. Firstly, the Cry protein crystal requires alkaline conditions, as in the larval insect gut, with pH values of 10 or above, to be soluble. The Cry protein, which is in the form of a protoxin, must then be partially digested by a specific protease in the insect gut to create its 'active' (toxic) form (core toxin). The active core toxin must then diffuse through the midgut membrane and bind with specific receptors found on the midgut epithelium surface in order to exert its toxic activity (Hofmann et al. 1988; Van Rie et al. 1989; Karim et al. 2000).

64. Binding of activated Cry toxin to the insect epithelial gut cell receptors leads to formation of pores in the cell membrane, allowing leakage of intracellular contents (for example potassium ions)

into the gut lumen and water into the cell (Sacchi et al. 1986; English & Slatin 1992; Knowles & Dow 1993). The larval gut epithelial cells swell due to osmotic pressure and rupture. The gut becomes paralysed because of changes in the electrolyte and pH balance and the insects stop eating and die (Goldburg et al. 1990).

65. The specific set of conditions required for Cry1 toxicity is only found in lepidopteran insect larvae. Specific proteases cleave off the carboxyl-terminal domain as well as approximately 28 amino acids from the amino-terminal end, of the Cry1Fa and Cry1Ac proteins, leaving an active protease-resistant core of approximately 600 amino acids (Bietlot et al. 1989; Choma & Kaplan 1990; Narva et al. 2001b; Narva et al. 2001a).

66. Non-target insects, mammals (including humans), birds and fish do not possess the specific receptors for the Cry proteins and therefore are not susceptible to the toxic effects (Federici 2003).

67. The biological activity of the Cry1Fa and Cry1Ac proteins expressed in the GM cotton lines has been demonstrated in the laboratory and in field trials in the United States of America (USA), on tobacco budworm (*Heliothis virescens*) and pink bollworm (*Pectinophora gossypiella*) (Pellow 2001).

### Section 3.4 The *pat* gene

68. The *pat* gene in the GM cotton lines is derived from the bacterium *Streptomyces viridochromogenes* (Strauch et al. 1988; Wohlleben et al. 1988). The coding sequence of the *pat* gene has been modified to achieve optimal expression in plants, without affecting the encoded protein sequence.

69. *Streptomyces* spp. are saprophytic, soil bacteria, not considered pathogens of plants, humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). In *S. viridochromogenes*., the *pat* gene provides resistance to the tripeptide antibiotics naturally produced by a small number of actinomycete bacteria (Organisation for Economic Co-operation and Development (OECD) 1999): Bialaphos (phosphinothricyl-L-alanyl-L-alanine), produced by *S. viridochromogenes* and *S. hygroscopicus* (the bacterial species from which the *pat* and related *bar* genes, respectively, are derived); and Phosalacine (phosphinothricyl-L-alanyl-L-leucine), produced by *Kitasatosporia phosalacinea*.

70. There are two versions of the *pat* gene in the GM cotton lines, each under control of a different promoter. These promoters are identical to those controlling the two *cry1* genes above, namely the Ubiquitin promoter of *Zea mays* (corn) (Christensen et al. 1992) and the (4OCS)Δmas 2' synthetic promoter, derived from the mannose synthase gene promoter and octopine synthase gene enhancer of *A. tumefaciens* (Ni et al. 1995). The mRNA termination region for both is provided by the bidirectional polyadenylation signal of *A. tumefaciens* open reading frame 25 (Barker et al. 1983). In the Cry1Ac cotton line (expressing of *cry1Ac* under control of the Ubiquitin promoter), *pat* is under control of the (4OCS)Δmas 2' promoter, whereas in the Cry1Fa cotton line (expressing of *cry1Fa* under control of the (4OCS)Δmas 2' promoter), *pat* is under control of the Ubiquitin promoter.

### Section 3.5 The PAT protein

71. The *pat* gene encodes the enzyme phosphinothricin acetyl transferase (PAT), which detoxifies the herbicide glufosinate ammonium (also referred to as phosphinothricin) (Wohlleben et al. 1988). The glufosinate ammonium tolerance trait was introduced into the GM cotton lines as a selectable marker to identify GM plant cells and GM plants during their development in the laboratory, as well as potentially enabling the use of glufosinate ammonium as a herbicide to control weeds in the crop. However, the applicant does not intend to apply glufosinate ammonium to the GM cottons in the proposed trial.

72. Glufosinate ammonium acts as a herbicide by inhibiting the plant enzyme glutamine synthetase, resulting in ammonia accumulation, inhibition of amino acid synthesis and inhibition of photosynthesis, leading to severe damage to plant tissues, ultimately killing the plant (Pline 1999). Glufosinate ammonium is the active ingredient of a number of proprietary herbicides including Basta®, Finale® and Liberty®.

## SECTION 4 METHOD OF GENE TRANSFER

73. The two chimeric *cry1* genes were each introduced separately into an American commercial cotton variety, GC510, in combination with one copy of the *pat* gene, by *Agrobacterium*-mediated DNA transformation (Zambryski 1992).

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

75. 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 and rapid callus growth (Klee & Rogers 1989).

76. The plasmids used contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984). *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing any biosafety problems.

77. Following co-cultivation with *A. tumefaciens* carrying the plasmid with the gene construct, cotton cells were cultured in the presence of glufosinate ammonium to select for those cells containing inserted gene construct (since the *pat* gene confers tolerance to glufosinate ammonium). Subsequently cotton plants containing the individual insecticidal genes were regenerated from these GM cells.

78. The chimeric *cry1Ac* gene (under control of the Ubiquitin promoter) with one *pat* gene (under control of the (4OCS)Δmas2' promoter) were introduced into cotton from plasmid pMYC3006, leading to a genetic modification or genetic 'transformation', referred to as transformation event 3006-210-23 or Cry1Ac cotton.

79. The chimeric *cry1Fa* gene (under control of the (4OCS)Δmas2' promoter) with one *pat* gene (under control of the Ubiquitin promoter) were introduced into cotton cells from plasmid pAGM281, leading to transformation event 281-24-236 or Cry 1Fa cotton.

80. The two GM cotton plants containing the single insecticidal traits, or their progeny from self pollination, were then crossed, and repeatedly backcrossed, to another elite American commercial cotton variety, PSC355 (the 'recurrent parent' in the breeding program). The two transformation events (281-24-236 and 3006-210-23) were then combined by conventional breeding to generate the third GM cotton line, referred to as WideStrike™ cotton. Thus the GM WideStrike™ cotton line contains two insecticidal genes, chimeric *cry1Fa* and chimeric *cry1Ac* and two copies of the herbicide tolerance *pat* gene.

## SECTION 5 CHARACTERISATION AND STABILITY OF THE INSERTED GENETIC MATERIALS

81. Southern blot analysis, using probes from each gene and from regulatory sequences (promoters and termination region), demonstrates that the transformation event 3006-210-23 contains one intact copy of the insecticidal gene, chimeric *cry1Ac*, and one intact copy of the herbicide tolerance gene, *pat*. Transformation event 281-24-236 contains one intact copy of the insecticidal gene, chimeric *cry1Fa*, and one intact copy plus an additional small fragment of the *pat* gene (Green 2002; Green et al. 2002b; Green et al. 2002a).

82. The DNA sequences from each transformation event have also been confirmed by DNA sequence analysis (Song 2002a; Song 2002b).

83. The gene construct in each transformation event have been shown to be stable over several generations, both by phenotype (insecticidal and glufosinate ammonium tolerance) and Southern blot analysis, adhering to Mendelian inheritance ratios (Narva et al. 2001b; Narva et al. 2001a).

84. The GM WideStrike™ cotton contains all of the introduced genetic material of transformation events 281-24-236 and 3006-210-23 (Green 2002).

## SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS

85. Expression levels of Cry1Fa, Cry1Ac and PAT proteins in various plant tissues, as well as in processed cottonseed fractions, from plants of these three cotton lines grown in the field in the USA, have been determined by enzyme linked immunosorbent assay (ELISA) (Phillips et al. 2002).

86. Data for mean protein levels from samples collected from these GM cotton lines grown in 2001 at six sites in five States of the USA (only two sites for nectar) is presented in Tables 1 and 2. The Cry1Fa protein was detected in Cry1Fa cotton and WideStrike™ cotton at a low level in all tissues and processed fractions except nectar, meal and oil. The highest mean value of its expression was 25.3 µg protein/g tissue dry weight (equivalent to ppm) found in whole plants (combined above ground tissues) of WideStrike™ cotton at pollination stage. The Cry1Ac protein in Cry1Ac cotton and WideStrike™ cotton was expressed at lower levels than Cry1Fa protein in all tissues and fractions except for pollen. The PAT protein was detected in most of the samples of Cry1Fa cotton and WideStrike™ cotton, but was not detectable in most tissues and fractions of Cry1Ac cotton (Tables 1 and 2).

87. Expression of the introduced proteins in these GM cotton lines throughout the season was also examined at two sites (Table 3). Equivalent tissue was collected at different stages of plant growth (eg. young leaves collected in areas of new plant growth at approximately 1, 2, 3 and 4 months, or open flowers every 2 – 3 weeks from first flowering). The Cry1Ac and PAT proteins were expressed throughout the season without any significant changes in expression level. At only one site the Cry1Fa protein was found at substantially higher levels in young leaves of Cry1Fa cotton and WideStrike™ cotton later in the season (Table 3).

## SECTION 7 INSECTICIDAL EFFICACY OF THE GM COTTONS

88. The insecticidal efficacy of the GM cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) has been assessed in field trials conducted at different locations in the USA in 2001 (Pellow 2001).

89. Three trials used either artificial or natural infestation with tobacco budworm (*Heliothis virescens*). The performance of these GM cottons was compared to non-GM cotton with and without chemical spray control for the insect pest. Numbers of insect larvae and damage to squares and bolls were assessed throughout the growing season.

90. Each of these cotton lines was found to perform better than non-GM cotton that was not sprayed to control lepidopteran insect pests, and at least as well as sprayed non-GM cotton. In some cases the GM cottons out-performed sprayed non-GM cotton.

91. One trial was conducted using artificial infestation with pink bollworm (*Pectinophora gossypiella*). Larvae, pupae and exit holes were counted 2 weeks after inoculation with eggs. The Cry1Ac cotton and the WideStrike™ cotton provided nearly complete control of larvae, while Cry1Fa cotton was similar to non-GM cotton.

92. Later trials conducted with WideStrike™ cotton in comparison sprayed non-GM cotton, variety PSC355, showed that this GM cotton can provide effective control of major insect pests of cotton at various locations in the USA, including tobacco budworm *(Heliothis virescens*), cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*). WideStrike™ cotton was also effective against beet armyworm (*Spodoptera exigua*), southern armyworm (*S. eridania*) fall armyworm (*S. frugiperda*), soybean looper (*Pseudoplusia includens*) and cabbage looper (*Trichoplusia ni*). This suggests that the WideStrike™ cotton can provide effective control of a wide range of lepidopteran pests of cotton (Pellow 2002).

**Table 1 Expression levels of the introduced proteins in various tissues of the three GM cotton lines**

| Tissue | Protein Expression (Mean µg protein/g[a] dry weight or fresh weight[b] ± standard deviation) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cry1Ac | | Cry1Fa | | PAT | | |
| | WideStrike™ | Cry1Ac cotton | WideStrike™ | Cry1Fa cotton | WideStrike™ | Cry1Ac cotton | Cry1Fa cotton |
| Young leaf (3-6 week) | 1.82 ± 0.6 | 1.92 ± 0.7 | 6.81 ± 3.6 | 6.48 ± 3.3 | 0.43 ± 0.13 | ND | 0.43 ± 0.12 |
| Terminal leaf (9-13 week) | 1.31 ± 0.4 | 1.44 ± 0.5 | 8.19 ± 3.5 | 7.67 ± 5.3 | 0.23 ± 0.11 | ND | 0.21 ± 0.12 |
| Flower | 1.83 ± 0.4 | 1.92 ± 0.3 | 5.44 ± 1.8 | 5.71 ± 2.1 | 0.35 ± 0.07 | ND | 0.29 ± 0.11 |
| Square | 1.82 ± 0.5 | 1.84 ± 0.5 | 4.88 ± 1.8 | 5.04 ± 1.8 | 0.52 ± 0.18 | ND | 0.51 ± 0.15 |
| Boll | 0.64 ± 0.2 | 0.77 ± 0.2 | 3.52 ± 1.7 | 4.02 ±2.0 | 0.27 ± 0.10 | ND | 0.22 ± 0.09 |
| Whole plant[c] (seedling) | 1.37 ± 0.4 | 1.59 ± 0.4 | 14.1 ± 5.6 | 11.5 ± 4.3 | 0.35 ± 0.06 | ND | 0.31 ± 0.07 |
| Whole plant[c] (pollination) | 1.05 ± 0.2 | 1.15 ± 0.5 | 25.3 ± 11.0 | 22.8 ±7.2 | 0.30 ± 0.07 | ND | 0.23 ± 0.07 |
| Whole plant[c] (defoliation) | 0.6 ± 0.2 | 0.81 ± 0.3 | 22.0 ± 11.0 | 21.1 ±9.9 | 0.34 ± 0.19 | 0.11 ± 0.05 | 0.19 ± 0.13 |
| Root (seedling) | 0.17 ± 0.05 | 0.20 ± 0.1 | 0.88 ± 0.7 | 0.72 ± 0.6 | (0.06)[d] ± 0.05 | ND | (0.07)[d] ± 0.05 |
| Root (pollination) | (0.07)[d] ± 0.06 | 0.10 ± 0.07 | 0.54 ± 0.4 | 0.36 ± 0.1 | ND | ND | ND |
| Root (defoliation) | ND | (0.05)[d] ± 0.04 | 0.51 ± 0.2 | 0.61± 0.5 | (0.05)[d] ± 0.04 | ND | ND |
| Pollen[b] | 1.45 ± 0.5 | 1.44 ± 0.5 | (0.06)[d] ± 0.15 | (0.09)[d] ± 0.30 | (0.05)[d] ± 0.11 | ND | (0.09)[d] ± 0.15 |
| Nectar[b] | ND | ND | ND | ND | ND | ND | ND |
| Seed[b] | 0.55 ± 0.07 | 0.57 ± 0.09 | 4.13 ± 1.1 | 5.13 ± 1.1 | 0.54 ± 0.21 | (0.06)[d] | 0.47 ± 0.17 |

[a] µg /g is equivalent to parts per million (ppm).

[b] Results based on fresh tissue weight for pollen, seed and nectar, dry weight for all other tissues, representing the mean of samples collected from six sites.

[c] Whole plant refers to combined above-ground plant tissue.

[d] Figures in brackets represent values below the validated limit of quantitation for the assay.

ND: not detected.

**Table 2 Levels of the introduced proteins in processed GM cottonseed fractions**

| **Cottonseed Fraction** | **Protein Expression** (mean µg protein/g[a] fresh weight) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cry1Ac | | Cry1Fa | | PAT | | |
| | WideStrike™ | Cry1Ac cotton | WideStrike™ | Cry1Fa cotton | WideStrike™ | Cry1Ac cotton | Cry1Fa cotton |
| Cottonseed | 0.46 | 0.62 | 3.1 | 3.3 | 0.53 | (0.09)[b] | 0.42 |
| Kernel | 0.51 | 0.41 | 3.9 | 3.0 | 0.78 | (0.23)[b] | 0.67 |
| Hulls | ND | ND | 0.16 | 0.22 | ND | ND | ND |
| Toasted meal | ND | ND | ND | ND | ND | ND | ND |
| Refined oil | ND | ND | ND | ND | ND | ND | ND |

[a] µg /g is equivalent to parts per million (ppm).

[b] Figures in brackets represent values below the validated limit of quantitation for the assay.

ND: not detected.

