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 into the environment

DIR 049/2004

Title: GM cotton field trial – Evaluation under field conditions of the cotton rubisco small subunit promoter driving a reporter gene

Applicant: CSIRO

October 2004

Abbreviations

ANZFA	Australia New Zealand Food Authority (now FSANZ)
APVMA	Australian Pesticides and Veterinary Medicines Authority (formerly NRA)
bar	gene encoding bialaphos or phosphinothricin resistance
bp	basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
cry	gene encoding Cry
Cry	crystal insecticidal proteins of Bt
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EMBL	European Molecular Biology Laboratory
EPA	Environmental Protection Agency
epsps	gene encoding 5-enolpyruvylshikimate-3-phosphate synthase
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	gram
GFP	green fluorescent protein
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
GUS	β-glucuronidase
ha	hectare
hph	gene encoding HPH
HPH	hygromycin phosphotransferase
IgE	immunoglobulin E
kDa	kilodalton
km	kilometre
m	metre
mRNA	messenger ribonucleic acid
nos	gene encoding nopaline synthase
nptII	gene encoding NPTII
NPTII	neomycin phosphotransferase II
NRA	National Registration Authority for Agricultural and Veterinary Chemicals (now APVMA)
ocs	gene encoding octopine synthase
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PCR	polymerase chain reaction
rbcL	gene encoding Rubisco large subunit
rbcS	gene encoding Rubisco small subunit
RNA	ribonucleic acid
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
T-DNA	transfer deoxyribonucleic acid
uidA	gene encoding GUS
US EPA	United States Environmental Protection Agency

US FDA	United States Food and Drug Administration
μg/g	micrograms per gram
WHO	World Health Organisation
X-gluc	5-bromo-4-chloro-3-indolyl β-D-glucuronide

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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 and 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 049/2004 from CSIRO.

THE APPLICATION

CSIRO has applied for a licence (application number DIR 049/2004) for the intentional release, under limited and controlled conditions, of 60 genetically modified (GM) cotton lines[1]. CSIRO proposes to conduct a small field trial over two summer growing seasons (between October 2004 and May 2006) on one site covering a total area of 0.1 hectares in each season in New South Wales. The aim of the proposed release is to assess the efficacy of a new promoter, compared to a commonly used promoter, in controlling the expression of the *uidA* reporter gene under field conditions. This new promoter is a candidate for controlling the expression of potential commercially useful introduced traits in cotton. The GM cotton lines proposed for release are for research purposes only and are not suitable for commercial development.

Promoters are short regulatory sequences that control the expression of genes that they are linked to. They control the location, timing and level of expression of the gene and thus where, when and how much of the protein it encodes is produced. The promoters under evaluation in this field trial are linked to the *uidA* reporter gene from *Escherichia coli* (a

[1] The term "line" has been used throughout this RARMP to denote cotton containing a specific genetic modification derived from a single transformation event.

common gut bacterium) that encodes the enzyme[2] β-glucuronidase (GUS). GM tissues expressing GUS turn an easily visible blue colour when treated with a simple biochemical stain. Thus the strength and distribution of the blue colour in the plant tissues after staining will provide an indication of the activity of the promoters being evaluated. Therefore, expression of the GUS enzyme is for research purposes only and no trait of commercial interest has been introduced.

In 30 of the GM cotton lines, the expression of the *uidA* reporter gene is being controlled by a new promoter, the Rubisco small subunit (*rbcS*) promoter, which is derived from cotton itself. This promoter normally controls the expression of a Rubisco small subunit gene that encodes a subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme that is involved in the photosynthetic pathway in plant green tissues. It is expected that this promoter from cotton will direct the expression of the *uidA* reporter gene in the photosynthetic green tissues of the GM cotton plants throughout the life of the plants.

In the other 30 GM cotton lines, the expression of the *uidA* reporter gene is being controlled by the *35S* promoter, derived from Cauliflower mosaic virus, that is commonly used to control the expression of introduced genes in GM plants. It is expected that this will direct the expression of the *uidA* reporter gene in most tissues of the GM cotton plants, as has been shown in previous releases of other GM cottons containing the *35S* promoter.

All of the GM cotton lines also contain an antibiotic resistance gene (*nptII*) from *E. coli* (a common gut bacterium), a selectable marker which confers resistance to the antibiotics kanamycin and neomycin. This marker gene was used in the laboratory during the development of the GMOs for identification and selection of plant tissues in which the *uidA* reporter gene was also present. Due to the genetic modification process used to develop the GM cotton lines containing the introduced *rbcS* promoter, these lines also contain a second antibiotic resistance gene (*hph*) from *E. coli* that confers resistance to the antibiotic hygromycin B. None of the antibiotics are currently in clinical use in Australia.

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 possible future trials (subject to approval) or research will be destroyed. Following hand-harvesting of selected GM material for research purposes, the applicant proposes that plant materials remaining at the site will be slashed and incorporated into the soil by cultivation. Any regrowth will be destroyed. Seed cotton from the pollen trap will be harvested by a commercial harvester and burnt on the site. The GMOs and material from the GMOs will be transported in accordance with the OGTR guidelines.

There have been no previous releases of GM cotton lines containing the *rbcS* promoter in Australia. However, licences for the intentional release of GM cottons containing various combinations of the *35S* promoter and/or the introduced genes (*uidA, nptII* and *hph*) have been issued under the current regulatory system (DIRs 005/2001, 006/2001, 008/2001, 009/2001, 012/2001, 017/2002, 022/2002, 023/2002, 025/2002, 034/2003, and 036/2003). There have been no reports of adverse effects on human health or the environment resulting from these releases.

[2] Enzymes are proteins which catalyse specific biochemical reactions.

THE EVALUATION PROCESS

A RARMP has been prepared in relation to licence application DIR 049/2004 from CSIRO 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 6 of the RARMP. The complete RARMP can be obtained from the OGTR by contacting the Office on 1800 181 030 or from the OGTR's website at *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 60 GM 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 and other organisms:** could these GM cottons be more toxic or allergenic than non-GM cotton to humans or harmful to other organisms as a result of the introduction of the new genetic material?
- **weediness:** could the genetic modifications be harmful to the environment by increasing the potential for these GM cottons to establish as problem weeds? and
- **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?

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator has concluded that the proposed limited and controlled release of the 60 GM cotton lines will 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 and the introduced genetic material in the environment. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans and other organisms

The 60 GM cotton lines are unlikely to prove more toxic or allergenic to humans than conventional cotton. Cotton pollen is unlikely to be an airborne allergen and exposure to the

introduced proteins through working with the GM cottons is expected to be very low. Humans are already exposed to the proteins produced from the introduced genes, as these proteins are naturally produced by the bacterium *E. coli* and are therefore already present in the environment. A number of other organisms contain similar genes that confer resistance to the same antibiotics or encode GUS proteins and are widespread in the environment. None of the introduced proteins are known to be toxins or allergens and there have been no reports of toxic or allergenic effects from previous releases of GM cotton lines containing the same introduced genes derived from the same bacterium. The proposed release is limited in scale and licence conditions have been imposed to limit unintended exposure to the GMOs.

The applicant does not intend to use any material 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.

Weediness

The germination and persistence of both GM and non-GM cottons in Australia are limited by the availability of adequate soil moisture, nutrients, herbivory (vertebrate and invertebrate), fire, plant competition and/or frost. It is highly unlikely that the genetic modifications would affect the response of the GM cottons to these variables and, thereby, alter the weediness of the GM cottons. Other GM cottons containing the same introduced proteins, grown commercially or under limited and controlled conditions in Australia, have not become problematic weeds.

However this is the first proposed field trial of these GM cottons and there may be unintended or secondary effects resulting from the genetic modification that could alter their potential for weediness under field conditions.

The applicant has not observed any unintended or secondary effects in the GM cotton lines grown under glasshouse conditions and reports that the growth characteristics of the GM cotton lines are the same as for conventional cotton.

Therefore, the risk of the GM cottons establishing as problematic weeds in the proposed release area is considered very low and not likely to be greater than that of non-GM cotton. Licence conditions have been imposed to minimise the spread and persistence of these GM cottons in the environment (refer to key licence conditions below).

Transfer of introduced genes to other organisms

Some gene transfer from the GM cottons to other cultivated cottons would be likely under uncontrolled conditions, although the overall frequency of out-crossing would be low as cotton is primarily self-pollinating. Transfer of introduced genes to other cultivated cotton would pose the same very low risk posed by the GM cottons themselves. Licence conditions have been imposed to minimise the risk of transfer of the introduced genes to plants outside the release site (refer to key licence conditions below).

The risk of transfer of the introduced genes to feral/naturalised cotton is negligible due to geographic isolation. The risk of transferring the introduced genes to native cotton is also negligible because of geographic isolation and genetic incompatibility. Similarly, 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 and the environment.

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 and other organisms

Licence conditions have been imposed which require the applicant to:

- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- prevent GM cottonseed being used as stockfeed;
- limit the scale and duration of the release;
- destroy all GM materials not required for possible future trials or research;
- securely transport and store the GMOs; and
- report adverse effects.