**Table 3 Expression levels of the introduced proteins in the three GM cotton lines over time**

| Tissue | Stage | Site | Protein Expression (Mean µg protein/g[a] dry weight[b]) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cry1Ac | | Cry1Fa | | PAT | | |
| | | | WideStrike™ | Cry1Ac cotton | WideStrike™ | Cry1Fa cotton | WideStrike™ | Cry1Ac cotton | Cry1Fa cotton |
| Young leaf | 3-6 weeks | 1 | 2.6 | 2.9 | 7.3 | 8.7 | 0.51 | ND | 0.51 |
| | | 2 | 1.8 | 2.4 | 9.4 | 11 | 0.41 | 0.12 | 0.36 |
| | + 1 month | 1 | 1.9 | 2.5 | 6.7 | 5.4 | 0.40 | ND | 0.38 |
| | | 2 | 1.4 | 1.8 | 6.7 | 7.1 | 0.41 | (0.08)[c] | 0.44 |
| | + 2 month | 1 | 1.1 | 0.77 | 9.8 | 7.5 | 0.36 | (0.07)[c] | 0.35 |
| | | 2 | 2.0 | 1.6 | 30 | 19 | 0.40 | (0.07)[c] | 0.30 |
| | + 3 month | 1 | 0.67 | 1.2 | 7.7 | 8.6 | 0.35 | 0.12 | 0.18 |
| | | 2 | 2.3 | 2.7 | 41 | 28 | 0.48 | 0.20 | 0.36 |
| Flower | First flower | 1 | 2.1 | 2.1 | 4.8 | 4.3 | 0.34 | ND | 0.27 |
| | | 2 | 2.2 | 2.2 | 5.6 | 5.3 | 0.43 | ND | 0.41 |
| | + 2-3 weeks | 1 | 2.0 | 1.9 | 4.4 | 4.5 | 0.39 | ND | 0.31 |
| | | 2 | 1.8 | 1.7 | 6.5 | 5.3 | 0.59 | ND | 0.59 |
| | + 4-6 weeks | 1 | 0.91 | 1.3 | 2.6 | 2.7 | 0.28 | ND | 0.28 |
| | | 2 | 1.9 | 2.0 | 5.7 | 6.5 | 0.46 | ND | 0.46 |
| Boll | Early boll | 1 | 0.56 | 0.75 | 3.5 | 4.4 | 0.21 | ND | 0.23 |
| | | 2 | 0.47 | 0.58 | 1.4 | 1.5 | (0.19) | ND | (0.17)[c] |
| | + 2-3 weeks | 1 | 0.44 | 0.66 | 6.3 | 6.7 | 0.42 | ND | 0.31 |
| | | 2 | 0.33 | 0.41 | 7.6 | 5.1 | 0.26 | ND | 0.20 |
| | + 4-6 weeks | 1 | 0.37 | 0.46 | 5.0 | 7.3 | 0.52 | ND | 0.44 |
| | | 2 | 0.40 | 0.43 | 4.8 | 4.6 | 0.34 | (0.06)[c] | (0.26)[c] |

[a] µg /g is equivalent to parts per million (ppm).

[b] Each figure represents the mean value of 9 replicate samples.

[c] Figures in brackets represent values below the validated limit of quantitation for the assay.

ND: not detected.

## SECTION 8 COMPARISON OF GM AND NON-GM COTTON CHARACTERISTICS

93. The demonstrated agronomic and compositional similarity between the GM WideStrike™ cotton, two single insecticidal gene lines (Cry1Fa or Cry1Ac cotton) and non-GM cotton indicates that no significant pleiotropic or epistatic effects (that is, unintended effects of a genetic change on other, apparently unrelated, plant genes or plant characteristics) have occurred.

### Section 8.1 Agronomic performance

94. Agronomic performance and varietal selection trials were conducted in the USA during 2002. The three GM cotton lines were grown at 32 locations alongside the non-GM recurrent parental variety (PSC355) used in its breeding (Pellow 2003).

95. Thirty agronomic characteristics were analysed from up to 20 locations. Growth characteristic measures included germination, field emergence, vegetative vigour, growth habit (plant height, number of branches, fruiting positions etc.), flowering period, reproductive potential (boll retention, open bolls, seed cotton [seed embedded in the lint] weight per boll etc.) and lint yield. Fibre quality indicators included length, strength, micronaire (a fineness indicator), uniformity and colour.

96. For most of these measurements there was no statistically significant difference between the GM cotton lines and the PSC355 cotton variety. For some there was a small but statistically significant difference, however the values for the GM cotton were well within the normal range for non-GM cottons.

97. Fibre characteristics were measured from 14 sub-lines of the GM WideStrike™ cotton, each grown at 11 locations. All sub-lines contained the same GM events (ie. the combined events, 3006-210-23 and 281-24-236) and were crossed with the same recurrent parent (PSC355) but kept separate for three subsequent generations of breeding.

98. There was considerable variation between the GM cotton sub-lines, presumably reflecting differences in the background genetic make-up, arising from the combination of the original genetically modified parental cotton variety (GC510) and the recurrent parent in the breeding program (PSC355). The report states that this level of variation is typical for sub-lines within a given pedigree in a cotton breeding program. However most fibre characteristics measurements were not significantly different between the sub-lines of GM cotton lines and the non-GM recurrent parent.

99. It should be noted that the genetic difference between the original parent and the recurrent parent is considerable. GC510 is a high fibre quality variety adapted to completely irrigated Californian conditions, while PSC355 is a moderate fibre quality variety adapted to non-irrigated mid-south region of the USA.

100. It should also be noted that should Dow AgroSciences decide to attempt the commercialisation of these GM cottons, the GM insecticidal traits would need to be bred into cultivars more suitable for the Australian conditions. These trials would require additional applications and approvals.

## Section 8.2 Compositional analysis

101. The results of extensive compositional analyses of cottonseed, processed cottonseed fractions and fresh cotton tissue (Phillips et al. 2002) in a study in the USA demonstrated that the levels of the important nutritional and anti-nutritional components in the three cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) are comparable to those in the similar non-GM variety and to established values for commercial cotton varieties.

### 8.2.1 Cottonseed

102. Ash, total fat, moisture, protein, carbohydrate, calories and fibre in cottonseed were analysed for all three lines. The only statistically significant difference between WideStrike™ cotton seed and non-GM seed was for crude fibre (15.3% for WideStrike™ cotton and 17.0% for non-GM cotton). Both of these values are within the range reported in the literature for non-GM cotton seed.

103. The levels of the minerals calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, sulfur and zinc were analysed. No statistically significant differences between the three GM cottonseed and non-GM cottonseed were found, and all minerals were within reported literature ranges.

104. No statistically significant differences in amino acid content between the three GM and non-GM cottonseed were found, and all values were within or close to reported literature ranges (Berberich et al. 1996).

105. No statistically significant differences in fatty acid content between the three GM and non-GM cottonseed were found. Published values are only available for a few fatty acids, and are slightly higher than those found in both the GM cotton lines and non-GM cottonseed in this study.

106. Cotton tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the presence of anti-nutritional and toxic factors including gossypol and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). No statistically significant differences in these components between the three GM and non-GM cottonseed were found, and all values were within reported literature ranges. No aflatoxins, toxicants which may be produced by infecting fungi, were detected in any sample.

### 8.2.2 Processed fractions

107. Ash, total fat, moisture, protein, carbohydrate, calories and fibre were analysed in cottonseed processed fraction samples, including kernels, hulls, toasted meal and refined oil. Individual analysis results were comparable for all three GM cotton lines and non-GM samples and were within reported ranges.

108. Hulls and meal were analysed for minerals (calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, sulfur and zinc). No statistically significant differences between fractions from these three GM cotton lines and non-GM fractions were found.

109. No statistically significant differences in amino acid content of toasted meal from the three GM cotton lines and non-GM cotton were found, and all values were within or close to reported literature ranges.

110. Refined cottonseed oil was analysed for fatty acid content. No statistically significant differences between these three GM cotton lines and non-GM oil were found, and all values were within reported literature ranges. Refined oil was also analysed for tocopherol isomers, which are naturally occurring anti-oxidants. Results for GM and non-GM oil were very similar. The level of alpha tocopherol (vitamin E) in the cottonseed oil for the GM cotton lines Cry1Fa, Cry1Ac and WideStrike™ cottons (501, 502 and 515 µg/g respectively), and non-GM cotton (549 µg/g) was higher than the available literature value (320 µg/g).

111. Kernel, meal and refined oil were analysed for gossypol. Results for these three GM cotton lines and non-GM fractions were comparable and similar to literature values. Refined oil was also analysed for anti-nutritional cyclopropenoid fatty acids. Levels in oil from these GM lines and non-GM cottonseed were comparable and similar to or below literature values.

### 8.2.3 Cotton leaves and squares

112. Anti-nutrients gossypol and polyphenols were analysed in terminal leaves and squares from the three GM cotton lines and non-GM cotton grown at two sites. Total gossypol was higher in the terminal leaves of Cry1Ac cotton and WideStrike™ cotton lines than that of non-GM cotton at both sites but very similar in squares. Total polyphenols were higher in terminal leaves of Cry1Fa cotton and WideStrike™ cotton lines than that of non-GM cotton at only one site. Polyphenols were very similar for GM and non-GM squares.

## SECTION 9 RESEARCH REQUIREMENTS

113. The proposed release of three GM cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) is a small scale, multi-site trial over four cotton growing seasons. Research conditions have been imposed in the licence issued for this release, including a requirement to collect data on the levels of expression of the insecticidal and herbicide tolerance genes in cotton tissues under Australian field conditions.

114. If the applicant makes any applications for future larger scale releases of the GM cotton lines, more data would be required to demonstrate the absence of unintended effects of the genetic modification under Australian field conditions.

# APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS

115. 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 to human health and safety as a result of any toxicity or allergenicity of the GMOs or its novel proteins.

116. It should be noted that GM cottons containing *cry* insecticidal genes derived from the same bacterium have been extensively trialed and commercially released in Australia since 1996 and 2002 with no reported adverse effects to humans (refer to DIRs 022/2002 and 012/2002 respectively, *www.ogtr.gov.au*). Currently, one of the GM cotton lines proposed for release, WideStrike™ cotton, is being trialed under limited and controlled conditions in New South Wales (DIR 040/2003).

117. In addition, GM cottons containing the herbicide tolerance *pat* gene have been trialed under licence DIR 015/2002 and are being trialed under limited and controlled conditions under licence DIR 038/2003.

## SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

118. A toxic response to a chemical is shown by 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 (FAO & WHO 2000; Taylor & Lehrer 1996). Allergic responses have a well-defined etiology (ie. biochemical cause) that is quite different from toxicity.

119. An allergic response can have severe consequences for an individual, for example, anaphylaxis is a shock syndrome caused by a massive release of histamine and other allergic mediators from even minute exposures to an allergen in a sensitised individual. Food proteins are common causes of anaphylaxis, especially peanut and shell fish (Frick 1995; Frick 1995).

120. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein is sufficiently stable to reach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion.

121. The GM insecticidal/herbicide tolerant cotton lines differ from conventional cotton in the expression of two or three additional proteins. These are either one or both of the insecticidal Cry1Fa and Cry1Ac proteins, and the PAT enzyme that 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 or allergenic to humans due to either expression of the novel gene products or because of unintended effects of the genetic modification is considered in this Appendix.

122. The APVMA has a complementary regulatory role in respect of this application due to its responsibility for agricultural chemical use in Australia, including insecticides and herbicides, under

the *Agricultural and Veterinary Chemicals Code Act 1994* (refer to Chapter 1 for details). The GM insecticidal/herbicide tolerant cottons are subject to regulation by the APVMA due to their production of insecticidal proteins. Any use of herbicide on the GM cottons is also subject to APVMA regulation. 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. Dow AgroSciences does not intend to apply glufosinate ammonium herbicide (eg. Liberty® and Basta® herbicides) to the GM cottons in the proposed release. Thus risks associated with the use of the herbicides in connection with the GMOs are not considered here.

## SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

123. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) on human health and safety, the following factors were considered:

- the inherent toxicity and allergenicity of non-GM cotton (OGTR, 2002);
- the potential exposure to the GM cottons, to their products and to the new proteins which are expressed in the GM cottons, the Cry1Fa, Cry1Ac and PAT proteins;
- the potential exposure to the Cry1Fa, Cry1Ac and PAT 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 insecticidal/herbicide tolerant cottons.

### Section 2.1 Toxicity and allergenicity of conventionally bred non-GM cotton

124. Cotton is a well established field crop with a long history of safe use. A comprehensive review of conventional non-GM 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 base line for comparison with the GM cottons being considered in this risk assessment.

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

126. 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 (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).

127. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore its potential to act as an airborne allergen is extremely low. However, inhalation of cotton dust by mill

workers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition.

## Section 2.2 Exposure of people to the GM insecticidal/herbicide tolerant cottons

128. The applicant proposes to destroy the GM cottons produced in the release, apart from some seed which will be retained for further planting as part of this proposed release or for possible future releases (subject to separate approvals). Since it is not intended that any product of the release will be used in human food or animal feed, there will be no opportunity for human exposure to these GM cottons through food. Therefore potential hazards to humans through food do not warrant detailed discussion here. If products from these GM cottons were proposed to be used in food, approval would need to be obtained from FSANZ.

129. It should be noted that FSANZ is currently evaluating an application for approval for food use of oil and linters derived from one of the GM cotton lines, WideStrike™ cotton, for release.

130. There will also be no opportunity for humans to be exposed to the GM cottons through cotton lint in clothing or other household products, or through cottonseed oil in cosmetics. Therefore potential risks to humans as a result of such exposure to GM cotton products will not be discussion further here.

131. Potential exposure of people to the GM cottons will be 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);

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

132. 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 proteins. 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 the 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).

133. 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, including cotton pollen (see Appendix 1 for details). Dermal exposure of workers to cotton pollen is possible, but the amounts to which workers may be exposed is expected to be very low.

134. The primary processing of seed cotton (seed embedded in the lint) 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. Since cotton lint contains no DNA or protein and fibre characteristics of the GM cottons are the same as non-GM cotton (see Appendix 1), lint from the GM cottons is not

more likely to induce adverse responses in workers than is lint from non-GM cotton. Processing of the GM seed cotton will only occur on a small scale, for preparation of seed for possible future trials.

135. After harvest, seed cotton may be dispersed beyond the limits of the farms where it is grown during transportation to ginning facilities. Specific licence conditions have been imposed to require cleaning of equipment used in connection with the release, and harvested material to be securely wrapped before transporting away from the release sites, so as to prevent the GM cotton seed escaping into the wider environment (see Appendix 7 for details). Seeds not required for future releases must be destroyed.

### Section 2.3 Other sources of Cry1Fa, Cry1Ac and PAT proteins in the environment

136. The Cry1Fa, Cry1Ac and PAT proteins are widespread in the environment, through the presence of the bacteria to which they are native. Although the introduced *cry1* genes in the GM cotton lines for release are chimeric, the encoded Cry proteins are very similar to the native Cry proteins (99.3% similarity in amino acid sequence of core toxin between native and chimeric Cry1Fa and 99.6% between native and chimeric Cry1Ac), and retain the species specific insecticidal toxicity to lepidopteran caterpillar pest. As discussed in Section 3.3 of Appendix 1, all the Cry1 toxins are highly specific to lepidopteran insects (Macintosh et al. 1990; Chambers et al. 1991; Federici 2003).

137. The native Cry1Fa and Cry1Ac proteins are naturally produced by the bacterium *Bacillus thuringiensis* (Bt), varieties *aizawai* (Bta) and *kurstaki* (Btk), respectively. Related Cry proteins are also produced by other varieties of Bt (see Appendix 1), as well as in other GM cottons which are being grown commercially in Australia (Bollgard® II and INGARD® cottons, under licences DIR 012/2001 and DIR 023/2002, see *www.ogtr.gov.au*). Bt spores and their crystal (Cry) toxins are found widely in soils, on plant leaves and in grain stores (Meadows 1993).

138. The presence of Cry1 proteins in food has increased over the past 30 years due to the commercial use of Bt microbial sprays to protect food crops, including organic crops, from insect attack (ANZFA 1999). Insecticidal products containing Btk or Bta as the active ingredient are registered in Australia, including for use on cotton, vegetables, vines and fruit trees (APVMA, see *www.apvma.gov.au*). Thus residues of Bt proteins, including Cry 1Fa and Cry1Ac, can be present on a wide variety of fresh foods such as cabbage, lettuce and tomato, with no reported toxic or allergic responses.

139. PAT enzymes are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by *pat* and *bar* genes respectively (Wohlleben et al. 1988; Strauch et al. 1988). *Streptomyces spp*. are saprophytic, soil-borne bacteria and are not considered pathogens of plants, humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). A search of the GenBank database reveals that other genes encoding PAT or similar enzymes are present in a wide variety of bacteria. The class of enzymes to which PAT belongs, acetyltransferases, are common enzymes in all microorganisms, plants and animals. PAT enzymes have also been expressed in GM crop plants trialed in Australia (eg. cotton, under licences DIR 015/2002 and DIR 038/2003; and canola, under DIR 010/2001).

## Section 2.4 Toxicity and allergenicity of the introduced proteins

### 2.4.1 Toxicity

140. Studies using the purified forms of the introduced proteins have been conducted, as the very low expression of these proteins in GM cotton means it is generally not possible to feed test animals the quantity of the plant material necessary to produce a specific effect. However, it is possible to test the mammalian toxicity of the purified proteins at much higher concentrations than present in the GM plants.

141. As discussed in Section 3.1 of Appendix 1 and in Section 2.3 above, the Cry1Fa and Cry1Ac proteins encoded by the introduced chimeric genes are similar to the native Cry1Fa and Cry1Ac proteins and retain the specificity of the native proteins.

CRY1AC

142. Purified native Btk Cry1Ac protein, at single oral doses of up to 4300 mg/kg body weight, produced no adverse effects in mice (Naylor 1993a; Naylor 1993b). Several studies on acute oral toxicity of Bt microbial preparations, containing Cry1Ac, in rats and rabbits revealed no adverse effects at doses of up to thousands of milligrams per kilogram (Barbera 1995; Carter & Ligget 1994; McClintock et al. 1995; Spencer et al. 1996). These studies reported no treatment-related effects on survival, body weight, food consumption, clinical observations or gross pathology findings.

143. A two-year chronic rat feeding study was undertaken with Btk microbial products at doses of up to 8400 mg/kg of body weight/day. A decrease in weight gain was observed in female rats at the highest dose but, in the absence of any other adverse findings (eg. survival, clinical observations or pathology), this was not considered to indicate Cry protein toxicity (McClintock et al. 1995).

144. Two separate studies on humans found no observable health effect of an oral dose of 1000 mg of Btk microbial spores per day for 3 or 5 days (McClintock et al. 1995; Betz et al. 2000).

145. In a recent review, based on the information from the acute toxicity/pathogenicity studies on Bt strains, Kough (Kough 2003) also concluded that the Bt microorganisms are not pathogenic or toxic to mammals.

146. The United States Environment Protection Agency (US EPA) considers Cry1Ac protein to be non-toxic to mammals and has established an exemption from residue tolerance requirements (EPA 2000). In Australia, the APVMA has also determined that a maximum residue limit (MRL) for human food and animal feed is not necessary for the Cry1Ac protein expressed in GM cottons or on fruit and vegetables sprayed with Btk microbial products, indicating that it is of no toxicological significance (see The MRL Standard, table 5 at: *www.apvma.gov.au/residues/mrl_standard.shtml*).

CRY1FA

147. Given the very close amino acid sequence similarity between Cry1Fa and other Cry proteins, such as Cry1Ac, Cry1Fa is expected to have a similar toxicological profile. Some of the studies above related to Bta, the strain of Bt, which naturally expresses Cry1Fa (McClintock et al. 1995; Betz et al. 2000). .

148. Data submitted to the US EPA indicates no adverse effects in rats after oral administration of greater than $10^8$ Bta microbial spores (McClintock et al. 1995).

149. The US Department of Agriculture (USDA-APHIS 2001), Health Canada (Health Canada 2002) and FSANZ (FSANZ 2003) have reported that the purified Cry1F protein is of low acute oral toxicity in mice (having an LD50, the dose found to kill 50% of test animals, of greater than 576 mg/kg body weight). The US EPA also reported chimeric Cry1F protein expressed in Widestrike™ cotton to be of low acute oral toxicity and considered it unnecessary to establish a maximum permissible level for residues produced in cotton (US EPA 2003). In Australia, the APVMA has also determined that an MRL is not necessary for Bta (expressing native Cry1Fa) microbial products on food producing and non-food producing crops (see The MRL Standard, table 5 at: *www.apvma.gov.au/residues/mrl_standard.shtml*). FSANZ has approved corn expressing the core toxin of Cry1Fa for food use since 2003 (see *Food Standards Code* 1.5.2 at *www.foodstandards.gov.au/foodstandardscode/*)

PAT

150. Evidence indicates that the PAT protein is not toxic to either humans or other animals. The potential for PAT to be toxic has been addressed via acute toxicity studies using PAT protein having several extra amino acids at the N-terminal end of the protein as an aid in its purification (Merriman 1996). Mice (5 male and 5 female) were given a single oral gavage dose of PAT protein at 2500 mg/kg bodyweight. Body weights of the test animals were determined prior to dosing (day 0) and on days 7 and 14 after dosing, and the animals were observed daily for any clinical abnormalities or mortality. No mortality occurred during the study. Following scheduled euthanasia of test animals on day 14, no gross internal findings were observed. Based on this test, the acute oral LD50 was estimated to be greater than 2500 mg of PAT/kg body weight.

151. In addition, a study by Pfister et al (1996, cited in Bremmer & Leist 1996) investigated the toxicity of purified PAT protein in a repeat dose oral toxicity study in rats. Groups of five male and five female rats were fed PAT protein for 14 days at levels of 0, 0.5 or 5% of their diet (equivalent to 0, 707 and 7792 mg/kg body weight/day). The highest concentration is approximately 100,000 times the PAT concentration in the GM cotton tissue. No clinical signs of toxicity or mortality were observed during the study and no significant differences were observed in white blood cell counts or spleen and thymus weights, and there were no histological changes in the organs of the immune system that were examined.

152. PAT protein, as expressed in a variety of GM plants, has been assessed by a number of regulatory bodies in Australia, USA, Canada, and Europe (ANZFA 2001c; Canadian Food Inspection Agency 1996; European Scientific Committee on Plants 2000; FSANZ 2003; Health Canada 1997; Health Canada 2000; FDA 1995; FDA 1997; FDA 2001). The PAT enzyme was found to be of very low toxicity. The United States Environmental Protection Agency, for example, has determined that PAT is exempt from the requirement to establish a maximum permissible level for residues in plants (EPA 1997). FSANZ has approved a corn expressing the PAT protein for food use (see *Food Standards Code* 1.5.2 at *www.foodstandards.gov.au/foodstandardscode/*)

### 2.4.2 Allergenicity

153. Although there are no predictive assays available to assess the allergenic potential of proteins, much is known about the biochemical events associated with allergic reactions, as well as the kinds of proteins that cause problems (Taylor & Lehrer 1996; Metcalfe et al. 1996).

154. Predictions of allergenicity have been based on sequence, structural and biochemical comparisons with known allergens. Protein allergens usually share a number of characteristics (Davies 1986; Flavell et al. 1992; Fuchs et al. 1993a; Fuchs et al. 1993b; Fuchs et al. 1993a; Taylor 1995; Metcalfe et al. 1996; Fuchs & Astwood 1996; ANZFA 2001b; Kimber et al. 1999), including the following:

- molecular weight ranges between 15-70 kD;
- typically glycosylated;
- stable in the mammalian digestive system;
- stable during high temperatures involved in cooking or processing; and
- present as the major protein component in the specific foods.

155. None of the introduced proteins in the GM insecticidal/herbicide tolerant cottons are derived from known sources of allergens, nor are they present as major components of the GM cotton plants (see Appendix 1).

156. Taylor (1992) has shown in double-blind placebo-controlled food challenges that individuals who are allergic to peanuts or sunflower seeds are able to consume oil derived from these seeds without it eliciting an allergic response. Taylor (1992) also suggests that when a protein is present in food at levels well below 1 mg per serving, the hazard for allergenic consumers is minimal. Thus consumers would be highly unlikely to develop allergic responses as a result of the use of oil or linters derived from GM insecticidal/herbicide tolerant cottons in food.

CRY1AC

157. The Cry1Ac protein is heat labile and rapidly degraded, in 30 seconds, under simulated mammalian gastrointestinal conditions (Fuchs et al. 1993a). The Cry1Ac protein does not display characteristics common to known food allergen proteins. Searches of allergen sequence databases have shown no significant matches of the Cry1 proteins to known allergens (Metcalfe et al. 1996).

158. While there have been reports in the US claiming allergic reactions to Bt microbial products in topical insecticidal sprays, these are not due the Cry1Ac protein. A survey conducted among farm workers who picked vegetables treated with Bt microbial products indicated that exposure to Bt products may lead to allergic skin sensitisation, however there was no clinical allergic disease in any of the workers. Most reactions in these workers were shown to be due to other constituents of the Bt sprays, and there was no evidence of antibodies specific to the endotoxin proteins of the Bt sprays (Bernstein et al. 1999). The US EPA has also determined that reports of reactions to Bt microbial products have been due to non-Cry proteins produced during fermentation or to other ingredients added to the insecticidal formulations (EPA 2001).

CRY1FA

159. The Cry1Fa protein is expected to have a similar toxicological profile, including potential for allergenicity, as Cry1Ac and other related Cry proteins.

160. FSANZ (FSANZ 2003), the USEPA (US EPA 2003) and the USDA-APHIS (USDA-APHIS 2001) have reported that the Cry1F protein as expressed in GM cotton and corn was rapidly degraded *in vitro* under conditions simulating mammalian digestion and the amino acid sequence of the protein has no significant homology with known allergens or protein toxins. Therefore, the Cry1Fa protein is unlikely to elicit an allergenic response.

PAT

161. The PAT protein is not a known allergen and is not derived from a known source of allergens. Although its molecular weight of ~22 kD is within the range of molecular weights usually shown by allergens, it lacks glycosylation sites (Bremmer & Leist 1996) and many of the other characteristics which are common to plant food allergens (EPA 1997; Canadian Food Inspection Agency 1998a; ANZFA 2001a).

162. A study in which PAT protein was subjected to simulated gastric conditions (low pH plus the proteolytic enzyme pepsin) reported that the protein was degraded within seconds (Wehrmann et al. 1996). Other studies have shown that the PAT enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of GM canola seed (expressing the PAT enzyme) into feed ingredients (European Scientific Committee on Plants 1998).

163. In addition, a study by Bremmer & Leist (Bremmer & Leist 1996) investigated the allergenicity of purified PAT protein in a repeated high-dose study in rats. The study did not reveal any immunotoxic allergenic effects based on a number of screening parameters.

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

164. The insecticidal and the herbicide tolerance genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the two genes, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur.

165. The composition of cottonseed from these three GM insecticidal/herbicide tolerant cotton lines is within the normal range for cottonseed in terms of the concentration of protein, oils, carbohydrate and ash, and amino acid and fatty acid profiles. The levels of known toxic and anti-nutritional factors in the GM cotton seed kernel, meal and oil, including gossypol and cyclopropenoid fatty acids, are also within the range of non-GM cotton controls (see Appendix 1). This suggests that no unintended effects have occurred as a result of the genetic modifications in the GM cottons.

166. The available information for the introduced proteins shows that they are of low acute oral toxicity and unlikely to be allergens. Furthermore, exposure to the GM cottons as a result of the proposed release would be low. Thus, the risk of adverse effects as a result of toxicity or allergenicity of the GM cottons are likely to be low.

## SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

167. It is considered that the risk of GM insecticidal/herbicide tolerant cottons (Cry1Fa, Cry1Ac and WideStrike™ cottons) being toxic or allergenic for humans is low because:

- products of the GM cotton lines will not be used for human food or animal feed, or processed for lint or oil production;
- exposure to the introduced proteins through working with cotton plants is very low;
- cotton pollen is not wind dispersed and therefore not likely to be an airborne allergen;
- processing of the GM seed cottons will only occur on a small scale (for preparation of seed for future plantings) and lint from GM cottons is not more likely to induce adverse responses in workers than is lint from non-GM cotton;
- the introduced core proteins are very similar to native bacterial proteins which are already widespread in the environment and present in human food;
- the introduced proteins are of very low oral toxicity;
- the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; and
- compositional analyses have not indicated any differences between the GM and non-GM cotton, other than the presence of the introduced proteins and the intended plant traits.

168. The licence holder is required to report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the GM cottons) or to the environment.

# APPENDIX 3 TOXICITY TO NON-TARGET ORGANISMS

169. 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 GMOs or their novel proteins to non-target organisms.

## SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

170. The GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) differ from conventional cotton in the expression of two or three additional proteins. These are Cry1Fa, Cry1Ac insecticidal proteins and PAT enzyme that confers herbicide tolerance (see Appendix 1 for details of protein expression in these GMOs). The potential for the GM cottons to be toxic to organisms, other than the target Lepidopteran insect pests, is considered. This could occur either due to expression of the novel gene products or because of unintended effects of the genetic modifications.

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

- livestock and wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (pollinators, parasitoids or predators of target insect pests); and
- microorganisms, particularly soil microorganisms, with direct impact on growth of crops on farms.

172. Toxicity for the lepidopteran insects 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
- populations of organisms that are preyed on by non-target lepidopteran insects.

173. As noted in Chapter 1 and discussed in more details in Chapter 6, the APVMA has responsibility for agricultural chemical use in Australia, including insecticides and herbicides, under the *Agricultural and Veterinary Chemicals Code Act 1994*. GM insecticidal cottons are subject to regulation by the APVMA due to their production of insecticidal substances. Any use of herbicide on the GM cottons is also subject to APVMA regulation. As part of their assessment of chemical use, the APVMA considers potential environmental effects, for example residues and toxicity to other organisms. Dow AgroSciences does not intend to apply glufosinate ammonium herbicide (eg. Liberty® and Basta® herbicides) to the GM cottons in the proposed release. Thus risks associated with the use of the herbicides in connection with the GMOs are not considered here.

## SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

174. In assessing the likelihood of adverse impacts due to toxicity of these GM cotton lines, a number of factors were considered including:

- the inherent toxicity of non-GM cotton (OGTR 2002);
- the potential exposure to Cry1Fa, Cry1Ac and PAT proteins from other sources in the environment;
- information about the likely routes of exposure to these GM cottons and to the introduced proteins, the Cry 1Fa, Cry1Ac and PAT proteins;
- the potential toxicity of the new proteins expressed in the GM cottons for particular species; and
- the potential toxicity of the GM cottons for particular species.

## Section 2.1 Toxicity of non-GM cotton

175. 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, 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 base line for comparison with the GM cottons being considered in this risk assessment.

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

177. 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.

178. 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

179. As discussed in Section 3.1 of Appendix 1 and Section 2.3 of Appendix 2, Cry1Fa, Cry1Ac and PAT proteins are widespread in the environment, through the presence of the bacteria to which they are native. Although the introduced *cry1* genes in GM cotton lines proposed for release are chimeric, the encoded core toxins are very similar to the native Cry proteins (99.3% similarity in amino acid sequence of core toxin between native and chimeric Cry1Fa and 99.6% between native and chimeric Cry1Ac), and retain the species specific insecticidal toxicity to lepidopteran caterpillar pest.

180. Cry1Fa and Cry1Ac proteins are naturally produced by the common bacterium *B. thuringiensis*, varieties *aizawai* (Bta) and *kurstaki* (Btk), respectively. Related Cry toxins are also produced by other varieties of *B. thuringensis* (see Appendix 1), as well as by other GM cottons which are being grown commercially in Australia (Bollgard® II and INGARD® cottons, under licences DIR 012/2001 and DIR 022/2002 respectively, see *www.ogtr.gov.au*). Commercial Btk and Bta microbial formulations (produced by the fermentation of the same strains of bacteria from which these genes were derived) also contain these proteins, and have been used over the past 40 years to protect food crops (see Appendix 1, Section 3.2), including organic crops from insect attack. Bt spores and their Cry toxins are found widely in both agricultural and natural environments, including in soils, on plant leaves, in grain stores and in dead insects (Meadows 1993) and on a variety of fresh foods (such as lettuce and tomato) (ANZFA 1999).

181. PAT proteins as enzymes are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by *pat* and *bar* genes respectively (Wohlleben et al. 1988; Strauch et al. 1988). *Streptomyces spp*. are saprophytic, soil-borne bacteria and are not considered pathogens of plants, human or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). A search of the GenBank DNA sequence database reveals that other genes encoding PAT or similar enzymes are present in a variety of other bacteria. The class of enzymes to which PAT belongs, acetyltransferases, are common enzymes in all microorganisms, plants and animals.

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

### 2.3.1 Exposure to the GM cottons

182. None of the cotton plants from the proposed release or their by-products will be used as stockfeed. As discussed in section 2.1, most mammals avoid feeding on cotton, non-GM or GM, due to its production of toxic, anti-nutrient substances and plant morphology. The applicant proposes to destroy all materials produced in the release, apart from some cottonseed for use in subsequent seasons under the proposed release or in possible future releases (subject to further application and assessment processes).