Weediness

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- prevent cottonseed being used as stockfeed;
- surround the GM cottons by a 20 m pollen trap of non-GM cotton;
- prevent the GM cottons being grown within 50 metres of a natural waterway;
- securely transport and store the GM cottons;
- clean the release site after harvest and equipment used at the site; and
- monitor the release site after harvest and destroy volunteers.

Transfer of introduced genes to other organisms

Licence conditions have been imposed which require the applicant to:

- limit the scale and duration of the release;
- surround the GM cottons by a 20 m pollen trap of non-GM cotton;
- securely transport and store the GM cottons;
- clean the release site after harvest and equipment used at the site; and
- monitor the release site after harvest and destroy volunteers.

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 persons covered by the licence;
- a requirement that the applicant allows access to the release site 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 or 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 5.

Identification of issues to be addressed for future releases

The proposed limited and controlled release is a small scale, single-site 'proof of concept' trial over two cotton growing seasons to test the efficacy of the *rbcS* promoter from cotton under field conditions. If the applicant makes an application for any future use of the *rbcS* promoter to control the expression of potential commercially useful introduced traits in any GMOs, data would be required to be collected on:

- the level of expression of the introduced genes and encoded proteins, and the plant tissues in which they are being expressed, by the *rbcS* promoter under Australian field conditions;
- stability of the introduced genes and modified traits under Australian field conditions;
- genetic segregation and molecular characterisation of the introduced genetic materials;
- agronomic characteristics relating to fitness; and
- 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 unintended effects.

CHAPTER 1 BACKGROUND

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

2. The OGTR has received an application (licence application number DIR 049/2004) from CSIRO for the intentional release of genetically modified (GM) cottons into the environment, on a limited scale and under controlled conditions. Key information on the application is given below:

SECTION 1 THE APPLICATION

Project Title:	**GM cotton Field Trial – Evaluation under field conditions of the cotton rubisco small subunit promoter driving a reporter gene**
Applicant:	CSIRO GPO Box 1700 Canberra ACT 2601
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	*Gossypium hirsutum* L.
Modified trait(s):	One reporter gene (enables detection and quantification of gene expression) linked to one of two promoters, and either one or two selectable marker genes (antibiotic resistance)
Identity of the genetic elements responsible for the modified trait(s):	• *uidA* (β-glucuronidase or GUS) gene from the bacterium *Escherichia coli* (reporter gene) • *nptII* and *hph* genes from the bacterium *Escherichia coli* (antibiotic resistance) • *rbcS* (Rubisco small subunit) promoter from cotton • *35S* promoter from Cauliflower mosaic virus
Proposed Location:	Shire of Narrabri in New South Wales
Proposed Release Size:	One site covering an area of up to 0.1 hectares in each of two summer growing seasons
Proposed Time of Release:	October 2004 – May 2006

Section 1.1 The proposed dealings

3. CSIRO proposes to conduct a small scale, limited and controlled release of 60 GM cotton lines on one site covering an area of up to 0.1 hectares in each of two summer growing seasons at the Australian Cotton Research Institute (ACRI) in the shire of Narrabri, New South Wales. The release is planned for October 2004 to May 2006.

4. CSIRO proposes to study a new promoter that is a candidate for controlling the expression of potential commercially useful introduced traits, for example insect resistance, in cotton. Promoters are short regulatory sequences that control the expression of genes that they are linked to. They control the location, timing and level of expression of the gene and thus where, when and how much of the protein it encodes is produced. The new promoter is a Rubisco small subunit (*rbcS*) promoter derived from cotton itself. In unmodified cotton plants, *rbcS* promoters control the expression of Rubisco small subunit genes that are involved in the photosynthetic pathway in plant green tissues. In the GM cotton plants proposed for release, the introduced gene construct comprises the *rbcS* promoter linked to a reporter gene, *uidA*.

5. A reporter gene is a gene that encodes an easily detectable protein and can therefore be used to study the activity of a promoter of interest. The *uidA* reporter gene enables visual identification of plant tissues in which this gene is being expressed and provides an indication of the level of activity of the promoter being tested.

6. The aim of the proposed release is to test the efficacy of a *rbcS* promoter from cotton in controlling the expression of a reporter gene (*uidA*) derived from a common gut bacterium (*Escherichia coli*). The applicant proposes to do this by comparing the performance of the *rbcS* promoter from cotton (30 GM cotton lines) to the performance of the commonly used *35S* promoter from Cauliflower mosaic virus (30 GM cotton lines), in controlling the expression of the same reporter gene (*uidA*). In addition, the applicant proposes to harvest and retain a small quantity of seed for research or possible future trials (subject to further approvals).

7. None of the cotton plants from the release, or their by-products, will be used for animal feed or human food, nor will lint or linters be sold for processing.

8. The cotton lines proposed for release have been generated by transformation of the Coker 315 cultivar developed as a part of a CSIRO research and development program and are not suitable for commercial development in Australia. The Coker cultivar is a US cultivar used widely in research because it can be readily cultured and regenerated in the laboratory.

Section 1.2 Parent organism

9. 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' that was produced in order to inform the risk assessment processes for licence applications involving GM cottons. This document is available at *www.ogtr.gov.au/ pubform/riskassessments.htm.*

Section 1.3 Genetic modification and its effect

10. CSIRO proposes to release 60 GM cotton lines in total. Thirty lines contain the *uidA* reporter gene controlled by the *rbcS* promoter from cotton and two selectable antibiotic resistance marker genes, the neomycin phosphotransferase gene (*nptII*) and the hygromycin phosphotransferase gene (*hph*). The other 30 lines contain the same reporter gene controlled by the commonly used *35S* promoter from Cauliflower mosaic virus and one selectable antibiotic resistance marker gene, *nptII*.

11. In unmodified cotton plants, *rbcS* promoters control the expression of Rubisco small subunit genes that encode subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase that is involved in the photosynthetic pathway in plant green tissues. It is expected that the *rbcS* promoter from cotton will direct the expression of the *uidA* reporter gene in the photosynthetic green tissues of GM cotton plants, whereas the *35S* promoter from Cauliflower mosaic virus is a constitutive promoter that is expected to drive expression of the *uidA* reporter gene in most tissues of the GM cotton plants.

12. The *uidA* reporter gene encodes the enzyme β-glucuronidase (GUS) that enables visual identification of plant tissues in which this gene is being expressed. Tissues containing GUS will turn a blue colour after adding a simple biochemical stain to a tissue sample in the laboratory. The strength and distribution of the blue colour in plant tissue after staining indicates the activity of the promoter that is being used to control the expression of the *uidA* reporter gene.

13. The *nptII* selectable marker gene is the most widely used antibiotic resistance marker in genetically modified plants. It encodes an enzyme, neomycin phosphotransferase II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. The *hph* selectable marker gene encodes a hygromycin phosphotransferase enzyme (HPH) conferring resistance to the antibiotic hygromycin B. The *nptII* marker gene was used during the laboratory stages of development of the GM cotton lines to identify and select plant tissues containing the genetic modification. None of the antibiotics are currently in clinical use in Australia.

14. The reporter gene and the antibiotic resistance marker genes are derived from the common gut bacterium *Escherichia coli*. Although certain strains of *E. coli* can be pathogenic, the three genes comprise only a small part of the bacterial genome and are not in themselves capable of causing disease.

15. In addition to the two promoters that are being evaluated, other short regulatory sequences that control expression of the genes are also present in the GM cottons. A short sequence (intron) derived from the castor bean (*Ricinus communis*) catalase gene is present within the *uidA* reporter gene construct to prevent expression of the *uidA* gene until after integration into the plant genome. Promoter and terminator sequences derived from the common soil bacterium *Agrobacterium tumefaciens* are also present. Although *A. tumefaciens* and Cauliflower mosaic virus are plant pathogens, their regulatory sequences comprise only a small part of their total genomes and are not in themselves capable of causing disease.

16. Further details on the introduced genetic materials, their products and mechanism of action are provided in Appendix 1, Section 3.

Section 1.4 Method of genetic modification

17. The gene construct comprising the *uidA* gene, controlled by the *rbcS* promoter, the *nptII* and *hph* genes, and other associated regulatory sequences were introduced into the cotton on a standard plasmid vector carried by *A. tumefaciens.* The *uidA* gene, controlled by the *35S* promoter, the *nptII* gene, and other associated regulatory sequences were introduced into the cotton in the same way. The standard plasmid vectors carried by *A. tumefaciens* are 'disarmed' since they lack the genes that encode the tumour-inducing functions of *A. tumefaciens* (See Appendix 1, Section 4 for details).

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian releases of similar GM cottons

18. There have been no previous releases of GM cottons containing the *rbcS* promoter controlling the expression of an introduced gene. However, various combinations of the *35S* promoter and/or the introduced genes have previously been approved for release. CSIRO conducted a limited and controlled release of GM cotton containing the *uidA* reporter gene controlled by the *35S* promoter (PR100 and PR100X) under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

19. Licences for the intentional release of GM cottons containing the *uidA* reporter gene controlled by the *35S* promoter, and/or containing the *nptII* or *hph* antibiotic resistance marker genes, have been issued under the current regulatory system as listed in Table 1 below. Bollgard II® cotton containing the *nptII* and *uidA* genes, both controlled by the *35S* promoter, was approved for commercial release under DIR 012/2002. INGARD® cotton and Roundup Ready®/INGARD® cotton, both containing the *nptII* gene controlled by the *35S* promoter, were also approved for commercial release under DIR 022/2002 and DIR 023/2002 respectively.