183. 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 insecticidal protein expressed in the seeds of the GM cottons.

184. Cottonseed or pollen does not enter aquatic habitats in any significant quantity (OGTR 2002), and therefore the level of exposure of aquatic species to the GM cottons will be low.

185. In addition, the proposed release is small in size and limited in duration, further limiting the potential for exposure of stock and wildlife to the GM cottons.

186. After harvest, seed cotton may be dispersed beyond the limits of the farms where it is grown during transportation to ginning facilities. Specific licence conditions have been imposed to require cleaning of equipment used in connection with the release, and harvested material to be securely wrapped before transporting away from the release sites, so as to prevent the GM cotton seed

escaping into the wider environment (see Appendix 7 for details). Seeds not required for future releases must be destroyed.

### 2.3.2 Toxicity of the GM cottons for livestock and wildlife

187. As discussed above, the native Cry1Fa, Cry1Ac and other related Cry proteins are widespread in the environment. To exert a toxic effect, the Cry toxins, which are expressed in the form of protoxins, must first be digested by specific proteases in the insect gut and then diffuse through the midgut membrane and bind to specific receptors on the midgut epithelium surface (for more details of the specificity and mode of action refer to Appendix 1, Section 3). Mammals, birds and fish do not possess these receptors and therefore are not susceptible to the specific toxic effects of the Cry toxins.

188. Studies with high doses of purified Cry1Fa and Cry1Ac proteins, or of Bt microbial preparations containing these proteins, in mice, rats and rabbits have revealed no adverse toxic effects, as discussed in detail in Appendix 2, Section 2.4.

189. Feeding studies with other GM insecticidal cottons have also indicated no adverse effects of the expressed Cry proteins. Northern Bobwhite Quail fed raw cottonseed meal, derived from either INGARD® (containing *cry1Ac* gene) or conventional cotton, at up to 10% (w/w) of diet, equivalent to 100 seeds/bird/day, for five days showed no significant differences in feed consumption or body weight (Gallagher et al. 2000, Monsanto Unpublished). Commercial catfish fed a 20% processed cottonseed meal, either Bollgard® II (containing *cry1Ac* and *cry1Ab* genes) or conventional, showed no differences in survival, weight gain, feed conversion ratio or fillet composition (Li & Robinson 2000).

190. The performance of cows fed controlled diets including cottonseed from either non-GM cotton, INGARD® or Bollgard® II cottons have been compared (Castillo et al. 2001b; Castillo et al. 2001a; Hartnell et al. 2001, Monsanto Unpublished). There were no significant differences in body condition, milk yield or milk composition between cows fed the alternative diets. Western blot assays of the milk tested negative for the new proteins expressed in INGARD® cotton (Hartnell et al. 2001, Monsanto Unpublished). A study of dairy cows fed silage of GM corn containing the Cry1Ab and PAT proteins found no measurable impact on short-term lactational performance and ruminal fibre degradation relative to cows fed non-GM corn silage (Folmer et al. 2000).

191. GM corn containing Cry1Fa core toxin has been approved for use in food in Australia since 2003. No adverse effect of the GM crop on humans has been reported. FSANZ concluded that corn containing this *cry1F* gene is as safe and wholesome as from other commercial corn varieties (*http://www.foodstandards.gov.au/_srcfiles/ACF18.pdf*).

192. Evidence shows that the PAT protein is not toxic to any animal. As discussed above, acetyltransferases, the class of enzymes to which PAT belongs, are present in all microorganisms, plants and animals. The potential for PAT to be toxic has been addressed via acute and repeat dose toxicity studies in mice and rats, without any adverse findings (see Appendix 2, Section 2.4).

193. The demonstrated agronomic and compositional similarity of the GM cotton lines to non-GM cotton (see Appendix 1, Section 8) indicates that no significant unintended effects have occurred.

## Section 2.4 Potential toxicity hazard for invertebrates, including beneficial insects

### 2.4.1 Exposure to the GM cottons

194. Non-target invertebrates may be exposed to the GM cottons and the introduced proteins, either directly through feeding on the GM plants, or indirectly through eating other organisms, including the lepidopteran target organisms, that feed on the plants. Relative exposure will be greatest for other herbivorous species feeding on the cotton plants. Pollinator species and various adult insects that feed on the pollen will be exposed to the proteins. Sap feeders, such as aphids, will have minimal exposure, as the sap is composed primarily of sugars and mineral salts dissolved in water.

195. Non-target lepidopteran species may also be exposed to the GM cottons and may be affected by the Cry1Fa and Cry1Ac proteins. As cotton is not the preferred food source for non-target Lepidopteran species, their populations would be sustained on other types of plants found around the release locations. Furthermore, the proposed release is small in size and limited in duration, further limiting the potential for exposure of non-target invertebrates to the GM cottons.

### 2.4.2 Toxicity of the GM cottons for invertebrates

196. The Cry1Fa and Cry1Ac proteins are toxic specifically to a range of lepidopteran insect larvae, including pest species of cotton: *Helicoverpa zea* (cotton bollworm), *Helicoverpa virescens* (tobacco budworm), *Pectinophora gossypiella* (pink bollworm), *Spodoptera exigua* (beet armyworm), *S. eridania* (southern armyworm), *S. frugiperda* (fall armyworm), *Pseudoplusia includens* (soybean looper) and *Trichoplusia ni* (cabbage looper).

197. Data provided by the applicant show that there is no significant negative impact of the bacterially expressed Cry1Fa and Cry1Ac proteins on various non-target arthropods (Table 1). Reproductive activity in *Folsomia candida* (springtails or collembola) was reduced when fed Cry1Ac (22.6 µg/ml diet) in two experiments, by about 50% and 20%. However, feeding Cry1Ac with Cry1Fa (22.6 + 709 µg/ml diet respectively), Cry1Fa alone (709 µg/ml diet), or leaves of GM cotton expressing only Cry 1Ac (Cry1Ac cotton, at 5% or 50% of diet) did not affect reproduction. The highest mean level of expression of these proteins in the GM cottons, observed in young leaves, was 2.6 µg/g Cry1Ac and 41 µg/g Cry1Fa (see Appendix 1, Table 3). Since there was no adverse effect from feeding Cry1Ac with Cry1Fa, or Cry1Ac expressed in GM cotton leaves, it is unlikely that GM cottons will have a significant negative impact on collembola reproduction.

198. Field surveys of the GM Widestrike™ cotton line conducted in the US indicate no significant adverse effects on nearly 200 non-target arthropod species in sweep net and aerial trap samples (Mahill & Storer 2002). However, currently there is no information available on the potential toxicity or other adverse effects of these GM cottons to non-target organisms under Australian field conditions. As noted in Chapter 1, Dow AgroSciences proposes to test the effects of the GM cotton lines on non-target organisms. Further, research conditions have been imposed for collection of this information during the proposed field trial.

**Table 1 Non-target species found to be insensitive to the Cry1Fa and Cry1Ac proteins and GM material in laboratory analysis**

| Species | Common Name | Proteins tested | |
|---|---|---|---|
| | | Source | Dose/unit diet |
| *Apis mellifera* | Honey bee (larvae) | Pollen expressing Cry1Ac/Cry1Fa | 200 mg pollen/ml |
| | | Bacterial derived protein | 11.94 µg Cry1Ac + 1.98 µg Cry1Fa/ml |
| *Eisenia fetida* | Earthworm | Bacterial derived protein | 247 µg Cry1Ac/g<br>107 µg Cry1Fa/g<br>247 µg Cry1Ac + 107 µg Cry1Fa/g |
| *Hippodamia convergens* | Ladybird beetle | Bacterial derived protein | 22.5 µg Cry1Ac/ml<br>300 µg Cry1Fa/ml<br>22.5 µg Cry1Ac + 300 µg Cry1Fa/ml |
| *Nasonia vitripennis* | Parasitic hymenoptera | Bacterial derived protein | 46.8 µg Cry1Ac/ml<br>5.2 µg Cry1Fa/ml<br>46.8 µg Cry1Ac + 5.2 µg Cry1Fa/ml |
| *Chrisoperia carnea* | Green lacewing | Bacterial derived protein | 46.8 µg Cry1Ac/g<br>5.2 µg Cry1Fa/g<br>46.8 µg Cry1Ac + 5.2 µg Cry1Fa/g |
| *Daphnia magna* | Daphnid | Bacterial derived protein | 2.5 µg Cry1Ac/ml<br>0.51 µg Cry1Fa/ml<br>2.5 µg Cry1Ac + 0.51 µg Cry1Fa/ml |

199. As discussed above (Section 2.2), acetyltransferases, the class of enzymes to which PAT belongs, are present in all microorganisms, plants and animals. This enzyme has been extensively studied, and there is no indication that it is toxic to any organism (e.g. earthworms or honeybees) (USDA-APHIS 1998). Further detail is given in the risk assessment for DIR 021/2002, available at *www.ogtr.gov.au*

## Section 2.5 Potential toxicity hazard for microorganisms, particularly soil microorganisms

### 2.5.1 Exposure to the GM cottons

200. Microorganisms, particularly soil microorganisms, will be exposed to the GM cotton plants and the introduced proteins during growth and decomposition of plant material. While cotton plants are living, exposure of soil microorganisms to the introduced proteins may occur as a result of root exudations, as has been observed in Bt corn expressing Cry1Ab (Saxena et al. 1999); (Stotzky 2000) and INGARD cotton expressing Cry1Ac (Gupta et al. 2002). Root breakage could also lead to the release of the introduced proteins into soil. After the cotton is harvested, the remaining plant residues will be tilled into the soil. As noted in Chapter 1, the applicant proposes to measure the expression levels of the insecticidal proteins in cotton leaves and squares and residues of these proteins in soil

201. The proposed release is small in size and limited in duration, which will further limit exposure of microorganisms to the GM cottons. Research conditions have been imposed to require collection of more information on the persistence of insecticidal proteins in the soil.

### 2.5.2 Toxicity of the GM cottons for microorganisms

202. A number of studies on the effect of related Cry1 proteins, expressed by Bt or in GM crops expressing Cry1 proteins (e.g. INGARD® cotton), on soil microorganisms have shown no detrimental effects, as discussed in detail in Appendix 3 of the risk assessment and risk management plan for DIR 022/2002, available at *www.ogtr.gov.au*.

203. As discussed above (Section 2.2), acetyltransferases, the class of enzymes to which PAT belongs, are present in all microorganisms, plants and animals. This enzyme has been extensively studied, and there is no indication that it is toxic to any organism (USDA-APHIS 1998).

## SECTION 3 CONCLUSIONS REGARDING TOXICITY TO NON-TARGET ORGANISMS

204. It is considered that the risk of the GM cottons being toxic to non-target organisms is low because:

- the introduced core proteins are very similar to native bacterial proteins which are already widespread in the environment, including in soil, on plants and on fresh produce, in the microorganisms from which the genes were derived;
- the release is small in size and limited in duration;
- the toxicity of Cry1Fa and Cry1Ac proteins is specific to lepidopteran caterpillar larvae;
- the PAT protein is not known to be toxic to any organism;
- exposure of livestock and wildlife to the GM cotton lines would be low, and no materials from the release are proposed to be used in stockfeed;
- toxicity studies with purified proteins and Bt microbial preparations indicate that the Cry1Fa and Cry1Ac proteins are not toxic to mammals, birds or fish; and
- laboratory and field studies done in the United State of America indicate that populations of non-target invertebrates are unlikely to be affected by Cry1Fa, Cry1Ac and PAT proteins.

## SECTION 4 RESEARCH REQUIREMENTS

205. The proposed release of three GM cotton lines is a small scale, multi-site trial over four cotton growing seasons. The licence holder is required to collect data on the potential toxicity or other adverse effects of these GM cotton lines on non-target organisms and on persistence of the insecticidal proteins in soil under Australian field conditions.

# APPENDIX 4 WEEDINESS

206. 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 release to the environment are considered in relation to the potential for the GMOs to become problematic weeds.

## SECTION 1 NATURE OF THE WEEDINESS HAZARD

207. There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their 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.

208. 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(Roy 1990; Williamson & Fitter 1996); . 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 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.

209. The GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) differ from conventional cotton in the expression of two or three additional proteins, the Cry1Fa and/or Cry1Ac toxin and the PAT enzyme, which confers tolerance to the herbicide glufosinate ammonium (see Appendix 1 for details of protein expression in the GMOs).

210. The possibility was considered that the GM 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.

211. 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 a weed, this could result in impacts such as loss of native biodiversity or adverse effects on agricultural systems.

## SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

212. 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 non-GM cotton;
- the potential selective advantage conferred by the introduced proteins; and
- the potential weediness of GM insecticidal/herbicide tolerant cottons.

### Section 2.1 Inherent weediness of conventional non-GM cotton

213. 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, temperature and roadside management practices limit the establishment and/or persistence of cotton seedlings. Information on the weediness of non-GM cotton is included here to establish a baseline for comparison with the GM cottons being considered.

214. 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).

215. Another important element in prediction of weediness is taxonomic relationship, considering weediness within a taxon, including its 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* (Craven et al. 1994; Fryxell 1992) of which only one (*G. tomentosum*) is listed as a weed in the USA (Holm et al. 1997).

### Section 2.2 Potential selective advantage conferred by the introduced proteins

#### 2.2.1 Cry1Fa and Cry1Ac toxins

216. The Cry1Fa and Cry1Ac proteins could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton.

217. Another GM insecticidal cotton, INGARD® cotton, that is resistant to lepidopteran insect pests, due to expression of a different version of the *cry1Ac* gene, has been commercially grown in Australia since 1996. The potential weediness of INGARD® cotton has been considered in the risk assessment for application DIR 022/2002. Surveys of volunteer cotton in Australia and experimental research on the weedy potential of GM insecticidal/herbicide tolerant cottons in Australia consistently suggest that major factors limiting both the GM cottons and non-GM 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).

### 2.2.2 PAT enzyme

218. The PAT protein could only confer a selective advantage in the presence of glufosinate ammonium herbicide application. In an agricultural setting, the GM cottons lines proposed for release will have increased fitness where glufosinate ammonium is applied for weed control. However, glufosinate ammonium is not generally used to control volunteer cotton plants in agricultural systems, and has limited effectiveness on established cotton (ie. beyond the seedling stage). Cultivation or other herbicides are the main control strategies employed (Australian Cotton Cooperative Research Centre 2002).

### 2.2.3 Cry1Fa, Cry1Ac and PAT Combination

219. The insecticidal and herbicide tolerance genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the genes, their proteins or their metabolic pathways, and no reason to expect that this is likely to occur. Agronomic qualities of all three GM cotton lines are similar to those of non-GM cotton, apart from the intended herbicide tolerance and insecticidal characteristics, suggesting that no unintended effects have occurred as a result of the genetic modifications (see Appendix 1, Section 8).

## Section 2.3 Potential weediness of the GM insecticidal/herbicide tolerant cottons

220. 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. Growth habit, germination, emergence in the field, vigour, flowering period and reproductive potential of these three GM cotton lines are very similar to those of the non-GM parental cotton variety used in its breeding, and are within the range typical for non-GM cotton varieties (Pellow 2003). These data suggest that the genetic modifications in these GM cotton lines have not led to any unintended effects on characteristics typically associated with weediness.

## Section 2.4 Spread of the GM cottons beyond the release sites

221. During and after planting and harvesting, cottonseed may be dispersed beyond the limits of the trial sites on equipment or during transportation of harvested seed cotton. Specific licence conditions have been imposed to require cleaning of equipment used in connection with the release, and harvested material to be securely wrapped before transporting away from the release sites, so as to prevent the GM cotton seed escaping into the wider environment (see Appendix 7 for details). Seeds not required for future releases must be destroyed.

## Section 2.5 Persistence of the GM cottons at the release sites

222. 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. 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 (November to March) following harvest.

223. Post harvest inspections of the release sites are required as a condition of the licence, to ensure volunteer cotton plants are destroyed before flowering and that the GM cottons are unable to persist in the environment.

## SECTION 3 CONCLUSIONS REGARDING WEEDINESS

224. It is concluded that the risk of the GM cottons establishing as environmental weeds as a result of the proposed release is low because:

- cotton does not possess characteristics commonly associated with weediness, and is not known to be a problematic weed in any environment;
- the genetic modifications in the GM cotton lines are not likely to affect these characteristics;
- other GM insecticidal cottons grown (targeted to lepidopteran caterpillar pests) commercially in Australia have not become problematic weeds;
- major constraints on weediness of both GM and non-GM cottons are water availability, nutrient availability, plant competition, herbivory by non-lepidopteran species, frost and fire;
- the insecticidal gene(s) in the GM cotton may confer a selective advantage if the cotton is limited by lepidopteran insects, however, the risk is low; and
- although the two traits (insecticidal and herbicide tolerance) may have an additive effect on competitiveness, neither of these traits individually has been found to be significant for weediness.

225. It is considered that the low risk of the GM cotton lines establishing as weeds can be managed by applying various strategies to limit the spread and persistence of the GM cottons from the release sites. Licence conditions have been imposed to manage this risk, including a requirement to conduct post harvest inspections of the release sites and destroy volunteer cotton plants before they flower (see Chapter 2 and Appendix 7 for details).

## SECTION 4 FURTHER RESEARCH REQUIREMENTS

226. If the applicant makes any applications for future larger scale releases of these GM cottons, more data would be required to be collected on agronomic characteristics of the GM cottons in relation to potential weediness under Australian field conditions and the additive effects of the two traits (insecticidal and herbicide tolerance) on weediness.

# APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

227. 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/herbicide tolerant cottons to other organisms.

228. 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 sometimes be produced between closely related species through sexual reproduction although this may require significant assistance. For example, in plants cross pollination of wheat and rye in the laboratory produced triticale. In animal, 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 new genes into a population.

229. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except for bacteria and viruses. 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 functional genes from plants to other types of organisms.

230. The likelihood of a hazard arising as a result of gene transfer from the GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideSrike™ cottons) is dependent on a number of factors, which must form a chain for a hazard to be realised, including:

- **opportunity** for gene transfer to occur such that the recipient organism is exposed to the genetic material of the donor in the form of pollen, plant cells or DNA;
- **occurrence** of the genetic material of the donor being incorporated into the genetic material of the recipient organism at a site and in a configuration that allows the gene to be functional;
- **persistence** of the transferred genetic material such that the recipient 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.

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

- **Section 1** details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM cottons to other plants, including other cotton crops;
- **Section 2** details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM cottons to microorganisms;

- **Section 3** details the nature and likelihood of a hazard arising through transfer of the introduced genes from the GM cottons to animals, including humans; and
- **Section 4** draws together the conclusions from these sections.

232. A detailed assessment of the likelihood of gene transfer from GM cottons to other organisms is also presented in the risk assessments for licence applications DIRs 005/2001, 006/2001, 008/2001, 009/2002, 012/2002, 015/2002, 016/2002, 017/2002, 022/2002, 023/2002, 025/2002, 034/2003, 035/2003, 036/2003, 038/2003 and 040/2003.

## SECTION 1 GENE TRANSFER FROM THE GM COTTONS TO OTHER PLANTS

### Section 1.1 Nature of the gene transfer hazard

233. Transfer of the introduced genes (*cry1Fa*, *cry1Ac* and *pat*) or regulatory sequences (promoter and terminator regions) from the GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideSrike™ cottons) to other cultivated cotton plants, volunteer cotton or naturalised (feral) cotton would present the same hazards, and have the same potential impacts, as the presence of the genes in the GM cottons proposed for release. These risks are considered in Appendices 2 - 6. However, if such a transfer occurred, it would increase the possibility that these genes could further spread in the environment.

234. 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 cry1Fa* AND *cry1Ac* (INSECTICIDAL) GENES

235. Plants expressing these genes 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 *pat* (HERBICIDE TOLERANCE) GENE

236. 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 (eg. Liberty® and Basta® herbicides).

THE INTRODUCED PROMOTERS AND OTHER REGULATORY SEQUENCES

237. 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.

238. 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.

239. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to new genetic contexts occurs naturally in all plant genomes and could also result in unpredictable effects. Thus the potential hazards from the introduced sequences are not different from those 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 transfer of the introduced genes to other plants

### 1.2.1 Transfer to other cultivated cotton and volunteer cotton

240. Cotton is primarily self-pollinating, however in a cropping situation a low level of pollen transfer, by insect pollinators, to nearby cotton plants is likely. Cotton pollen dispersal studies consistently show that outcrossing is localised around the pollen source and decreases significantly with distance. Studies carried out in southern Australia by Llewellyn and Fitt (1996) and Thomson (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 weedier or more 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 would present the same low hazards as their presence in the GM cottons proposed for release (see Appendices 2 - 6). However, if such a transfer occurred, it would increase the possibility that these genes could persist and further spread in the environment.

243. The likelihood of a hazard arising through transfer of the introduced genes would be further minimised because of the small size and limited duration of the proposed release. Licence conditions have been imposed to manage this risk. These include a requirement to either surround the GM cottons with a 20 m pollen trap of non-GM cotton or to ensure that there are no other cotton crops or naturalised cotton populations within 450 m of the GM cotton crops. Conditions have also been imposed to establish a 400 m wide area of land (called a 'Research Zone') around release sites in excess of 1 ha for the purpose of conducting research on gene flow. Development of an agreed

research program to inform the ongoing review of data on gene flow and to validate the efficacy of containment measures is a condition of the licence (see Chapter 2 and Appendix 7 for details).

### 1.2.2 Transfer to naturalised cotton

244. Transfer of the introduced genes to naturalised cotton could also increase the likelihood that the genes could spread and/or persist in the environment (away from cotton farming systems). 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, in northern Northern Territory and northern Western Australia. The remnants of these populations, a few of which may be within pollinating distance of commercial cotton crops, has not been confirmed. As part of the licence conditions for DIR 022/2002, a survey of naturalised cotton populations in Queensland, in locations suggested by herbarium records, is being conducted.

245. Transfer of the introduced genes or regulatory sequences to naturalised cotton plants would present the same low hazards as their presence in the GM cottons proposed for release (see Appendices 2 - 6). However, if such a transfer occurred, it would increase the possibility that these genes could persist and further spread in the environment.

246. Licence conditions are imposed to limit cross-pollination to plants outside the release sites (see Section 1.2.1 above or Chapter 2 and Appendix 7 for details).

### 1.2.3 Transfer to native cottons and other plant species

247. 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 (OGTR 2002).

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

249. The failure of cross-pollination due to well established genetic incompatibility also prevents gene transfer from these GM cotton lines to other plant species.

## SECTION 2 GENE TRANSFER FROM THE GM COTTON TO MICROORGANISMS

### Section 2.1 Nature of the gene transfer hazard

250. 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 (Nielsen 1998; Ochman et al. 2000; Smalla et al. 2000; Stanhope et al. 2001).

251. The potential hazards associated with the introduced genes of the GM 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.

### 2.1.1 Potential hazards from the introduced genes

THE *cry1Fa* AND *cry1Ac* (INSECTICIDAL) GENES

252. Microorganisms expressing these genes 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 *pat* (HERBICIDE TOLERANCE) GENE

253. Microorganisms expressing this gene would gain resistance to glufosinate ammonium and to the tripeptide antibiotics naturally produced by a small number of actinomycete bacteria (Organisation for Economic Co-operation and Development (OECD) 1999): Bialaphos (phosphinothricyl-L-alanyl-L-alanine), produced by *S. viridochromogenes* and *S. hygroscopicus* (the bacterial species from which the *pat* and related *bar* genes, respectively, are derived); and Phosalacine (phosphinothricyl-L-alanyl-L-leucine), produced by *Kitasatosporia phosalacinea*.

254. Neither bialaphos or phosalacine are used in human or veterinary therapy. *Streptomyces* spp. are saprophytic soil bacteria, and not considered pathogens 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.

255. Antibiotic production by non-pathogenic bacteria has been implicated in suppression of some plant diseases (Brimecombe et al. 2001). Thus transfer of the *pat* gene to new bacterial species, including plant pathogens, could potentially impact on microbial populations or plant disease susceptibility. However, expression of the *pat* or the related *bar* gene in a variety of crop plants (for example canola and corn), over several years of agronomic performance testing and commercial cultivation, has not been linked to increased susceptibility to disease, as assessed by regulatory authorities of other countries, including the Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture (eg. USDA-APHIS 2003) and the Canadian Food Inspection Agency (eg. Canadian Food Inspection Agency 1998b), also see Gene files (2002) for links to further safety assessments.

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256. 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.

257. 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.

258. 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.

### 2.1.2 Other sources of the introduced genes in the environment

259. 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 in the absence of the GM cotton.

260. All of the introduced genes in the GM cotton are already widespread in the environment, being derived from common soil bacteria. The regulatory sequences are derived from a plant and a common bacterium.

THE *cry1Fa AND cry1Ac* (INSECTICIDAL) GENES

261. Although the introduced *cry1* genes in GM cotton lines proposed for release are chimeric, the encoded Cry proteins are very similar to the native Cry proteins (99.3% similarity in amino acid sequence of core toxin between native and chimeric Cry1Fa and 99.6% between native and chimeric Cry1Ac), and retain the species specific insecticidal toxicity to lepidopteran caterpillar pest. The *cry1Fa* and *cry1Ac* genes occur naturally in the common soil bacteria *B. 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).

262. Many Cry 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 processes known as conjugation and transformation. The native *cry1Ac* gene has been identified on a plasmid of Bt variety *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 *pat* (HERBICIDE TOLERANCE) GENE

263. The herbicide tolerance *pat* gene was originally isolated from the common soil bacterium *S. viridochromogenes*, and another gene, *bar*, which also encodes a PAT enzyme is naturally present in *S. hygroscopicus*. These bacteria are not considered pathogenic to plants, humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999) and are widespread in natural and agricultural environments.

264. A search of the GenBank DNA sequence database reveals that other genes encoding PAT or similar enzymes are present in a variety of other bacteria.

## Section 2.2 Likelihood of a hazard arising through transfer of the introduced genes to microorganisms

265. 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.

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

267. In contrast, transfers of plant genes to other organisms such as bacteria, fungi or viruses are exceedingly rare (Greene & Allison 1994; Nielsen et al. 1998; Nielsen et al. 2000; Pittard 1997; Schoelz & Wintermantel 1993; Worobey & Holmes 1999; Aoki & Syono 1999; Harper et al. 1999; Mayo & Jolly 1991). The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments (Nielsen et al. 1998; Pittard 1997; Worobey & Holmes 1999; Greene & Allison 1994; Nielsen et al. 2000; Schoelz & Wintermantel 1993). 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.

### 2.2.1 Bacteria

268. Three different mechanisms of horizontal gene transfer in bacteria have been described: transduction, conjugation, and transformation (Nielsen 1998).

269. Transduction is a bacterial cell-virus interaction that can mediate gene transfer between bacteria in the environment (e.g. on plant leaf surfaces, in soil or water). Viruses that function in more than one species are known, but viruses that function in both plants and bacteria, and thereby facilitate horizontal gene transfer from plants to bacteria have not been identified (Nielsen et al. 1998).

270. Conjugation is a mechanism of cell-to-cell interaction that can mediate gene transfer between bacteria in the environment (e.g. in soil, on plant surfaces, in water etc).

271. Conjugation is known to occur frequently between compatible bacteria with the transferable genes usually residing on plasmids. Transfer of chromosomal genes is much less frequent, except for some high frequency recombination strains. Conjugative gene transfer has been regarded as the most frequently occurring mechanism of horizontal gene transfer between bacteria (Sprague 1991; Amabile-Cuevas & Chicurel 1993). However, mechanisms that support conjugative gene transfer from higher plants to bacteria (e.g. transposons that function in both plants and prokaryotes) are not known (Nielsen 1998).

272. Gene transfer by transformation results from the uptake of naked DNA by bacteria, and has been shown to occur in environments such as in soil and in water (Lorenz & Wackernagel 1994; Streips 1991). Most studies describing natural transformation have been conducted *in vitro*

(Lorenz & Wackernagel 1994; Streips 1991) but often are of little relevance to most natural terrestrial environments.

273. 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 GM 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 for gene transfer from divergent donor organisms (Nielsen 1998).

274. 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 state;
- **uptake** of DNA fragments;
- **chromosomal integration** via recombination or autonomous replication of the transforming DNA;
- **expression** of the genes by the recipient bacterium; and
- **selective advantage** to fix (maintain) the transferred DNA in the gene pool of the recipient species.

275. 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 (eg. 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 (Mercer et al. 1999; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001), and even then only at a very low frequency.

### 2.2.2 Viruses

276. 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.

277. Thus the likelihood 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 2.1.2).

### 2.2.3 Fungi

278. 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 *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by *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 1998).

279. Thus the likelihood 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 2.1.2).

## SECTION 3 GENE TRANSFER FROM THE GM COTTON TO ANIMALS

### Section 3.1 Nature of the gene transfer hazard

280. The potential hazards associated with the introduced genes in the GM 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.

### 3.1.1 Potential hazards from introduced genes

THE *cry1Fa* AND *cry1Ac* (INSECTICIDAL) GENES

281. 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 or disadvantage to the animal.

THE *pat* (HERBICIDE TOLERANCE) GENE

282. The expression of the PAT enzyme in an animal would not be expected to produce any adverse metabolic effects, since PAT has extremely high substrate specificity for L- phosphinothricin (L-PPT), and cannot acetylate any other amino acids or proteins (Wehrmann et al. 1996; Canadian Food Inspection Agency 1995).

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283. If these sequences were to be transferred to animals without the associated genes of the 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.

284. 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.

285. 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 from the GM cottons is no different to that arising from non-GM plants.

### Section 3.2 Likelihood of a hazard arising through transfer of the introduced genes to animals

286. The most significant route for entry of foreign DNA into animals, including humans, would be 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. However, the proportion of DNA which may derive from the introduced genes of GM plants in the animal diet is extremely low. For example, Beever and Kemp (2000) estimated that in a diet comprising 40% GM maize, the introduced genes would represent 0.00042% of total dietary DNA intake.

287. A recent study showed that the *cry1Ab* gene for GM maize was not detecable in the peripherical blood mononuclear cells and tissues of calves 5 to 18 hours after the calves were fed with the GM maize, indicating the gene was not being transferred into those cells or tissues (Chowdhury et al. 2004).

288. There are few published studies evaluating the survival of transgene in the humans. A study conducted by Netherwood (2004) with GM soya showed that the introduced gene (*epsps*) did not survive passage through the intact gastrointestinal tract of human subjects fed GM soya, suggesting the gene was not being transferred to humans (Netherwood et al. 2004).

289. The fate of DNA in the digestive tract of various animals has been studied and is discussed in detail in the risk assessments for DIR 021/2002 and DIR 22/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and no greater than the likelihood of transfer from other sources of the introduced genes in the environment (see Section 2.1.2 of this Appedix).

290. No products from the GM cottons in the proposed field trial will be used for human food or animal feed. Most animals avoid feeding on cotton due to its natural toxicity and morphology (OGTR 2002). Thus the likelihood of gene transfer to animals, including humans, is negligible. Furthermore, any uptake of plant DNA is likely to occur in non-reproductive (somatic) cells such as immune system of gut epithelium cells, and the introduced gene would be transmitted to the cells of any progeny. It is worth noting that cottonseed oil and linters are the only fraction of cotton plants used in human food. Since these 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 DNA of these GM cottons via food, excluding the possibility of gene transfer to human cells in the gut.

# SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS

## Section 4.1 Conclusions regarding gene transfer to other plants

291. It is considered that risks arising through gene transfer from the GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) to other plants are low because:

- gene transfer to other cotton crops, volunteer or naturalised cotton would not pose any risks additional to the low risks posed by the GM cottons themselves;
- genetic incompatibility prevents gene transfer to native cotton species and other plant species.

292. The licence conditions have been imposed to further limit gene transfer to other cultivated, volunteer and naturalised cotton. These include a requirement to either surround the GM cottons with a 20 m pollen trap of non-GM cotton or to ensure that there are no other cotton crops or naturalised cotton populations within 450 m of the GM cotton crops. Additional conditions have been imposed to establish a 400 m wide area of land (called a 'Research Zone') around release sites in excess of 1 ha for the purpose of conducting research to inform the ongoing review of data on gene flow and to validate the efficacy of containment measures (see Chapter 2 and Appendix 7 for details).

## Section 4.2 Conclusions regarding gene transfer to microorganisms

293. It is considered that risks arising through transfer of the introduced genes from GM cottons to microorganisms are negligible, and do not require management, because:

- the introduced genes in the GM cottons are derived from, and are similar to, native bacterial genes that 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 microorganisms 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.