20. The *35S* promoter was also used to control the expression of other genes such as *cry1Ab*, *cry1Ac*, *cry2Ab*, *bar* and *cp4 epsps* in GM cottons approved under DIR 015/2002, DIR 016/2002, DIR 035/2003, DIR 036/2003 and DIR 038/2003 for limited and controlled releases.

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

Table 1: Previous licences for the release of similar GM cottons in Australia.

Introduced genes	DIR	Applicant	Type of release
35S/uidA/nptII	005/2001	Cotton Seed Distributors Ltd	Limited and controlled
	006/2001	CSIRO	Limited and controlled
	009/2001	Department of Agriculture WA	Limited and controlled
	012/2001	Monsanto	Commercial (Bollgard II®)
35S/nptII	008/2001	Department of Agriculture WA	Limited and controlled
	022/2002	Monsanto	Commercial (INGARD®)
	023/2002	Monsanto	Commercial (Roundup Ready®/INGARD®)
hph	017/2002	CSIRO	Limited and controlled
	025/2002	CSIRO	Limited and controlled
	034/2003	Syngenta	Limited and controlled
	036/2003	CSIRO	Limited and controlled

Section 2.2 Approvals by other Australian government agencies

22. The OGTR is responsible for assessing the risks to human health and safety and the environment associated with development and use of gene technology. Other government regulatory requirements would also have to be met in respect of the release of the GMOs, including the requirements of Food Standards Australia New Zealand (FSANZ), if material from the GM cottons was proposed for use in human food.

2.2.1 Food Standards Australia New Zealand

23. FSANZ is responsible for human food safety assessment and food labelling, including food derived from GMOs. Currently, the applicant has not applied to FSANZ for evaluation of material from the GM cottons proposed for release for use in human food. The applicant does not intend to use materials from the GM cottons proposed for release in food. FSANZ's approval would need to be obtained before such material could be used for this purpose.

24. Further information about food safety and food labelling 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

Section 2.3 International approvals

25. The GM cotton lines proposed for release under the current application have not been released in other countries. However, Bollgard II® cotton containing the *nptII* and *uidA* genes, both controlled by the *35S* promoter, as well as INGARD® cotton and Roundup Ready®/INGARD® cotton, both containing the *nptII* gene controlled by the *35S* promoter, have been approved for commercial release in other countries such as:

- The United States - the US Department of Agriculture and the Food and Drug Administration approved the commercial release and use in food of INGARD® cotton in 1995 and Bollgard II® cotton in 2002;
- Canada - the Canadian Food Inspection Agency and Health Canada authorised the commercial release and use in food of INGARD® cotton in 1996, and the use of Bollgard II® cotton and cottonseed oil for livestock feed and use in human food, respectively, in 2003; and
- Japan – INGARD® cotton was approved for commercial release and use in food in 1997, and Bollgard II® cotton was approved for use in food and animal feed in 2002 and 2003 respectively.

26. The NPTII protein is approved for food use in the USA (FDA 1994) and in Australia (FSANZ applications A355, A346, A379 and A372). The US Environmental Protection Agency (EPA) does not consider the GUS protein to be toxic to mammals and has approved its exemption from the requirement to establish tolerance levels (EPA 2001). The US EPA has also established an exemption for the HPH protein in cotton (US EPA 2004). According to the field trial database 'Biotrack' administered by the Organisation for Economic Cooperation and Development (OECD), a large number of genetically modified crop plants

(e.g. potato, maize, sugar beet, alfalfa, rice, soybean and rapeseed) containing the HPH protein as a selectable marker have been approved for field trials in New Zealand, the Netherlands, Germany, Canada and the USA (available at: *http://webdomino1.oecd.org/ehs/ biotrack.nsf*).

27. There have been no reports of adverse effects on human health or the environment resulting from any of the releases of similar GM cottons containing the same introduced genes in other countries.

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 6).

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 6), 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 049/2004 received from the agencies and authorities suggested the following issues relating to human health and safety or the environment, which have been addressed in the RARMP:

- the potential toxicity and allergenicity of the GM cottons (Appendix 2 refers);
- the potential for increased weediness of the GM cottons (Appendix 3 refers);
- the potential for, and management of, gene transfer to other cotton crops, naturalised cotton populations and native cottons (Appendices 4 and 5 refer);
- the potential for adverse impacts arising from gene transfer to other organisms (Appendix 4 refers);
- the potential for dissemination of GM cotton pollen or seed beyond the release site (Appendix 3, 4 and 5 refer); and
- the use of products derived from the GM cotton in food (Chapter 1 and Appendix 5 refer).

31. The Regulator received one submission from the public on this application. A summary of the written submission is provided in Appendix 7. This submission identified the benefits of trialing new promoters in GM cottons. As benefits are excluded from consideration in the assessment of applications conducted under the Act, they were not considered in the evaluation process.

32. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to the protection of human health and safety and to the environment in finalising the RARMP. 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

33. 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.

34. The following table (Table 1) lists each of the potential hazards that were considered during the risk assessment process in the *Hazard Identification* column and summarises the assessment of each hazard under the column headed *Risk*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 4, as cross-referenced in the column headed *Summary of Risk Assessment*.

35. Where it is considered, on the basis of a combination of possible adverse impacts and likelihood of occurrence, that risk management may be required to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the methods selected to limit the potential for risk exposure and the reasons they were chosen. The risk management plan for the proposed dealings is given effect by specific conditions within the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 5.

SECTION 3 IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES

36. The proposed limited and controlled release is a small scale, single-site 'proof of concept' trial over two cotton growing seasons to test the efficacy of the *rbcS* promoter from cotton under field conditions. If the applicant makes an application for any future use of the *rbcS* promoter to control the expression of potential commercially useful introduced traits in any GMOs, data would be required to be collected on:

- the level of expression of the introduced genes and encoded proteins, and the plant tissues in which they are being expressed, by the *rbcS* promoter under Australian field conditions;
- stability of the introduced genes and modified traits under Australian field conditions;
- genetic segregation and molecular characterisation of the introduced genetic materials;
- agronomic characteristics relating to fitness; and
- unintended effects of the genetic modification.

37. 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 from the proposed release. The risk management measures summarised in Table 1 of this Chapter and given effect by the licence conditions will achieve this purpose. It should also be noted that the use of these GM cottons in food would require approval from FSANZ.

SECTION 4 DECISION ON THE APPLICATION

38. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 6. 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.

39. The Regulator has concluded that the proposed limited and controlled release of the 60 GM cotton lines will 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 more data is gathered on the behaviour and interactions of the GMOs in the environment. Detailed risk analyses based on the available scientific information are provided in Appendices 2-4 in support of this conclusion.

40. Therefore, the Regulator has issued licence DIR 049/2004 in respect of this application.

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

GM cottons: the genetically modified cotton lines proposed for release.
GUS: β-glucuronidase (enzyme), encoded by the reporter gene (*uidA*), which enables visualisation of plant tissues in which this gene is being expressed.
NPTII: neomycin phosphotransferase II (enzyme), encoded by an antibiotic resistance gene (*nptII*), which provides resistance to the antibiotics kanamycin and neomycin.
HPH: hygromycin phosphotransferase (enzyme), encoded by an antibiotic resistance gene (*hph*), which provides resistance to the antibiotic hygromycin B.
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 Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Food	Negligible	See Appendix 2 • none of the GM cotton materials from the release will be used in human food or animal feed, or processed for lint production; • FSANZ approval would be required before material from GM cotton could be used for human food; • the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment; • very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment and present in human food; • toxicity studies with the purified GUS, NPTII and HPH proteins indicate that these proteins are not toxic to mammals; • the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; and • no toxic or allergic effects have been reported from GM cottons expressing the same proteins that have been extensively field trialed or commercially released in Australia.	Yes	• **Prevent seed from entering human food supply:** prevents exposure through food. • **Destroy all seed not required for possible future trials or research:** prevents unintended exposure. • **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 animal feed. • **Destroy seed**: destroy all seed not required for possible future trials or research. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within.

Hazard Identification	Risk (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management ?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	**Negligible**	**See Appendix 2** • cotton pollen is not wind-dispersed and therefore unlikely to be an air-borne allergen; • exposure to the introduced proteins through working with cotton plants is very low; • processing of the GM seed cotton will only occur on a small scale; • GM cotton lint is no more likely to induce adverse responses in workers than is lint from non-GM cotton; • the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment; • very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment and present in human food; • the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins; and • no toxic or allergic effects have been reported from similar GM cottons expressing the same proteins that have been extensively field trialed or commercially released in Australia.	Yes	• **Limit scale of release:** decreases likelihood of exposure. • **Destroy all seed not required for possible future trials or research:** prevents unintended exposure. • **Ensure secure transport and storage of GM material:** prevents unintended exposure. • **Report any adverse impacts on human health and safety:** ensures identification of unexpected adverse impacts.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons. • **Destroy seed**: destroy all seed not required for possible future trials or research. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within. • **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 Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY FOR OTHER ORGANISMS: Mammals and wildlife, including birds and fish	**Negligible**	See **Appendix 2** • the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment; • very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment including in soil and on plants; • the release is small in size and limited in duration; • exposure of livestock and wildlife to the GM cotton lines will be very low, and no material from the release is proposed to be used in stockfeed; and • toxicity studies with the purified GUS, NPTII and HPH proteins indicate that these proteins are not toxic to mammals.	Yes	• **Limit scale of release:** decreases likelihood of exposure. • **Prevent seed and plant material from being used as stockfeed:** prevents exposure of animals. • **Destroy all seed not required for possible future trials or research:** prevents unintended exposure. • **Ensure secure transport and storage of GM material:** prevents unintended exposure.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons. • **Prevent seed from being used as stockfeed**: no material from the GM cotton to be used in stockfeed. • **Destroy seed**: destroy all seed not required for possible future trials or research. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within.
TOXICITY FOR OTHER ORGANISMS: Invertebrates, including beneficial insects	**Negligible**	See **Appendix 2** • the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment; • very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment including in soil and on plants; • the release is small in size and limited in duration; and • the introduced proteins are not known to be toxic to any organisms.	Yes	• **Limit scale of release:** decreases likelihood of exposure.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons.

Hazard Identification	Risk (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management ?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
TOXICITY FOR OTHER ORGANISMS: Micro-organisms	**Negligible**	**See Appendix 2** • the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment; • very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment including in soil and on plants; • the release is small in size and limited in duration; and • the introduced proteins are unlikely to have adverse effects on microorganisms.	Yes	• **Limit scale of release:** decreases likelihood of exposure.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons.
WEEDINESS	**Very Low**	**See Appendix 3** • the release is small in size and limited in duration; • cotton has a low potential for dispersal by natural means; • 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 unlikely to affect these characteristics; • other GM cottons containing the same proteins, grown commercially or under limited and controlled conditions in Australia, have not become problematic weeds; and • major constraints on weediness of both GM and non-GM cottons are water availability, nutrient availability, plant competition, herbivory, frost and fire.	Yes	• **Limit scale of release:** decreases likelihood of escape. • **Surround the GM cotton with a pollen trap:** minimises spread of the introduced genes beyond the release site via pollen flow. • **Ensure secure transport and storage of GM material:** prevents escape of viable GM plant material outside the release site. • **Clean equipment used at the release site:** prevents escape of viable GM plant material. • **Prevent cottonseed being used as stockfeed**: prevent dispersal of cottonseed. • **Destroy all seed not required for possible future trials or research**: prevents unintended spread. • **Destroy any volunteers**: prevents persistence.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons. • **Surround the GM cotton with a pollen trap**: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within. • **Clean equipment used at the release site**: equipment must be cleaned before it is used for any other purpose. • **Destroy seed**: destroy all seed not required for possible future trials or research. • **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.

Hazard Identification	Risk (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management ?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
GENE TRANSFER: Plants Other cotton crops	**Very Low**	**See Appendix 4** • cotton is mostly self-pollinated; • the introduced genes do not confer a selective environmental advantage; and • although some gene transfer from the GM cotton lines to cultivated cotton is possible, the risk posed by such gene transfer is very low because gene transfer will not pose any risks additional to the very low risks posed by the GM cotton lines themselves.	Yes	• **Limit scale of release:** decreases potential transfer. • **Surround the GM cotton with a pollen trap:** minimises spread of the introduced genes beyond the release site via pollen flow. • **Ensure secure transport and storage of retained seed:** prevents escape of viable GM plant materials outside the release site. • **Clean equipment used at the release site:** prevents escape of viable GM plant material. • **Destroy all seed not required for possible future trials or research**: prevents unintended spread. • **Destroy any volunteers**: prevents persistence.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons. • **Surround the GM cotton with a pollen trap**: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outer edge of the release site. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within. • **Clean equipment used at the release site**: equipment must be cleaned before it is used for any other purpose. • **Destroy seed**: destroy all seed not required for possible future trials or research. • **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: Plants Feral (naturalised) cotton	**Negligible**	**See Appendix 4** • cotton is mostly self-pollinated; and • gene transfer to naturalised (feral) cotton populations is unlikely due to geographic isolation.	Yes	• **Limit scale of release:** decreases potential exposure. • **Surround the GM cotton with a pollen trap:** minimises spread of the introduced genes beyond the release site via pollen flow. • **Ensure secure transport and storage of retained seed:** prevents escape of viable GM plant material outside the release site. • **Clean equipment used at the release site:** prevents escape of viable GM plant material. • **Destroy all seed not required for possible future trials or research**: prevents unintended spread. • **Destroy any volunteers**: prevents persistence.	Yes	• **Limit scale**: restrict area to 0.1 hectares per season over two growing seasons. • **Surround the GM cotton with a pollen trap**: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outer edge of the release site. • **Secure transport and storage**: the GM cottonseed material must be transported within a primary, sealed container that is packed in a secondary unbreakable container; stored in a sealed container within a locked facility that is signed to indicate GM cotton is stored within. • **Clean equipment used at the release site:** equipment must be cleaned before it is used for any other purpose. • **Destroy seed:** destroy all seed not required for possible future trials or research. • **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.

Hazard Identification	Risk (combines 'likelihood' & 'impact'	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management ?	Risk Management Method(s) and Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
GENE TRANSFER: Plants Native cottons	**Negligible**	See **Appendix 4** • genetic incompatibility and geographical isolation from native populations prevent the production of fertile hybrids.	No	N/A	N/A	None Required
GENE TRANSFER: Plants Other genera	**Negligible**	See **Appendix 4** • well established genetic incompatibility prevents successful cross-pollination with other plant species.	No	N/A	N/A	None Required
GENE TRANSFER: Micro-organisms	**Negligible**	See **Appendix 4** • all of the introduced genetic materials in the GM cottons are already present in the environment and are readily available for transfer from these sources via demonstrated natural mechanisms; and • gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other sources of these genes.	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 Reasons(s) for selection	Is Risk Managed ?	Licence conditions (see Appendix 5 for detailed licence conditions)
GENE TRANSFER: Animals, including humans	**Negligible**	**See Appendix 4** • all of the introduced genetic materials in the GM cotton lines are already present in the environment; • most animals avoid feeding on GM or non-GM cotton plants; • products from the GM cotton lines are not intended for stockfeed or human food; • FSANZ approval would be required before the GM materials (oil and linters) could be used for human food; • limited probability of occurrence: The chance of interaction, uptake and integration of intact plant genes by other organisms is extremely low, especially if it involves unrelated sequences (non-homologous recombination); • natural events of horizontal gene flow from plants to distantly related organisms are extremely rare; and • in the extremely unlikely event of gene transfer occurring, human health and safety and the environment are unlikely to be adversely affected.	No	N/A	N/A	None Required

APPENDIX 1 INFORMATION ABOUT THE GMOs

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

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

SECTION 1 SUMMARY INFORMATION ABOUT THE GMOs

43. In application DIR 049/2004, CSIRO proposes to release a total of 60 GM cotton lines to assess the efficacy of a new promoter, compared to a commonly used promoter, in controlling expression of the *uidA* reporter gene under field conditions. Thirty GM cotton lines (the 'RbcS-GUS' lines) contain a Rubisco small subunit (*rbcS*) promoter from cotton linked to the *uidA* reporter gene. The other 30 GM cotton lines (the '35S-GUS' lines) contain the commonly used *35S* promoter from Cauliflower mosaic virus (CaMV) linked to the *uidA* reporter gene.

44. Promoters control the expression of genes that they are linked to. They control the location, timing and level of expression of the gene and thus where, when and how much of the protein it encodes is produced. Section 3.4 of this Appendix provides details on the function of the *rbcS* and *35S* promoters.

45. The *uidA* reporter gene from *Escherichia coli* encodes the enzyme[3] β-glucuronidase (GUS). GM tissues expressing GUS turn a blue colour when treated with a simple biochemical stain. This assay was used during the laboratory stages of development of the GM cotton lines to identify and select lines expressing GUS. The assay will also be used to identify GM cotton lines expressing GUS in the field, as some lines may still be segregating for the *uidA* reporter gene in the first planting season, depending on the generations available at the time of planting. To compare the performance of the *rbcS* and *35S* promoters in controlling *uidA* reporter gene expression under field conditions, *uidA* gene expression levels and GUS enzyme activity in tissues sampled from the GM cotton lines will be quantified in the laboratory using northern blot analysis and a fluorometric assay respectively.

46. All of the GM cotton lines contain the *nptII* antibiotic resistance selectable marker gene derived from *E. coli*. The *nptII* gene encodes an enzyme, neomycin phosphotransferase II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. The *nptII* gene was used during the laboratory stages of development of the GM cotton lines to identify and select plant tissues containing the genetic modification. Expression of the *nptII* gene in the GM cotton lines is controlled by the bacterial *nos* promoter. Therefore the applicant may also compare *nptII* and *uidA* gene expression levels within tissue samples taken from the RbcS-GUS lines, by northern blot analysis in the laboratory, as another measure of the performance of the *rbcS* promoter under evaluation.