## Section 4.3 Conclusions regarding gene transfer to animals, including humans

294. It is considered that risks through transfer of the introduced genes from GM cottons to animals, including humans, are negligible, and do not require management, because:

- the introduced genes in the GM cottons are derived from, and are similar to, native bacterial genes that are already widespread in the environment;
- transfer of the introduced genes would be unlikely to pose a hazard to human health and safety or to the environment;
- products from the GM cotton lines are not intended for stockfeed or human food;
- most animals avoid feeding on GM or non-GM cotton plants; and
- even if the GM cottons were approved by FSANZ for use in food, the cotton by-products used in food do not contain DNA.

## SECTION 5 RESEARCH REQUIREMENTS

295. The proposed release of the GM cotton is a small scale, multi-site trial over four cotton growing seasons. The licence holder is required to establish a 400 m wide area of land (called a 'Research Zone') around release sites in excess of 1 ha for the purpose of conducting research on gene flow. Development of an agreed research program to inform the ongoing review of data on gene flow and to validate the efficacy of containment measures is a condition of the licence (see Chapter 2 and Appendix 7 for details).

# APPENDIX 6 DEVELOPMENT OF INSECTICIDE RESISTANT PESTS

296. 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 release to the environment are considered in relation to the potential for development of insecticide resistance among target pests.

297. It should be noted that Dow AgroSciences does not intend to apply glufosinate ammonium herbicide (e.g. Liberty® and Basta® herbicides) to the GM cotton lines proposed for the release, therefore issues relating to risks of development of herbicide resistant weeds are not considered in this Appendix.

## SECTION 1 REGULATION OF AGRICULTURAL CHEMICALS IN AUSTRALIA

298. Regulation of agricultural chemicals, including herbicides and insecticides, is principally the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the *Agricultural and Veterinary Chemicals Code Act 1994* (the Ag Vet Code Act). The GM cotton lines proposed for release fall under the Ag Vet Code Act definition of an agricultural chemical product, due to its production of an insecticidal substance(s), and are thus subject to regulation by the APVMA.

299. The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to the use of 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 for experimental work that allow restricted use of an agricultural chemical, for example, for a limited period of time or for a limited area.

300. In considering applications for registration or permits, as well as considering potential health and environmental impacts, the APVMA also considers a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as efficacy and the trade implications of residues. The hazard of development of resistance to agricultural chemicals is part of the APVMA's assessment of agricultural chemical use. The APVMA can impose conditions on the use of chemical products in both registrations and permits. These conditions can include restrictions on use, implementation of a resistance management plan, and ongoing reporting on compliance.

301. The *Gene Technology Act 2000* requires the regulator to consult the APVMA in relation to the assessment of licence applications involving intentional release of GMOs to the Environment. The *Gene Technology (Consequential Amendments) Act 2000* places a reciprocal obligation upon the APVMA to consult the Gene Technology Regulator in relation to certain decisions regarding registrations and permits for an agricultural chemical that is or contains a genetically modified product.

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

information about the APVMA's assessment and approval processes can be obtained from *www.apvma.gov.au*.

## SECTION 2 NATURE OF THE INSECTICIDE RESISTANCE HAZARD

303. If the GM insecticidal/herbicide tolerant cotton lines (Cry1Fa, Cry1Ac and WideStrike™ cottons) were cultivated extensively, *Helicoverpa armigera*, *H. punctigera* and other susceptible lepidopteran insect pest species that feed on the GM cottons would be placed under selection pressure for resistance to the Cry1Fa and Cry1Ac insecticidal proteins. If resistance were to develop in target pests, the insecticidal efficacy of these GM cottons would be adversely affected, potentially attenuating any benefits of these GM cottons and of other applications of these insecticidal proteins.

## SECTION 3 LIKELIHOOD OF THE INSECTICIDE RESISTANCE HAZARD OCCURRING

304. Due to the small scale and limited duration of the proposed release, the likelihood of insects developing resistance to the insecticidal proteins in these GM cottons is negligible.

305. Dow AgroSciences has submitted an application to the APVMA for a research permit for the use of the insecticidal genes in GM cotton lines for the proposed release. The hazard of development of insecticide resistance in pests is also being assessed by the APVMA in considering Dow AgroSciences' permit application. The APVMA would impose conditions if it considered this necessary to manage any identified risk.

## SECTION 4 CONCLUSION REGARDING INSECTICIDE RESISTANCE

306. The risk of development of insecticide resistance in pests is negligible due to the small scale and limited duration of the proposed release. This risk is being assessed by the APVMA in considering Dow AgroSciences' permit application for the use of the insecticidal genes as insecticides in the GM cottons.

307. Therefore, the Regulator has not imposed specific conditions in relation to management of insecticide resistance, however the requirement to comply with any conditions imposed by the APVMA is noted in the licence.

# APPENDIX 7 LICENCE CONDITIONS

### *Gene Technology Regulation in Australia*

*The Gene Technology Act (2000)* and corresponding State and territory legislation form a substantial part of a range of integrated regulatory measures relevant to controlling genetically modified organisms (GMOs) and their use.

The Gene Technology Regulator is required to consult with, and take into account advice from, a range of regulatory authorities on risks to human health and safety and the environment in assessing applications for dealings involving the intentional release of GMOs into the Australian environment.

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

Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. FSANZ approval would need to be obtained before any parts of GM cottons, such as oil and linters derived from GM cottonseed, could be used as human food. This licence contains a condition that prohibits this use.

### *Note in relation to insecticide resistance management*

The *Gene Technology (Consequential Amendments) Act 2000* requires the Australian Pesticides and Veterinary Medicines Authority (APVMA), to consult the Gene Technology Regulator for the purposes of making certain decisions, including the imposition of conditions of use regarding registration or issuing a permit for a chemical product that is, or contains a genetically modified product.

The GMOs referred to in this licence fall under the *Agricultural and Veterinary Chemicals Code 1994* definition of agricultural chemical products, due to their production of insecticidal substances, and are therefore subject to regulation by the APVMA. The APVMA assesses the hazard of development of insecticide resistance as part of its evaluation process and would impose conditions to manage any identified risks. Therefore, the conditions of this licence do not relate to management of insecticide resistance, and do not replace any conditions set by the APVMA.

## SECTION 1 INTERPRETATIONS AND DEFINITIONS

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

In this licence:

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

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

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

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

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

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

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

In this licence:

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

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

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

(b) in relation to Equipment, the removal and Destruction of plants and Plant Material from the Equipment, to the reasonable satisfaction of the Regulator;

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

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

(a) stalk pulling; or

(b) uprooting by ploughing;

(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.*

**'Equipment'** includes machinery, harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), ginning facilities, clothing and tools used in connection with this licence**;**

**'GM'** means genetically modified;

**'GMOs'** means the genetically modified organism or organisms authorised for release by this licence;

**'Isolation Zone'** means the area of land, extending outwards 50 metres in all directions from the outer edge of a Location**;**

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

**'Plant Material'** means viable parts of GMOs and Pollen Trap plants, including seed, stubble, pollen, whether from the plant itself or derived from or produced by the plant;

**'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 the area of land extending outwards 20 metres in all directions from the outer edge of a Location;

**'Pollen Trap plant'** means Cotton from a Pollen Trap;

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

**'Regulator'** means the Gene Technology Regulator;

**'Seed'** means whole Cotton seed from the GMOs or Pollen Trap plants, including seed cotton, fuzzy seed and black seed;

**'Sign-off'** means a notice in writing from the Regulator, in respect of a place, that inspection conditions no longer apply in respect of that place;

**'Volunteer plants'** means progeny of the GMOs or Pollen Trap plants and regrowth of Cotton plants;

## SECTION 2 GENERAL CONDITIONS

### Duration of Licence

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

### Holder of Licence

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

### Project Supervisor

3. The Project Supervisor in respect of this licence is identified at Attachment A.

4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

### No dealings with GMOs except as authorised by this licence

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

### GMOs covered by this licence

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

### Permitted dealings

7. The permitted dealings with the GMOs are to plant and grow the GMOs and to conduct experiments on the GMOs that are grown. The permitted dealings includes the possession, storage, 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

8. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs or places that are referred to in this licence.

### Informing people of their obligations

9. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:

   (a) the particular condition (including any variations of it);

   (b) the cancellation or suspension of the licence;

   (c) the surrender of the licence.

10. 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**

11. The licence holder must immediately, by notice in writing, inform the Regulator of:

    (a) any relevant conviction of the licence holder occurring after the commencement of this licence;

    (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;

    (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of his licence to meet the conditions in it.

**Additional information to be given to the Regulator**

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

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

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

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

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

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

**Remaining an accredited organisation**

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

## SECTION 3 SPECIFIC CONDITIONS

1. The permitted dealings with the GMOs, other than disposal of the GMOs, may only be undertaken between May 2004 and July 2006.

2. The GMOs must only be grown in accordance with the restrictions set out in Attachment C. Attachment C sets out, for each growing season (in which the GMOs may be grown):

   (a) those shires where the GMOs may be grown;

   (b) the maximum number of Locations; and

   (c) the maximum combined area of all Locations.

3. The licence holder must be able to access and control a Location where the GMOs are grown to the extent necessary to comply with this licence, for the duration of the life of the licence.

**Notification of planting of the GMOs**

4. The licence holder must provide a notice in writing to the Regulator each time a crop of the GMOs are planted at a Location.

5. The notice must set out:

   (a) The date on which planting of the GMOs commenced;

   (b) The Location's GPS coordinates and either a street address or other directions to the Location;

   (c) The period during which the licence holder considers the GMOs are likely to flower; and

   (d) The period during which the licence holder considers the Location will be Cleaned (after the GMOs have been grown there).

6. The notice must be provided to the Regulator within 14 days of the date on which planting of the GMOs commenced.

**Notice of Cleaning of Location**

7. The licence holder must provide a notice in writing to the Regulator when a Location is Cleaned pursuant to this licence.

8. The notice must be provided to the Regulator within 14 days of the date on which Cleaning the Location concluded.

**Location must be surrounded by a Pollen Trap or an Isolation Zone**

9. Every Location where a crop of the GMOs is grown must be surrounded by either:

(a) a Pollen Trap; or

(b) an Isolation Zone.

**Isolation Zones may only be used in limited situations**

10. A Location may only be surrounded by an Isolation Zone if:

(a) There are no naturalised cotton populations (*Gossypium hirsutum* or *G. barbadense*) within 450 m of the Location; and

(b) There are no cotton crops (*Gossypium hirsutum* or *G. barbadense*) within 450 m of the Location (other than crops of the GMOs grown pursuant to this licence).

**Conditions about Pollen Traps**

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

12. The edge of the Pollen Trap that is farthest from the GMOs must not be within 50 metres of a Natural Waterway.

13. Once planted, Pollen Trap plants and Plant Material from those plants must be handled and controlled as if they are GMOs and Plant Material from the GMOs. (ie., once planted, Pollen Trap plants and Plant Material from those plants are GMOs for the purposes of this licence and 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.

**Conditions about Isolation Zones**

15. No cotton of any kind may be grown in an Isolation Zone.

16. No flowering cotton plants may be present in an Isolation Zone while the GMOs are being grown at the Location and any cotton plant that is found to be present in an Isolation Zone must be immediately Destroyed.

17. If any cotton crop not grown pursuant to this licence occurs within 450 m of a Location with an Isolation Zone while the GMOs are being grown at the Location, either the cotton crop or the GMOs in the Location must be Destroyed prior to flowering. If GMOs are Destroyed pursuant to this condition, they are taken to have been harvested for the purposes of this licence

**Locations of more than 1 hectare in size must be surrounded by a Research Zone**

18. Each Location of 1 hectare or more in size 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.

19. A 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.

**Seed and other Plant Material may be collected**

20. Leaf tissue from the GMOs may be collected from a Location for the purpose of conducting experiments on it.

21. Leaf tissue from the GMOs that is collected may only be transported off the Location to:

    (a) a locked facility on the same property as the Location that is signed so as to indicate GM Plant Material is stored within the facility; or

    (b) a facility certified by the Regulator to physical containment level 2 (PC2).

22. Leaf tissue that is collected from the Location may be stored in a locked facility on the same property as the Location that is signed so as to indicate GM Plant Material is stored within the facility. Leaf tissue stored in the facility must be stored in a sealed container.

23. After any experiments with leaf tissue from the GMOs are completed, the leaf tissue must be incinerated.

**Crops of the GMOs must be either harvested or Destroyed**

24. Within 9 months of being planted, crops of the GMOs must be either harvested or Destroyed.

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

**Conditions in relation to the Cleaning of Locations and Pollen Traps after each crop of GMOs is grown**

26. After the GMOs are harvested or Destroyed at a Location, the Location and the Pollen Trap around it (if any) must be Cleaned.

27. A Location must be Cleaned within 14 days of harvest or Destruction of the GMOs in it, whichever occurs first.

28. A Pollen Trap must be Cleaned within 14 days of the Cleaning of the Location within it.

**Harvested crops of the GMOs may be ginned**

29. Seed harvested from the GMOs and Pollen Trap plants may be ginned. If it is ginned, it must be ginned separately from any other Cotton. If it is not ginned it must be Destroyed.

30. Following ginning, Seed must be:

    (a) stored in a sealed container, within a locked facility that is signed so as to indicate that GM Material is stored within the facility;

(b) exported;

(c) Destroy it by burning; or

(d) transported to a facility certified by the Regulator to physical containment level 2 (PC2).

**General conditions in relation to the Cleaning of all other places and Equipment used in connection with this licence**

31. If:

(a) an area or place other than a Location or Pollen Trap is used in connection with this licence; or

(b) Equipment is used in connection with the GMOs, Pollen Trap plants or Plant Material;

then that area, place or Equipment must also be Cleaned.

32. Cleaning must occur immediately or as soon as practicable after the use and before it is used for any other purpose.

33. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned. (It is not necessary for Equipment to be Cleaned only at a Location.)

34. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with these Cleaning conditions.

**General conditions that apply wherever inspections must be undertaken for the existence of Volunteer plants**

35. After a Location is Cleaned, the following places must be inspected for the existence of Volunteer plants:

(a) the Location;

(b) the Pollen Trap (if any);

(c) the Isolation Zone (if any);

(d) irrigation channels and drains through which water flows to and from the Location and the Pollen Trap;

(e) any areas used to Clean Equipment.

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

37. 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 inspection 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.

38. Any Volunteer plant identified must be Destroyed prior to the plant flowering.

39. Unless this licence provides otherwise, a place must be inspected at least once every 60 days, either until the GMOs are once again planted at the Location pursuant to this licence, or until the Regulator has issued a sign-off.

40. If:

(a) inspections have been routinely completed in a place for a period of a year; and

(b) inspection records for that place show that no Volunteers have been observed in the most recent 6 month inspection period;

the licence holder may make written application to the Regulator that these inspection conditions no longer apply in respect of that place.

41. Inspection conditions do not apply in respect of a place if the Regulator has issued a sign-off in respect of that place.

**Restrictions in relation to areas and plants after the GMOs are grown**

42. From the time a Location has been harvested until such time as the licence holder has received a sign-off, no Cotton of any kind may be grown in the Location or its Pollen Trap unless it is grown pursuant to this licence.

43. From the time a Location has been harvested until such time as the licence holder has received a sign-off, no plants may be planted at the Location or its Pollen Trap except:

(a) GM Cotton grown pursuant to this licence;

(b) grasses (grass pastures);

(c) cereals (cereal crops);

(d) plants agreed to in writing by the Regulator.

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

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

45. Every container used to transport the GMOs, Pollen Trap plants and Plant Material 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.

46. Harvested GMOs, Pollen Trap plants and Plant Material my be transported to a ginning facility in a Cotton module that is:

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

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

    (c) contained within a Cotton module in an enclosed chain-bed truck specifically designed for the purposes of transporting Cotton modules.

47. Ginned seed from the GMOs or Pollen Trap plants may only be transported to the extent necessary to store it, export it, Destroy it by burning it or relocate it to a facility certified by the Regulator to physical containment level 2 (PC2).

48. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs, Pollen Trap plants and Plant that is sent is delivered. Routes, methods and procedures used for transportation in accordance with this licence must be documented.

**Contingency Plans**

49. 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, Pollen Trap plants or Plant Material, outside an area that must be inspected.

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

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

    (b) destroy any of the GMOs, Pollen Trap plants and Plant; and

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

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

**Compliance Management Plan**

52. 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**

53. 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, Pollen Trap plants or Plant Material.