47. The RbcS-GUS lines contain an additional antibiotic resistance selectable marker gene, *hph*, due to the genetic modification process used to develop these lines. However, the applicant does not intend to use this trait. The *hph* gene is derived from *E. coli* and encodes an enzyme,

[3] Enzymes are proteins which catalyse specific biochemical reactions.

hygromycin phosphotransferase (HPH), which confers resistance to the antibiotic hygromycin B. Since the RbcS-GUS lines also contain the *nptII* gene, the *hph* gene was not used to select for the presence of the genetic modification during the laboratory development of these GM cotton lines.

48. In addition to the *rbcS* and *35S* promoters, other short regulatory sequences (introns and terminators) that control expression of the *uidA* reporter gene, and promoters and terminators that control expression of the *nptII* and *hph* selectable marker genes, are also present in the GM cotton lines. These sequences are derived from castor bean (*Ricinus communis*), CaMV and *Agrobacterium tumefaciens*. Although CaMV and *A. tumefaciens* are plant pathogens, the regulatory sequences comprise only a small part of their total genomes and are not in themselves capable of causing disease.

49. There have been no previous approvals of GM cotton lines containing the *rbcS* promoter under the current regulatory system. However, various combinations of the *35S* promoter and/or the introduced genes have been previously approved for release (see Chapter 1 for details). CSIRO conducted a limited and controlled release of GM cotton lines (PR100 and PR100X) containing the *uidA* reporter gene controlled by the *35S* promoter under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).

50. Further details on the introduced genetic materials, their products and mechanism of action are provided in Section 3 of this Appendix.

SECTION 2 THE PARENT ORGANISM

51. 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' that was produced in order to inform the risk assessment processes for licence applications involving GM cottons. This document is available at *www.ogtr.gov.au/ pubform/riskassessments.htm.*

SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

Section 3.1 The *uidA* reporter gene and encoded protein

52. The *uidA* gene is derived from the common gut bacterium *E. coli* and it encodes the enzyme β-glucuronidase (GUS) (Jefferson et al. 1986). The GUS protein is a monomer with a molecular mass of 68 kDa, and the GUS enzyme is active in the form of a tetramer. GUS catalyses the hydrolysis of β-glucuronides and, less efficiently, some β-galacturonides. A large variety of β-glucuronides exist and they have been described as the detoxified excretion forms of xenobiotics (foreign substances e.g. drugs) and endogenous compounds (e.g. steroids) in vertebrates (Jefferson & Wilson 1991). *E. coli* lives in the digestive tract of vertebrates, including humans, (Jefferson et al. 1986) and the GUS enzyme enables it to metabolise β-glucuronides as a main source of carbon and energy.

53. GUS is an exo-hydrolase and will not hydrolyse glucuronides in internal positions within polymers (Jefferson & Wilson 1991). GUS is inactive against β-glucosides, β-galactosides, β-mannosides, and glucosides in the alpha configuration (Jefferson & Wilson 1991).

54. GUS cleaves the chromogenic substrate X-gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide) and when exposed to this biochemical stain, cells that exhibit GUS enzyme activity produce an insoluble blue colour (Jefferson et al. 1987). Endogenous GUS enzyme activity is found in many other bacterial species, and also in vertebrates and invertebrates, but there is very little background activity in non-GM plants (Jefferson 1987; Gilissen et al. 1998; see also Appendix 2). Therefore, the production of a blue colour in particular plant cells after staining with X-gluc indicates that these cells have been successfully genetically modified and contain the *uidA* gene.

55. In addition to the qualitative biochemical stain assay described above, GUS enzyme activity may also be measured quantitatively using a fluorometric assay (Jefferson & Wilson 1991).

56. The *uidA* gene is the most widely used reporter gene in GM plants (Miki & McHugh 2004). Reporter genes encode enzymes that are easily assayed and are therefore used to 'report' on the activity of a promoter to which the reporter gene is linked. They can also be linked to a gene to report on the cellular location of the encoded protein, or used as a simple biochemical tag to identify GM tissues.

57. Thus the *uidA* gene serves as a reporter gene since its expression marks tissue in which other co-introduced regulatory sequences and genes (as part of the gene construct) are also present and are likely to be expressed. If *uidA* gene expression is controlled by a promoter derived from another gene, the strength and distribution of the blue colour in the plant tissue after X-gluc staining indicates the activity of the promoter and hence expression levels and patterns of the original gene can be inferred. Likewise, the expression levels and patterns of any other gene linked to the promoter can be inferred. In the proposed release, the *uidA* reporter gene will be used to compare the efficacy of the *rbcS* and *35S* promoters in the GM cotton lines under field conditions.

58. In the RbcS-GUS lines, *uidA* gene expression is controlled by a *rbcS* promoter from cotton and the nopaline synthase (*nos*) terminator from *A. tumefaciens*. In the 35S-GUS lines, *uidA* gene expression is controlled by the *35S* promoter from CaMV and the octopine synthase (*ocs*) terminator from *A. tumefaciens*. A short (100 bp) sequence (intron) derived from the castor bean (*R. communis*) catalase gene is present within the *uidA* gene to prevent its expression until after integration into the plant genome. Section 3.4 of this Appendix discusses each of these regulatory sequences.

59. Potential hazards relating to the toxicity and allergenicity of GUS are discussed in Appendix 2 and those of weediness and gene transfer in Appendices 3 and 4 respectively.

Section 3.2 The *nptII* antibiotic resistance gene and encoded protein

60. All of the GM cotton lines contain the *nptII* antibiotic resistance gene from *E. coli*. The *nptII* gene is commonly used as a selectable marker in the development of GM plants (Miki & McHugh 2004).

61. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. NPTII uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII-producing cell. The *nptII* gene functioned as a selectable marker during the laboratory stages of development of the GM cotton lines, allowing genetically modified cells to grow in the presence of kanamycin, which inhibited the growth of non-modified cells. Northern blot

analyses in the laboratory may also be used by the applicant to compare the expression levels of the *nptII* and *uidA* genes within the RbcS-GUS lines grown under field conditions, as another measure of the performance of the *rbcS* promoter under evaluation.

62. NPTII is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992) (see also Appendix 2). Currently, both kanamycin and neomycin are not in clinical use.

63. Expression of the *nptII* gene in the GM cotton lines is controlled by the promoter and terminator of the nopaline synthase (*nos*) gene from *A. tumefaciens* (see Section 3.4 of this Appendix).

64. Potential hazards relating to the toxicity and allergenicity of NPTII are discussed in Appendix 2 and those of weediness and gene transfer in Appendices 3 and 4 respectively.

Section 3.3 The *hph* antibiotic resistance gene and encoded protein

65. The RbcS-GUS lines also contain the *hph* antibiotic resistance gene from strains of *E. coli* (Rao et al. 1983) that encodes an enzyme, hygromycin phosphotransferase (HPH), which confers resistance to the antibiotic hygromycin B (Gritz 1983). HPH uses ATP to phosphorylate hygromycin B, thereby inactivating the antibiotic and preventing it from killing the HPH-producing cell.

66. Resistance to hygromycin B is also found naturally in the hygromycin B producing organism *Streptomyces hygroscopicus* (Leboul & Davies 1982). Hygromycin resistant bacteria have been isolated from human clinical sources in Europe (Salauze et al. 1990) and from bovine and porcine faeces in Japan (Ohmae et al. 1979). Hygromycin B is not in clinical use and is currently not used in veterinary medicine in Australia.

67. Two publications by the same authors claim that the *hph* gene may also confer some tolerance to the herbicide glyphosate (Penaloza-Vazquez et al. 1995a; Penaloza-Vazquez et al. 1995b). Tobacco cells that were transformed with the *hph* gene from a different bacteria, *Pseudomonas pseudomallei*, were able to grow as calli on medium containing twice as much glyphosate than untransformed cells are able to survive on (Penaloza-Vazquez et al. 1995b). However, no other publications on GM plants containing the *hph* gene, or articles considering the biosafety of selectable marker genes in GM plants, have reported any effects on herbicide tolerance relating to HPH.

68. The *hph* gene is commonly used as an antibiotic resistance marker in GM plants (Miki & McHugh 2004) and has not been used in any of the known releases of GM plants as a trait to confer glyphosate tolerance. The applicant does not intend to use this potential trait, and since the RbcS-GUS lines also contain the *nptII* selectable marker gene, the *hph* selectable marker gene was not used to select for the presence of the genetic modification during the laboratory development of these GM cotton lines.

69. Expression of the *hph* gene in the RbcS-GUS lines is controlled by the *35S* promoter from CaMV and the *nos* terminator from *A. tumefaciens* (see Section 3.4 of this Appendix).

70. Potential hazards relating to the toxicity and allergenicity of HPH are discussed in Appendix 2 and those of weediness and gene transfer in Appendices 3 and 4 respectively.

Section 3.4 Regulatory sequences

3.4.1 Rubisco small subunit (*rbcS*) promoter

ROLE OF RUBISCO SMALL SUBUNIT (*RBCS*) PROMOTERS IN HIGHER PLANTS

71. In higher plants, Rubisco small subunit (*rbcS*) promoters control the expression of *rbcS* genes that encode small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco is the key enzyme responsible for the initial photosynthetic fixation of atmospheric carbon dioxide into carbohydrates. It is composed of eight small subunits of 14 kDa (*rbcS*) encoded by a nuclear gene family and eight large subunits of 55 kDa (*rbcL*) encoded by a single chloroplast gene (for an overview, see Gutteridge & Gatenby 1995).

72. All *rbcS* genes within a family encode proteins of similar or identical sequence with no known functional differences. However, many *rbcS* genes are expressed at different levels in different tissues and at different developmental stages due to complex regulation of gene expression at the levels of transcription and post-transcription. In basic terms, transcription refers to the production of RNA from gene (DNA) sequences through the activity of a gene's promoter. Post-transcription refers to the series of processing events that RNA undergoes to form the mature mRNA molecule, which is subsequently translated into the protein encoded by the gene. For a detailed description of these processes, see Gilmartin (1993).

STRUCTURAL FEATURES OF *RBCS* PROMOTERS IN HIGHER PLANTS

73. Promoters are regions of DNA upstream of a gene's coding region that contain specific sequences recognised by proteins involved in initiating transcription. Variability in gene expression occurs when other diverse, semi-conserved sequence elements (*cis*-elements) are also present. These *cis*-elements bind proteins (*trans*-acting factors) that are involved in controlling the level, pattern and timing of a gene's expression. *Cis*-elements from a variety of higher plant *rbcS* promoters, especially tomato and pea, have been well studied (e.g. Gilmartin et al. 1990; Manzara et al. 1991). Common *cis*-elements found in plant *rbcS* promoters, and their suggested function(s), are listed in Table 1 of this Appendix.

Table 1: Common *cis*-elements found in plant *rbcS* promoters and their suggested function(s).

cis-element	Consensus Sequence	*trans*-factor	Function(s)
L box	AATTAACCAA		unknown
1	pyrimidine rich		unknown
2 or I-box	GATAAG	GA-1	high promoter activity
2 or GATA-box (I-box overlaps)	GATGAGATA	ASF-2	high level expression in leaf tissue in the light
3 or G-box	CACGTGGC	GBF	expression levels and response to light
5	TTAAATAGAGGGCGTAA		unknown
8 or Box II or GT-1 box	GGTTAA (core sequence)	GT-1	response to light
9	TTTGTAATGTCAA		unknown
10	GAGCCACA		unknown
12 or CAAT box	ATCCAAT		common sequence found in eukaryotic promoters
13	GGTTAC		unknown
15	AGATGAGG	LRF-1?	response to light
16	TTTGTGTCCGTTAGATG		unknown
18 or "LRE"	CCTTATCAT		unknown

cis-element	Consensus Sequence	*trans*-factor	Function(s)
19 or TATA box	TATA (core sequence)	TFIID	transcription initiation
MYB	CNGTTA (N = G, T, A or C)		binding site for plant MYB transcriptional factors involved in dehydration stress and flavonoid biosynthesis.
SURE	CACTTCCACATG		response to sucrose
3AF1	AAATAGATAAATAAAAACATT	3AF-1	transcriptional activity?
AT-1	AATATTTTTATT	AT-1	enhancer of expression

Table adapted from Gilmartin et al. (1990); Manzara et al. (1991); Outchkourov et al. (2003) and references therein.

74. The combination, copy number and spatial arrangements of *cis*-elements generally correlate with the activity of a given *rbcS* promoter. For example, promoters of all five of the characterised tomato *rbcS* genes contain CAAT and TATA boxes, the box II sequence element and a putative light-regulatory element ("LRE"), and all five genes are expressed at high levels in leaves and light-grown cotyledons. Two of the tomato *rbcS* genes, however, exhibit strict light-dependent expression in cotyledons and only these two *rbcS* genes contain the "9" and "10" sequence elements in their promoter regions (Sugita & Gruissem 1987; Manzara & Gruissem 1988; Wanner & Gruissem 1991). Figure 1 of this Appendix provides an example of these different *cis*-element combinations between two of the five tomato *rbcS* promoters.

Figure 1: Schematic arrangement of *rbcS* promoter *cis*-elements in two differently expressed tomato *rbcS* genes.

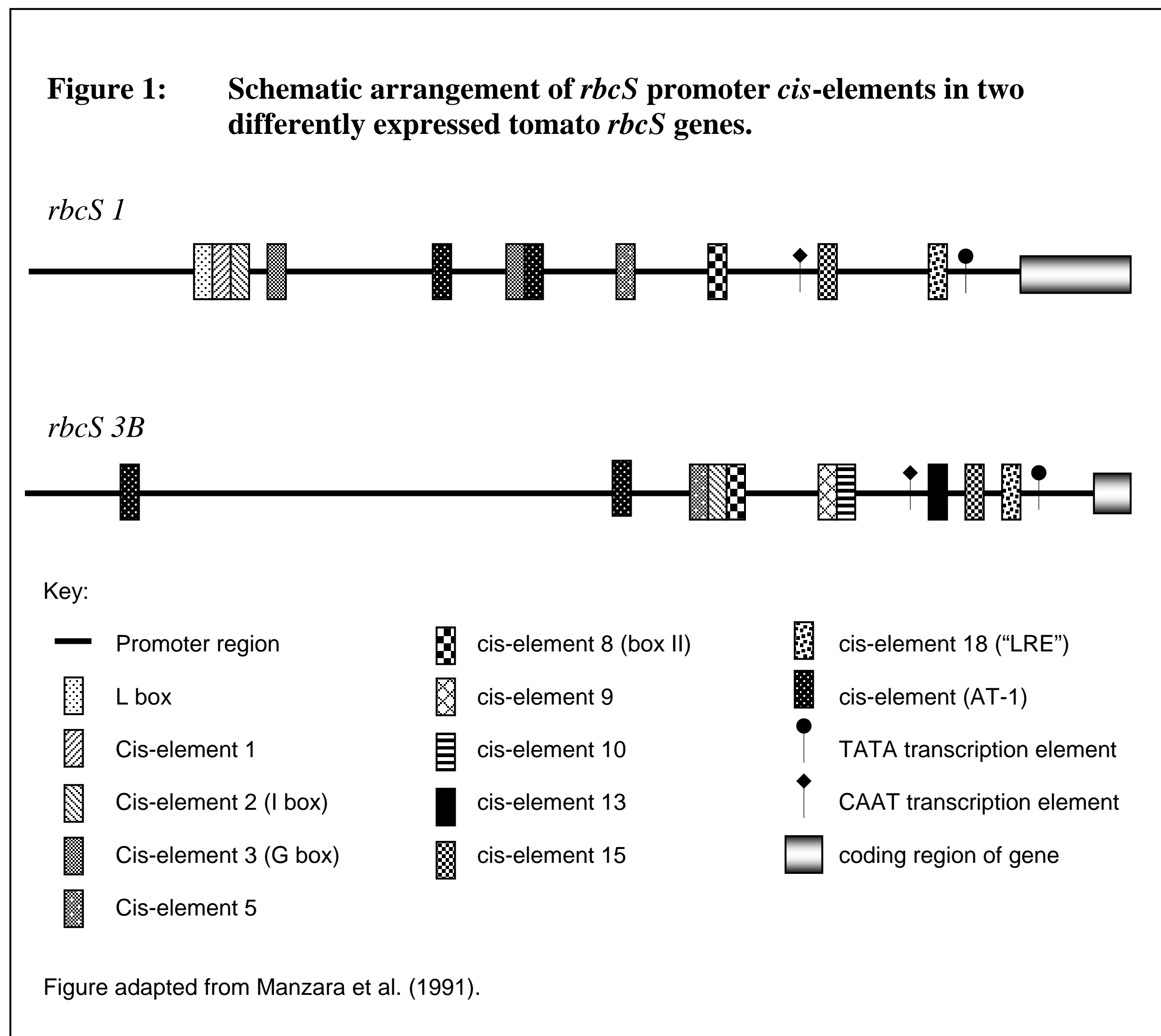

Figure adapted from Manzara et al. (1991).

USE OF *RBCS* PROMOTERS IN CONTROLLING TISSUE-SPECIFIC EXPRESSION OF INTRODUCED GENES IN GM PLANTS

75. In general, *rbcS* promoters direct the expression of *rbcS* genes in the green chloroplast-containing tissues of higher plants. The most abundant levels of *rbcS* mRNA are found in leaves, with *rbcS* mRNA also occurring in other photosynthetic tissues such as stems and immature fruit, while it is virtually undetectable in roots and mature fruit (e.g. Sugita & Gruissem 1987; Wanner & Gruissem 1991). The tissue-specificity of *rbcS* promoters has led to their increasing use in expressing genes that confer useful agronomic traits in more appropriate tissues in various crop species under laboratory test situations. These include expressing insect resistance (Christov et al. 1999) and herbicide tolerance (Stalker et al. 1988) genes in tobacco leaves as well as expressing enhanced nutritional genes in alfalfa leaves (Tabe et al. 1995).

76. Rubisco is arguably the most abundant gene product in plants, constituting some 50-60% of total leaf protein (Dean & Leech 1982). Thus there is interest in using Rubisco gene promoters to drive expression of genes of interest not only just in leaf tissue, but also at high levels in leaf tissue (Potenza et al. 2004). The level of expression of introduced genes when linked to *rbcS* promoters, as compared to the *35S* promoter (see Section 3.4.2 of this Appendix), may vary depending upon the *rbcS* promoter used, the plant species transformed, the copy number of the insertion and the position of the insertion in the genome.