**Research requirements**

54. The licence holder must, in consultation with the OGTR, develop an agreed research program to collect information regarding:

    (a) the levels of expression of the insecticidal and herbicide tolerance genes in GM Cotton tissues under Australian field conditions;

    (b) the effect of GM Cotton on non-target organisms under Australian field conditions;

    (c) the potential for the introduced proteins to accumulate in the soil under Australian field conditions; and

    (d) the efficacy of gene flow containment measures for any release sites in excess of one hectare, within the associated Research Zone, and for any release site for which a pollen trap is not required, due to having no other cotton crops or populations within 450 m.

**Testing methodology**

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

**GMOs, Pollen Trap plants and Plant Material must not be consumed**

56. The licence holder must ensure that the GMOs, Pollen Trap plants and products derived from these plants are not consumed by humans or used as stockfeed.

# APPENDIX 8 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

## SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

308. *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).

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

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

311. 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).

312. 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.

313. 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

314. 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.

315. 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.

316. 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.

317. 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 prescribe 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

318. 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 Australian Government Minister for Environment and Heritage; and
- relevant local council(s) where the release is proposed.

319. 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 news papers, in the *Gazette* and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

320. 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

321. 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, Australian 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.

322. 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, Australian Government agencies and the Australian Government Minister for Environment and Heritage and the public;
- advice from GTTAC;
- information from other national regulatory agencies; and
- current scientific knowledge and the scientific literature.

323. 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 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.

324. 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

325. 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.

326. 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.

327. 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.

328. 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

329. 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.

330. 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.

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

332. 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.

333. 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.

334. 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.

335. 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 unforseen problems.

# APPENDIX 9 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

**Submission from: A**: agricultural/industry organisation; **I**: individual

**Issues raised/consideration**: **App:** appendix; **APVMA:** issues dealt with by APVMA; **Ch:** chapter; **EN**: environmental risks; **FS:** feed safety; **H:** human health and safety; **HR**: herbicide resistance; **IR**: insecticide resistance; **W:** weediness.

| Sub. No. | Type | Summary of issues raised | Issue | Consideration of issue |
|---|---|---|---|---|
| 1 | I | Effects of cotton wastes [from GM cotton] in animal feeds have not been tested experimentally. Their effects on health of poultry and dairy animals have not been tested. | FS | Ch 2, App 3, 7 |
| | | The effects of human consumption of animals or products from animals fed [GM] cotton residues has not been tested, or at least no published evidence is available. | H | App 2, 3 |
| | | The effects of [GM] cotton on soil microorganisms have not been scientifically examined. "Evaluation" is no substitute for experimentations. | EN | App 3 |
| | | I believe this application is for commercial [purpose] and not [for] environmental or health testing. | EN, H | Ch 1, App 2-7 |
| | | I do not believe enough testing for health and environmental effects have been done to warrant proceeding with testing for commercial viability. | H, EN | App 2-6 |
| 2 | A | The introduction of genetically modified insecticidal cotton of this type (INGARD® and Bollgard® II) has shown a dramatic reduction in their pesticide use requirements, compared to conventional cotton. | APVMA | OSA |
| | | Stacking [combining] of different genes in commercial crops is seen as one of the best measures to improve efficacy of the plants as well as to reduce the risk of resistance developing to any one toxin. | APVMA, IR | App 6 |
| | | After 40 years of cotton growing and processing throughout the northwestern regions of New South Wales cotton has not been able to establish itself as a weed. | W | App 4 |

| Sub. No. | Type | Summary of issues raised | Issue | Consideration of issue |
|---|---|---|---|---|
| | | The likelihood of the trial cotton containing the *cry1Fa* gene or *cry1Ac* gene and marker gene conferring tolerance to glufosinate ammonium herbicide becoming a weed is absolutely minimal. | W | App 4 |
| | | strongly supports the proposed field trial of the GM cottons. | None | Noted |

# REFERENCES

Amabile-Cuevas, C.F., Chicurel, M. (1993). Horizontal gene transfer. *American Scientist* **81**: 332-341.

ANZFA (1999). Full assessment report and regulatory impact statement, A341: Oil and linters derived from insect resistant cotton. A341, Australia New Zealand Food Authority, Canberra, Australia. pp 1-59.

ANZFA (2001a). Final assessment report. Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 AND Oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines MS1, MS8, RF1, RF2 and RF3. 05/02, pp 1-88.

ANZFA (2001b). Final assessment report. Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 AND Oil derived from glufosinate-ammonium tolerant and pollination controlled canola lines MS1, MS8, RF1, RF2 and RF3. 05/02, pp 1-88.

ANZFA (2001c). Final assessment report. Application A380: Food from insect-protected and glufosinate ammonium-tolerant DBT418 corn. Australia New Zealand Food Authority,

ANZFA (2002). Draft assessment report (Full assessment - S.15) Application A436: Oil and linters derived from insect-protected cotton containing event 15985. Full assessment - S.15 Application A436, Australia New Zealand Food Authority, Canberra, Australia. pp 1-78.

Aoki, S., Syono, K. (1999). Horizontal gene transfer and mutation: N*grol* genes in the genome of *Nicotiana glauca*. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 13229-13234.

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

Australian Cotton Cooperative Research Centre (2002). WEEDpak - A guide for integrated management of weeds in cotton. Australian Cotton Cooperative Research Centre, Narrabri, NSW.

Barbera, P.W. (1995). Toxicity/pathogenecity testing of *Bacillus thuringiensis* strain EG 7826 following acute oral challenge in rats. IITRI Project Number L08574, IIT Research Institute, Chicago IL.

Barker, R.F., Idler, K.B., Thompson, D.V., Kemp, J.D. (1983). Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Molecular Biology* **2**: 335-350.

Beever, D.E., Kemp, C.F. (2000). Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts and Reviews, Series B: Livestock Feeds and Feeding* **70**: 175-182.

Berberich, S.A., Ream, J.E., Jackson, T.L. (1996). Safety assessment of insect-protected cotton: the composition of the cottonseed is equivalent to conventional cottonseed. *Journal of Agricultural and Food Chemistry* **41**: 365-371.

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

Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.K., Doerfler, D.L., Seligy, V.L. (1999). Immune responses in Farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environmental Health Perspectives* **107**: 575-582.

Bertolla, F., Simonet, P. (1999). Horizontal gene transfers in the environment: natural transformation as a putative process for gene transfers between transgenic plants and microorganisms. *Research in Microbiology* **150** (6): 375-384.

Betz, F.S., Hammond, B.G., Fuchs, R.L. (2000). Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regulatory Toxicology and Pharmacology* **32**: 156-173.

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

Bietlot, H., Carey, P.R., Kaplan, H., Lessard, T., Pozsgay, Z. (1989). Facile preparation and characterisation of the toxin from *Bacillus thuringiensis* var. *kurstaki*. *Biochemistry* **260**: 87-91.

Bremmer, J.N., Leist, K.H. (1996). Statement on the lack of allergenic potential of PAT-protein and glufosinate tolerant crops containing PAT-protein. Unpublished. Hoechst Schering AgrEvo GmbH Internal report 96.0351/A56695,

Brimecombe, M.J., De Leij, F.A., Lynch, J.M. (2001). The effect of root exudates on rhizosphere microbial populations. *In* "The rhizosphere: biochemistry and organic substances at the soil-plant interface", R Pinton, Z Varanini, P Nannipieri, eds. Marcel Dekker, Inc., New York, USA. pp 95-140.

Bryngelsson, T., Gustafson, M., Green, B., Lind, C. (1988). Uptake of host DNA by the parasitic fungus *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **33**: 163-171.

Buhariwalla, H., Mithen, R. (1995). Cloning of a *Brassica* repetitive DNA element from resting spores of *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **47**: 95-101.

Canadian Food Inspection Agency (1995). Decision Document DD95-04: Determination of environmental safety of Plant Genetic Systems Inc. (PGS) novel hybridization system for canola (*Brassica napus* L.). pp 1-8. http://www.inspection.gc.ca/english/plaveg/pbo/dd/dd9504e.shtml

Canadian Food Inspection Agency (1996). Supplement to Decision Document DD96-11 Suppl: Determination of Livestock Feed Safety of AgrEvo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola Line HCN28. *decision document* **DD96-11 Suppl.**

Canadian Food Inspection Agency (1998a). Decision Document (DD98-28): determination of the safety of AgrEvo Canada Inc's glufosinate-ammonium herbicide-tolerant *Brassica rapa* canola line HCR-1. http://www.inspection.gc.ca/english/plaveg/pbo/dd/dd9828e.shtml

Canadian Food Inspection Agency (1998b). Decision Document 98-23: Determination of Environmental Safety of Dekalb Genetics Corporation's European Corn Borer (ECB) Resistant Corn (*Zea mays* L.) Line DBT418.

Carter, J.N., Ligget, M.P. (1994). Acute oral toxicity and infectivity/pathogenicity to rats of EG 7841. HRC Study Report number ECO 6/942538, Huntingdon Research Centre Ltd., Huntington Cambridgeshire England.

Castillo, A.R., Gallardo, M.R., Maciel, M., Giordano, J.M., Conti, G.A., Gaggiotti, M.C., Quaino, O., Gianni, C., Hartnell, G.F. (2001a). Effect of feeding dairy cows with cottonseeds containing Bollgard(R) and Roundup Ready(R) genes or control non-transgenic cottonseeds on feed intake, milk yield and milk composition. International Animal Agriculture and Food Science Conference. http://www.asas.org/jas/jointabs/jntmttoc.htm *Journal of Dairy Science* **84** (Supplement 1): 413.

Castillo, A.R., Gallardo, M.R., Maciel, M., Giordano, J.M., Conti, G.A., Gaggiotti, M.C., Quaino, O., Gianni, C., Hartnell, G.F. (2001b). Effect of feeding dairy cows with either Bollgard®, Bollgard II®, RoundupReady® or control cottonseeds on feed intake, milk yield and milk composition. http://www.adsa.org/jointabs/jntmttoc.htm *Journal of Dairy Science* **84** (Supplement 1): 413.

Chambers, J.A., Jelen, A., Gilbert, M.P., Jany, C.S., Johnson, T.B., Gawron-Burke, C. (1991). Isolation and characterization of a novel insecticidal crystal protein gene from Bacillus thuringiensis subsp. aizawai. *Journal of Bacteriology* **173**: 3966-3976.

Choma, C.T., Kaplan, H. (1990). Folding and unfolding of the protoxin from *Bacillus thuringiensis*: evidence that the toxic moiety is present in an active conformation. *Biochemistry* **29**: 10971-10977.

Chowdhury, E.H., Mikami, O., Murata, H., Sultana, P., Shimada, N., Yoshioka, M., Guruge, K.S., Yamamoto, S., Miyazaki, S., Yamanaka, N., Nakajima, Y. (2004). Fate of maize intrinsic and recombinant genes in calves fed genetically modified maize Bt11. *Journal of Food Protection* **67**: 365-370.

Christensen, A.H., Sharrock, R.A., Quail, P.H. (1992). Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Molecular Biology* **18**: 675-689.

Coghlan, A. (2000). So far so good: for the moment, the gene genie is staying in its bottle. *New Scientist* **2231**: 4.

Conner, A.J., Glare, T.R., Nap, J.P. (2003). The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. *Plant Journal* **33**: 19-46.

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

Crickmore, N., Zeigler, D.R., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., Bravo, A., and Dean, D.H. *Bacillus thuringiensis* toxin nomenclature. (2002) Available from: http://www.biols.susx.ac.uk/home/Neil_Crickmore/Bt/

Davies, J.E. (1986). Aminoglycoside-aminocyclitol antibiotics and their modifying enzymes. *In* "Antibiotics in laboratory medicine", V Lorian, ed Ed. 2. Williams and Wilkins, Easton, MD. USA. pp 790-809.

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

De Vries, J., Wackernagel, W. (1998). Detection of *nptII* (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. *Molecular and General Genetics* **257**: 606-613.

Dulmage, H.T.a.A.K. (1982). Distribution of Bacillus thuringiensis in nature. *In* "Microbial and Viral Pesticides", E Kurstak, ed. Marcel Dekker, New York. pp 209-237.

Eastick, R. (2002). Evaluation of the potential weediness of transgenic cotton in northern Australia. Technical Bulletin no. 305, Northern Territory Government and Australian Cotton Cooperative Research Centre, pp 1-177. http://cotton.pi.csiro.au/Assets/PDFFiles/TB3051.pdf

English, L., Slatin, S.L. (1992). Mode of action of delta-endotoxins from *Bacillus thuringiensis*: a comparison with other bacterial toxins. *Insect Biochemistry and Molecular Biology* **22**: 1-7.

EPA (2001). Biopesticides registration action document: *Bacillus thuringiensis* plant-incorporated protectants. US EPA, IIA1-IIC109. http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm

EPA (1997). Phosphinothricin Acetyltransferase and the genetic material necessary for its production in all plants; exemption from the requirement of a tolerance on all raw agricultural commodities. *Federal Register* **62**: 17717-17720.

EPA (2000). Biopesticide fact sheet: *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac delta-endotoxin and its controlling sequences as expressed in cotton (006445) Issued: 4/00. pp 1-12. http://www.epa.gov/pesticides/biopesticides/factsheets/fs006445t.htm

European Scientific Committee on Plants (1998). Opinion of the Scientific Committee on Plants regarding the glufosinate tolerant, hybrid rape derived from genetically modified parental lines (MS8 x RF3) notified by Plant Genetic Systems (notification C/B/96/01). The European Commission, http://europa.eu.int/comm/food/fs/sc/scp/out09_en.html

European Scientific Committee on Plants (2000). Opinion of the Scientific Committee on Plants regarding submision for placing on the market of glufosinate tolerant oilseed rape transformation event Liberator PHOE 6/AC notified by the Agrevo company [now Aventis CropScience] (Notification C/DE/98/6). The European Commission, pp 1-10. http://europa.eu.int

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

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

FDA (1995). Glufosinate tolerant canola (Topas 19/2). United States Food and Drug Administration., http://www.cfsan.fda.gov/~lrd/biocon.html#list.

FDA (1997). Biotechnology Notification file No. 46. United States Food and Drug Administration, http://www.cfsan.fda.gov/~lrd/biocon.html#list.

FDA Biotechnology Consultation: Agency Response Letter: BNF No. 000073. (2001) Available from: http://www.cfsan.fda.gov/~rdb/bnfl073.html

Federici, B.A. (2003). Effects of Bt on non-target organisms. *In* "*Bacillus thuringiensis*: a cornerstone of modern agriculture", M Metz, ed. Food Products Press, Binghamton. pp 11-30.

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

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

Folmer, J.D., Grant, R.J., Milton, C.T., Beck, J.F. (2000). Effect of Bt corn silage on short-term lactational performance and ruminal fermentation in dairy cows. *Journal of Dairy Science* **83**: 1182.

Frick, O.L. (1995). The potential for allergenicity in transgenic foods. *In* "Genetically Modified foods; Safety Aspects", KH Engel, GR Takeoka, R Teranishi., eds. American Chemical Society, Washington DC.

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

FSANZ (2003). Final assessment report - Application A446: Insect-protected and glufosinate ammonium-tolerant corn line 1507. http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa446insectglufosinateresistantcornmaize/index.cfm

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

Fuchs, R.L., Berberich, S.A., Serdy, F.S. (1993a). Safety evaluation of genetically engineered plants and plant products: insect resistant cotton. *In* "Biotechnology and Safety Assessment", JA Thomas, LA Myers, eds. Raven Press Ltd, New York, pp 199-212.

Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruber, R.M., Berberich, S.A. (1993b). Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Bio/Technology* **11**: 1543-1547.

Gallagher, Grimes, and Beavers (2000). Insect protection protein 2 in cottonseed meal: a dietary toxicity study with the northern Bobwhite. Unpublished. Monsanto Company, St. Louis. Monsanto Report No. MSL:1678.

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

Gene Files (2002) Available from: http://www.genefiles.org/

Glare, T.R., O'Callaghan, M. (2000). *Bacillus thuringiensis*: Biology, Ecology and Safety. John Wiley & Sons, Chichester, UK.

Goldberg, L.J., Margalit, J. (1977). A bacterial spore demonstrating rapid larvicidalactivity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypsti* and *Culex pipens*. *Mosq News* **37**: 355-358.

Goldburg, R., Rissler, J., Shand, H., Hassebrook, C. (1990). Biotechnology's bitter harvest. A Report of the Biotechnology Working Group, pp 1-73.