77. For example, soybean and tomato *rbcS* promoters were both less effective than the *35S* promoter in driving high levels of *uidA* reporter gene expression in GM apple (Gittins et al. 2000). Multiple copies of the inserted *uidA* reporter gene under the control of the *rbcS* promoters resulted in both high and low levels of *uidA* reporter gene expression in some of the GM apple plants. Conversely, an alfalfa *rbcS* promoter directed up to 10-fold higher expression levels of the *uidA* reporter gene compared to the *35S* promoter in GM alfalfa leaf tissue (Khoudi et al. 1997).

THE COTTON *RBCS* PROMOTER

78. Cotton *rbcS* genes or their promoters have not been well studied. The *rbcS* promoter sequence used in the RbcS-GUS lines proposed for release is a 1827 bp region taken immediately upstream from the start codon of one member (Genbank Accession X54091) of the cotton *rbcS* gene family that may comprise 4 or 5 members (information supplied by the applicant). The *rbcS* promoter region under evaluation in this application is larger than the 520 bp promoter region reported for the same cotton *rbcS* gene (Sagliocco et al. 1991). This 520 bp region (and hence the 1827 bp promoter region) contains TATA and CAAT boxes typically required for eukaryotic gene transcription as well as the GATA-box, I-box and G-box *cis*-elements found in other plant *rbcS* promoters (see Table 1 of this Appendix).

79. The larger size of the promoter under evaluation should ensure that all regulatory elements of the cotton *rbcS* promoter are present. Due to time constraints, the applicant has not tested any of the promoter's *cis*-elements for response to regulatory cues (e.g. light). Sequence comparison of the cotton *rbcS* promoter with other *rbcS* promoter sequences (from rice, maize, chrysanthemum, potato and coffea) reveals sequence identities ranging from 10-18%, but the cotton *rbcS* promoter does not appear to be more similar to any particular plant *rbcS* promoter sequence (information supplied by the applicant). Thus the purpose of the proposed release is purely for research and proposes to evaluate the performance of this cotton *rbcS* promoter in controlling *uidA* reporter gene expression in cotton itself under field conditions.

3.4.2 *35S* promoter

ROLE OF THE *35S* PROMOTER IN CAULIFLOWER MOSAIC VIRUS

80. Cauliflower mosaic virus (CaMV) is a DNA virus that infects members of the *Cruciferae* (e.g. cabbages, cauliflowers, mustard). The virus has a circular genomic DNA of 8 kb and contains two promoters, *35S* and *19S*. Early in the virus replication cycle, CaMV delivers its genomic DNA to the nucleus, where it is assembled into a minichromosome by association with host proteins from the infected plant (Olszewski et al. 1982). Viral transcripts (35S RNA) are then produced by the *35S* promoter and are used as mRNAs for the production of viral proteins, or as templates to replicate the virus (Pfeiffer & Hohn 1983). Rapid viral replication in infected cells requires a copious supply of the 35S RNA, and the *35S* promoter drives high level production of the 35S RNA in infected plant cells (Guilley et al. 1982).

USE OF THE *35S* PROMOTER IN GM PLANTS

81. The *35S* promoter (Odell et al. 1985) is the most commonly used promoter for controlling the expression of introduced genes in GM plants (Potenza et al. 2004). It can drive high levels of gene expression in both dicotyledons and monocotyledons (e.g. Jefferson et al. 1987; Battraw & Hall 1990), and its activity when isolated from the viral genome suggests that transcription from the *35S* promoter does not depend on any *trans*-acting viral gene products (Odell et al. 1985). The *35S* promoter confers gene expression in most tissues and during most stages of development of GM plants and is thus considered to be a constitutive promoter, although some cell-type specificity has been found (e.g. Jefferson et al. 1987; Benfey & Chua 1989; Benfey et al. 1990).

82. The high constitutive activity of the *35S* promoter in GM plants is thought to result from interactions between its *cis*-elements (Fang et al. 1989). Individual *cis*-elements, Domain A and Domain B, are known to be involved in controlling tissue-specific gene expression (Benfey et al. 1989; Benfey et al. 1990). A tandemly repeated TGACG sequence motif in Domain A is known to bind a tobacco transcription factor, ASF-1 (Lam et al. 1989), and is involved in conferring gene expression in the root (Benfey et al. 1989; Lam et al. 1989). Domain B contains a conserved GATA motif similar to *cis*-elements found to be light responsive in plant light-inducible promoters (see Table 1 of this Appendix) and interacts with a transcription factor, ASF-2, from tobacco leaf tissue (Lam & Chua 1989). Domain B confers gene expression in cotyledons, stems and leaves (Benfey et al. 1989). Duplication of Domain B enhances activity of the *35S* promoter by 10-fold (Kay et al. 1987).

LIMITATIONS OF USING THE *35S* PROMOTER IN GM PLANTS

83. There are a number of limitations of using the *35S* promoter to control the expression of introduced genes in GM plants.

84. One limitation is the perceived risk to human health by using sequences derived from infectious plant viruses in GM plants. This is further addressed in Appendix 4.

85. Another limitation is the apparent ability of plants to recognise viral-derived sequences as foreign and inactivate them, a process that falls under the broad phenomena of 'gene silencing'. Gene silencing can result from a block in gene expression at the level of transcription or post-transcription. For a review of the mechanisms involved in gene silencing at these levels, see Chandler and Vaucheret (2001) and Vaucheret et al. (2001).

86. Gene silencing is unpredictable, but when it occurs it can lead to the stable shutdown of the introduced gene's activity throughout the plant or it can affect the specificity of an introduced promoter (e.g. Kloti et al. 2002). It may also affect the expression of similar host genes, and can have consequences for the plant's survival if it involves a housekeeping or a defence-related gene. Gene silencing resulting from the introduction of genes under the control of strong promoters, most often *35S*, has been frequently reported (e.g. Elmayan & Vaucheret 1996; Chalfun-Junior et al. 2003). It may, however, be less common when constitutive or tissue-specific promoters of plant origin are used.

87. The applicant notes that reduced transcription from the *35S* promoter in controlling expression of the *cry1Ac* insect resistance gene in INGARD® cotton contributed to late season decline in efficacy, although the INGARD® cotton varieties still reduced pesticide use on the areas in which they were planted (CRDC 2000). Other viral promoters, derived from Subterranean-clover stunt nanovirus, that share similar regulatory elements to *35S* also suffer from a late season decline in efficacy when controlling the expression of an insect resistance gene like *cry1Ab* in GM cotton (information collected by the applicant in previous field trials under PR100, PR100X, PR138, PR138X and DIR 016/2002). Hence the applicant is proposing to evaluate the performance of a strong, tissue-specific plant promoter derived from cotton itself (the *rbcS* promoter, see Section 3.4.1 of this Appendix) to explore its potential in controlling the expression of potential commercially useful introduced traits in cotton throughout the entire growing season.

3.4.3 Other regulatory sequences

88. The *nos* promoter and terminator sequences, and the *ocs* terminator sequence, are derived from the *A. tumefaciens* Ti plasmid-encoded nopaline synthase (Depicker et al. 1982; Bevan et al. 1983) and octopine synthase (De Greve et al. 1982) genes respectively. The *nos* promoter is considered to be a constitutive, but weak, promoter in GM plants (Sanders et al. 1987; Harpster et al. 1988). The *nos* promoter controls expression of the *nptII* gene in the GM cotton lines. Terminator sequences are located downstream of a gene's coding region and are also required for gene expression in plants.

89. A short (100 bp) sequence (intron) derived from the castor bean (*R. communis*) catalase gene (Ohata et al. 1990) is present within the *uidA* reporter gene. Insertion of a plant-derived intron into the *E. coli uidA* reporter gene prevents the synthesis of the functional GUS enzyme in prokaryotes, such as *Agrobacterium*, due to their lack of eucaryotic intron-splicing (i.e. intron-removing) machinery. In GM plants containing the *uidA* reporter gene with intron insertions, the intron is efficiently spliced (removed), giving rise to *uidA* mRNA and subsequently, GUS enzyme activity (Vancanneyt et al. 1990).

SECTION 4 METHOD OF GENETIC MODIFICATION

90. The gene construct comprising the *uidA* reporter gene, controlled by the *rbcS* promoter, the *nptII* and *hph* genes, and other associated regulatory sequences was introduced into the cotton by *A. tumefaciens*-mediated DNA transformation (Zambryski 1992). The gene construct comprising the *uidA* reporter gene, controlled by the *35S* promoter, and *nptII* gene, and other associated regulatory sequences was introduced into the cotton in the same way.

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

92. 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).

93. The binary vectors 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 biosafety problems.

94. The gene construct containing the *uidA* reporter gene controlled by the *rbcS* promoter was developed in the standard binary vector pIG121HM (Akama et al. 1992) by replacing the *35S* promoter controlling expression of the *uidA* gene with the *rbcS* promoter. This standard binary vector also contains the *nptII* and *hph* selectable marker genes in the T-DNA region. The gene construct containing the *uidA* reporter gene controlled by the *35S* promoter was developed in the standard binary vector pGA470 (An et al. 1985) which also contains the *nptII* selectable marker gene in the T-DNA region.