Green, S.B. (2002). Molecular characterisation of Cry1F (synpro)/Cry1Ac (synpro) stacked transgenic cotton line 281-24-236/3006-48-81. 010075.01, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Green, S.B., Ernest, A.D., Bevan, S.A. (2002a). Molecular characterisation of Cry1Ac (synpro) transgenic cotton event 3006-210-23. 010053.01, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Green, S.B., Ernest, A.D., Bevan, S.A. (2002b). Molecular Characterization of Cry1F(synpro) Transgenic Cotton Event 281-24-236. 010007.01, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Greene, A.E., Allison, R.F. (1994). Recombination between viral RNA and transgenic plant transcripts. *Science* **263**: 1423-1425.

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

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

Gupta, V. V. S. R., Roberts, G. N., Neate, S. M., McClure, S. G., Crisp, P., and Watson, S. K. (2002). Impact of Bt-cotton on biological processes in Australian soils. Akhurst, R. J., Beard, C. E., and Hughes, P. A. eds, CSIRO pp. 191-194.

Harper, G., Osuji, J.O., Heslop-Harrison, J.S., Hull, R. (1999). Integration of banana streak badnavirus into the Musa genome: Molecular and cytogenetic evidence. *Virology* **255**: 207-213.

Hartnell, Gianni, Videla, Castillo, and Maciel (2001). 2001 Sponsor Summary Report: Effect of feeding cottonseed produced from cotton containing Bollgard®, Bollgard II® or Roundup Ready® on feed intake, milk production and composition in lactating dairy cows in Argentina. Unpublished. Monsanto Company, St. Louis. Monsanto Report No. MSL:17294.

Health Canada (1997). Novel food information - food biotechnology. Glufosinate ammonium tolerant canola (T45). FD/OFB-97-03,

Health Canada (2000). Novel food information - food biotechnology. Glufosinate ammonium tolerant canola (HCN92).

Health Canada (2002). NOVEL FOOD INFORMATION– FOOD BIOTECHNOLOGY: Cry1F INSECT-RESISTANT/GLUFOSINATE-TOLERANT MAIZE LINE 1507. http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_cry1f_insect.html

Ho, M.W., Ryan, A., Cummins, J. (2000). Cauliflower mosaic viral promoter - a recipe for disaster? *Microbial Ecology in Health and Disease* **11**: 194-197.

Hodgson, J. (2000a). Reply to hazardous CaMV promoter? *Nature Biotechnology* **18**: 363.

Hodgson, J. (2000b). Scientists avert new GMO crisis. *Nature Biotechnology* **18**: 13.

Hoffman, T., Golz, C., Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* **27**: 70-76.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S., Van Mellaert, H. (1988). Specificity of *Bacillus thuringiensis* δ-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. *Proceedings of the National Academy of Sciences of the United States of America* **85**: 7844-7848.

Hofte, H., Whiteley, H.R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* **53**: 242-255.

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

Karim, S., Riazuddin, S., Gould, F., Dean, D.H. (2000). Determination of receptor binding properties of *Bacillus thuringiensis* delta-endotoxins to cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*) midgut brush border membrane vesicles. *Pesticide Biochemistry and Physiology* **67**: 198-216.

Keeler, K.H. (1985). Implications of weed genetics and ecology for the deliberate release of genetically engineered crop plants. *Recombinant DNA Technical Bulletin* **8**: 165-172.

Keeler, K.H. (1989). Can genetically engineered crops become weeds? *Bio/Technology* **7**: 1134-1139.

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

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

Knowles, B.H., Dow, J.A.T. (1993). The crystal delta-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut. *Bioassays* **15**: 469-476.

Kough, J. (2003). The safety of *Bacillus thuringiensis* for human consumption. *In* "Bacillus thuringiensis: A cornerstone of moden agriculture", M.Matthew, ed. Food Products Press, New York. pp 1-10.

Krieg, A., Huger, A.M., Langenbruch, G.A., Schnetter, W. (1983). *Bacillus thuringiensis* Var. *Tenebrionsis*: a new pathotype effectiveagainst larvar of Coleoptera. *Z Agrew Entomol* **96**: 500-508.

Lai, M.M.C. (1992). RNA recombination in animal and plant viruses. *Microbiological Reviews* **56** (1): 61-79.

Lawrence, J.G., Ochman, H. (1998). Molecular archaeology of the *Escherichia coli* genome. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 9413-9417.

Lereclus, D., Delécluse, A., Lecadet, M.M. (1993). Diversity of *Bacillus thuringiensis* toxins and genes. *In* "*Bacillus thuringiensis*, an environmental biopesticide: theory and practice", PF Entwistle, JS Cory, MJ Bailey, S Higgs, eds. John Wiley and sons, Chichester, UK. pp 37-69.

Li, M.H., Robinson, E.H. (2000). Evaluation of cottonseed meal derived from insect protected cotton lines 15813 and 15985 as a feed ingredient for catfish. MSL-16179, Trad Cochran National Warmwater Aquaculture Centre (Testing facility) Mississippi State University Stoneville MS 38776-0197. pp 1-22.

Lorenz, M.G., Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiological Reviews* **58** (3): 563-602.

Luthy, P., Ebersold, H.R. (1981). *Bacillus thuringiensis* delta-endotoxin: histopathology and molecular mode of action. *In* "Pathogenesis of invertebrate microbial desease.", E.W.Davidson, ed. Allenheld, Osmun and Co., Totowa, N.J. pp 235-267.

Macintosh, S.C., Stone, T.B., Sims, S.R., Hunst, P.L., Greenplate, J.T., Marrone, P.G., Perlak, F.J., Fischhoff, D.A., Fuchs, R.L. (1990). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *Journal of Invertebrate Pathology* **56**: 258-266.

Mahill, J.F., Storer, P.N. (2002). 2002 field survey to evaluate effects on non-target arthropods of Cry1F/Cry1Ac Bt Cotton MXB-13. GH-C 5578, Phytogen Seed Co. LLC, Corcoran , California, USA.

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

Mayo, M.A., Jolly, C.A. (1991). The 5'-terminal sequence of potato leafroll virus RNA: evidence of recombination between virus and host RNA. *Journal of General Virology* **72**: 2591-2595.

McClintock, J.T., Schaffer, C.R., Sjoblad, R.D. (1995). A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pesticide Science* **45**: 95-105.

Meadows, M.P. (1993). *Bacillus thuringiensis* in the environment: ecology and risk assessment. *In* "*Bacillus thuringiensis*, an environmental biopesticide: theory and practice", PF Entwistle, JS Cory, MJ Bailey, S Higgs, eds. John Wiley and sons, Chichester, UK. pp 193-220.

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

Merriman, T.N. (1996). An acute oral toxicity study in mice with Phosphinothricin Acetyltransferase (PAT) protein. Unpublished. DEKALB Study No. DGC-95-A18, DEKALB Genetics, Mystic, CT, USA.

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

Narva, K.A., Palta, A., Pellow, J.W. (2001a). Product characterisation data for *Bacillus thuringiensis* var. *aizawai* Cry1F (synpro) insect control protein as expressed in cotton. GH-C 5304, Dow AgroSciences LLC, San Diego, California, USA.

Narva, K.A., Palta, A., Pellow, J.W. (2001b). Product characterisation data for *Bacillus thuringiensis* var. *kurstaki* Cry1Ac (synpro) insect control protein as expressed in cotton. GH-C 5303, Dow AgroSciences LLC, San Diego, California, USA.

Naylor, M.W. (1993a). One month feeding study with insect-resistant cottonseed meal in Sprague-Dawley rats. Monsanto Company The Agricultural Group, St Louise Missouri USA.

Naylor, M.W. (1993b). Acute oral toxicity of *Bacillus thuringiensis* var. *kurstaki* [Cry1Ac] HD-73 protein in albino mice. The Agricultural group Monsanto Company, St. Louise Missouri USA.

Neppl, C.C. (2000). Management of resistance to *Bacillus thuringiensis* Toxins.

Netherwood, T., Martín-Orúe, S.M., O'Donnell, A.G., Gockling, S., Graham, J., Mathers, J.C., Gilbert, H.J. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnology* **22**: 204-209.

Ni, M., Cui, D., Einstein, J., Narasimhulu, S., Vergara, C.E., Gelvin, S. (1995). Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes. *The plant journal* **7**: 661-676.

Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *APMIS* **106**: 77-84.

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

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

Ochman, H., Lawrence, J.G., Grolsman, E. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**: 299-304.

OGTR (2002). The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia. http://www.ogtr.gov.au/pdf/ir/biologycotton.pdf

Organisation for Economic Co-operation and Development (OECD) (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. ENV/JM/MONO(99)13, pp 1-26.

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

Pellow, J.W. (2001). Field efficacy of Cry1F(synpro), Cry1Ac(synpro) and Cry1F+Cry1Ac stack insecticidal crystalline proteins from *Bacillus thuringiensis* var. *aizawai* strain PS811 and *Bacillus thuringiensis* subspecies *kurstaki* strain HD73 cotton events against tobacco budworm and pink bollworm. GH-C 5323, Dow AgroSciences LLC, Greenville, MS, USA.

Pellow, J.W. (2002). Efficacy of Cry1F/Cry1Ac cotton against a wide range of lepidopteran pests. Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Pellow, J.W. (2003). Agronomic prformance of MXB-7, MXB-9 and MXB-13 ad compared to PSC355. GH-C 5632, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

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

Phillips, A.M., Embrey, S.K., Shan, G., Korjagin, V.A. (2002). Field expression of Cry1F (synpro), Cry1Ac (synpro) and phosphinothricrin acetyltransferase (PAT) proteins in transgenic cotton plants, cottonseed and cottonseed processed products; and compositional analysis of cottonseed and cottonseed processed products. 010015.02, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Pittard, A. J. (1997). The use of antibiotic resistance markers in transgenic plants and microorganisms which are to be released into the environment. In "*Proceedings of Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations Conference"*, Canberra. pp. 173-178.

Roy, J. (1990). In search of the characteristics of plant invaders. *In* "Biological invasions in Europe and the Mediterranean Basin", F di Castri, AJ Hansen, M Debussche, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 335-352.

Sacchi, V.F., Parenti, P., Hanozet, G.M., Giordana, B., Luthy, P., Wolfersberger, M.G. (1986). *Bacillus thuringiensis* toxin inhibits K+-gradient-dependent amino acid transport across the brush border membrane of *Pieris brassicae* midgut cells. *FEBS Letters* **204**: 213-218.

Saxena, D., Flores, S., Stotzky, G. (1999). Insecticidal toxin in root exudates from *Bt* corn. *Nature* **402**: 480.

Schlüter, K., Fütterer, J., Potrykus, I. (1995). Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs- if at all- at an extremely low frequency. *Bio/Technology* **13**: 1094-1098.

Schoelz, J.E., Wintermantel, W.M. (1993). Expansion of viral host range through complementation and recombination in transgenic plants. *Plant Cell* **5**: 1669-1679.

Smalla, K., Borin, S., Heuer, H., Gebhard, F., van Elsas, J. D., and Nielsen, K. (2000). Horizontal transfer of antibiotic resistance from transgenic plants to bacteria - are there data to fuel the debate? Fairbairn, C., Scoles, G., and McHughen, A. eds, University Extension Press, Saskatchewan, Canada. pp. 146-154.

Song, P. (2002a). Cloning and Characterisation of DNA sequences in the insert and flanking border regions of B.t Cry1Ac cotton 3006-210-23. GH-C 5522, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Song, P. (2002b). Cloning and Characterisation of DNA sequences in the insert and flanking border regions of B.t Cry1F cotton 281-24-236. GH-C 5529, Dow AgroSciences LLC, Indianapolis, Indiana, USA.

Spencer, T.M., Orozco, E.M., Doyle, R.M. (1996). Petition for determination of non-regulated status: insect protected corn (*Zea mays* L.) with Cry1Ac gene from *Bacillus thuringiensis* subsp. *kurstaki*. DEKALB Genetics Corporation,

Sprague, G.F. (1991). Genetic exchange between kingdoms. *Current Opinions in Genetic and Development* **1**: 530-533.

Stanhope, M.J., Lupas, A., Italia, M.J., Koretke, K.K., Volker, C., Brown, J.R. (2001). Phylogenetic analysis do not support horizontal gene transfers from bacteria to vertebrates. *Nature* **411**: 940-944.

Starnes, R.L., Liu, C.L.a.M.P.G. (1993). History, use, and future of microbila insecticides. *Amer Entomol* **39**: 83--91.

Stotzky, G. (2000). Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* **29**: 691-705.

Strauch, E., Wohlleben, W., Puhler, A. (1988). Cloning of a phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes* Tu494 and its expression in *Streptomyces lividans* and *Escherichia coli*. *Gene* **63**: 65-74.

Streips, U.N. (1991). Transformation. *In* "Modern Bacterial Genetics", UN Streips, RE Yasbin, eds. Wiley-Liss, New York. pp 191-216.

Syvanen, M. (1999). In search of horizontal gene transfer. *Nature Biotechnology* **17**: 833.

Taylor, S. (1995). Evaluation of the allergenicity of foods developed through biotechnology. In "*Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms"*, University of California, Division of Agriculture and Natural Resources, Oakland, California, USA.

Taylor, S.L. (1992). Chemistry and detection of food allergens. *Food Technology* **May**: 146-152.

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

Teakle, R. E. (1991). Present use of, and problems with, Bacillus Thuringiensis in Australia. In "*Proceedings: Workshop on Bacillus Thuringiensis"*, CSRIO pp. 28-31.

Teycheney, P. Y. and Tepfer, M. (1999). Gene flow from virus-resistant transgenic crops to wild relatives or to infecting viruses. In "*Gene flow and agriculture: Relevance for transgenic crops, British Crop Protection Council Symposium Proceedings No. 72"*, pp. 191-196.

US EPA (1997). Bacillus Thuringiensis Subspecies Kurstaki CryIA(c) and the Genetic Material Necessary for Its Production in All Plants; Exemption From the Requirement of a Tolerance on All Raw Agricultural Commodities.

US EPA (2003). Bacillus thuringiensis Cry1F Protein in Cotton; Temporary Exemption from the Requirement of a Tolerance. http://www.epa.gov/fedrgstr/EPA-PEST/2003/April/Day-30/p10663.htm

USDA-APHIS (1998). Determination of Nonregulated Status for Glufosinate Tolerant Rice Transformation Events LLRICE06 and LLRICE62. United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Services, web site. pp 1-21. http://www.aphis.usda.gov/biotech/dec_docs/9832901p_det_ea.HTM

USDA-APHIS (2001). USDA/APHIS Decision on Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. Petition 00-136-01P Seeking a Determination of Nonregulated Status for *Bt* Cry1F Insect Resistant, Glufosinate Tolerant Corn Line 1507. http://www.aphis.usda.gov/biotech/dec_docs/0013601p_ea.HTM

USDA-APHIS (2003). USDA/APHIS Petition 96-291-01p for Determination of Nonregulated Status
for Insect-Protected Corn Line DBT418.

Van Rie, J., Jansens, S., Hofte, H., Degheele, D., Van Mellaert, H. (1989). Specificity of *Bacillus thuringiensis* delta -endotoxins: importance of specific receptors on the brush border membrane of the mid-gut of target insects. *European Journal of Biochemistry* **186**: 239-247.

Walker, K., Mendelsohn, M., Matten, S., Alphin, M., Ave, D. (2003). The role of microbial Bt products in U.S. crop protection. *In "Bacillus thuringiensis*: a cornerstone of modern agriculture", M Metz, ed. Food Products Press, Binghamton. pp 31-51.

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

Wehrmann, A., Van Vliet, A., Opsomer, C., Botterman, J., Schulz, A. (1996). The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. *Nature Biotechnology* **14**: 1274-1278.

Williamson, M., Fitter, A.H. (1996). The characters of successful invaders. *Biological conservation* **78**: 163-170.

Wohlleben, W., Arnold, W., Broer, I., Hillemann, D., Strauch, E., Puhler, A. (1988). Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from Streptomyces viridochromogenes Tu494 and its expression in Nicotiana tabacum. *Gene* **70**: 25-37.

Worobey, M., Holmes, E.C. (1999). Evolutionary aspects of recombination in RNA viruses. *Journal of General Virology* **80**: 2535-2543.

Zambryski, P. (1992). Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annual Review Plant Physiology and Plant Molecular Biology* **43**: 465-490.