95. Following co-cultivation with *A. tumefaciens* containing the gene constructs, cotton cells were cultured in the presence of kanamycin to select for those cells containing the inserted DNA (the *nptII* gene confers kanamycin resistance). Subsequently, cotton plants containing the inserted DNA were regenerated from these GM cells. It should be noted that *G. hirsutum* Coker 315 cultivar was used for transformation, since this cotton cultivar is used for research and development purposes and is not suitable for commercial development in Australia. The Coker cultivar is a US cultivar used widely in research because it can be readily cultured and regenerated in the laboratory.

96. T1, T2 and T3 generations of the GM cotton lines were obtained from primary transformants (TO) by self-pollination.

SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION

97. Primary transformants and their T1 progeny that showed positive GUS staining in leaves were further analysed for insertion copy number by Southern blot, using probes derived from either *nptII* or *uidA* genes. Copy numbers of the inserted DNA vary from one to eight copies at one or more genetic loci respectively. T2 or T3 plants were progeny tested to identify lines homozygous for the inserted genes. The inserted genes have been inherited as dominant Mendelian traits over at least two generations in the glasshouse.

98. The applicant states that the exact location of the inserted genes within the cotton genome is not known and will not be determined, since the GM cotton lines are for research purposes only and are not intended for commercial release.

99. The applicant has not thoroughly investigated the stability of the genotypes of the GM cotton lines proposed for release, other than to demonstrate GUS staining over two or more generations.

100. The GM cotton plants proposed for release under this application may be T1, T2 or T3 generation, depending on the timing of the trial and the stage of evaluation of the GM cotton plant material.

101. Similar 35S-GUS lines were previously released as T2 and T3 plants in 1998 and 1999 under PR100 and PR100X with approval from GMAC.

SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS

Section 6.1 GUS expression

102. It is expected that, in the 35S-GUS lines, GUS will be expressed in most tissues and during most stages of development of the plants. In the RbcS-GUS lines, it is expected that GUS will be expressed in the green tissues throughout the life of the plants.

103. In a previous study, northern blot analyses showed that the cotton *rbcS* gene, from which the *rbcS* promoter under evaluation in this application is derived, is expressed in germinated cotyledons, in leaves and at low levels in stems, but not in roots, young embryos or mature seeds of cotton (Sagliocco et al. 1991). The applicant's preliminary studies with the RbcS-GUS lines proposed for release show that, under glasshouse conditions, the 1827 bp cotton *rbcS* promoter can also direct tissue-specific expression of GUS. High levels of GUS staining were detected in leaves, stems, bracts and boll coats, but no GUS staining was detected in roots. One published study also describes a shorter region (520 bp) of the same cotton *rbcS* promoter controlling GUS expression in GM cotton under glasshouse conditions (Song et al. 2000). In this study, GUS staining was detected in young and old leaves, and to a lesser extent in the shoot cortex. No GUS staining was detected in roots, petals, anthers or 8-day postanthesis-developing ovules from flowering GM cotton plants. Thus the cotton *rbcS* promoter confers tissue-specific expression of the gene (and encoded protein) to which it is linked in GM cotton.

104. The applicant's preliminary studies with the 35S-GUS lines proposed for release show that, under glasshouse conditions, the *35S* promoter directs high levels of GUS expression (as indicated by GUS staining) in all vascularised tissues including the roots. A recent study has described the expression pattern of another reporter gene encoding green fluorescent protein (GFP) in GM cotton as controlled by the *35S* promoter (Sunilkumar et al. 2002). GFP fluorescence was not detected during the early stages of embryogenesis until around 13 days-postanthesis, when GFP fluorescence was observed at the junction of the hypocotyl and cotyledons. GFP fluorescence became stronger and expanded throughout the cotyledon and hypocotyl as the embryo developed. After germination, varying levels of GFP fluorescence were observed in all cell and tissue types in the hypocotyl, cotyledon, stem, leaf, petiole and root. GFP fluorescence was also detected in all floral parts, in some of the pollen and also in developing cotton fibres. Thus the *35S* promoter confers constitutive expression of the gene (and encoded protein) to which it is linked in GM cotton.

105. Levels of GUS protein expression, as controlled by the *35S* promoter, have been estimated in the insect resistant Bollgard II® cotton. GUS protein was detected at high levels in both young leaves (≤ 120 µg/g fresh weight of plant tissue) and seeds (≤ 104 µg/g fresh weight of plant tissue), but was not measured in other tissues (see Chapter 3 of the RARMP for licence application DIR 012/2002).

106. The applicant does not propose to measure levels of GUS protein expression, but proposes to measure the level of *uidA* reporter gene expression (by northern blot analysis) and to quantify GUS enzyme activity (by fluorometric assay) in the GM cotton lines throughout the growing season. This will allow a comparison of the efficacy of the *rbcS* and *35S* promoters in controlling *uidA* reporter gene expression under field conditions. These assays will be performed mostly on leaves and first flower buds (termed squares), although other tissues may be tested if enough plant material is available (information supplied by the applicant).

Section 6.2 NPTII and HPH expression

107. Since the *nptII* gene is under the control of the *nos* promoter, NPTII is expected to be weakly expressed in most tissues of the GM cotton lines (see Section 3.4.3 of this Appendix). Compared to the *35S* promoter, the *nos* promoter has been found to drive, on average, 30-fold less expression of *nptII* mRNA and resulted in 110-fold less NPTII enzyme activity in GM petunia (Sanders et al. 1987).

108. Expression of NPTII in the GM cotton lines has been confirmed through the ability of the plants to grow in the presence of kanamycin in the laboratory during development of the GM cotton lines. Expression of *nptII* mRNA has been confirmed in leaf tissue, using the Polymerase Chain Reaction (PCR) technique. NPTII protein or enzyme activity levels will not be quantified since the GM cotton lines are for research purposes only and are not intended for commercial release.

109. Since the *hph* gene is under the control of the *35S* promoter in the RbcS-GUS lines, HPH is expected to be expressed in most tissues and during most stages of development of these GM cotton lines. No analyses were performed on this selectable marker during the laboratory stages of development of these GM cotton lines (see Section 3.3 of this Appendix), and HPH protein or enzyme activity levels will not be quantified since the RbcS-GUS lines are for research purposes only and are not intended for commercial release.

SECTION 7 PLEIOTROPIC EFFECTS OF THE GENETIC MODIFICATION

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

111. No unintended or secondary effects have been observed in the GM cotton lines grown under glasshouse conditions. The applicant reports that the growth characteristics of the GM cotton lines are the same as for conventional cotton.

SECTION 8 RESEARCH REQUIREMENTS

112. The proposed release of the GM cotton lines is a small scale research trial to compare the performance of a new promoter from cotton to a commonly used promoter from CaMV in controlling expression of the bacterial *uidA* reporter gene under field conditions.

113. If the applicant makes an application for any future use of the *rbcS* promoter to control the expression of potential commercially useful introduced traits in any GMOs, data would be required to be collected on:

- the level of expression of the introduced gene(s) and encoded protein(s), and the plant tissues (including pollen and nectar) and developmental stages in which they are being expressed by the *rbcS* promoter being evaluated under Australian field conditions;
- stability of the introduced genes and modified traits under Australian field conditions;
- genetic segregation and molecular characterisation of the introduced genetic materials;

- agronomic characteristics relating to fitness; and
- unintended effects of the genetic modification.

APPENDIX 2 TOXICITY AND ALLERGENICITY TO HUMANS AND OTHER ORGANISMS

114. 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 (RARMP). This Appendix considers potential hazards that may be posed to the health and safety of humans and other organisms as a result of any toxicity or allergenicity arising from the introduction of the new genetic material.

115. It should be noted that other GM cottons containing the reporter gene, *uidA*, and the antibiotic resistance gene, *nptII*, have been extensively trialed and commercially released previously in Australia. GM cottons containing the antibiotic resistance gene *hph* have also been studied in a number of field trials (for details see Chapter 1, Table 1). There have been no reports of adverse effects on the health of humans or other organisms from dealings with these GMOs.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

116. 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).

117. Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies known as IgE or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO & WHO 2000). Allergic responses have a well-defined etiology (i.e. biochemical cause) that is quite different from toxicity. An allergic response can have severe consequences for an individual. Anaphylaxis, for example, 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).

118. 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 pass through the stomach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion.

119. The GM cotton lines proposed for release differ from non-GM cotton in the expression of either two or three additional proteins. These are the GUS protein and either one (35S-GUS lines) or both (RbcS-GUS lines) of the antibiotic resistance proteins, NPTII and HPH (see Appendix 1 for details of protein expression in the GM cottons). The potential for these cottons to be toxic or allergenic to humans or other organisms due to expression of the introduced genes, or due to unintended effects of the genetic modification, is considered in this Appendix.

120. If the GM cottons are toxic to humans and other organisms, the potential hazard could include adverse effects on:

- people (e.g. through food products, wearing cotton clothing or working with the GM cottons);
- livestock and wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (pollinators, or parasitoids/predators of insect pests); and

- microorganisms, particularly soil microorganisms, with direct impact on growth of crops on farms.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

121. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of the GM cotton lines (35S-GUS and RbcS-GUS) on the health and safety of humans and other organisms, the following factors were considered:

- the inherent toxicity and allergenicity of non-GM cotton;
- the potential exposure to the GM cottons, to their products and to the new proteins (GUS, NPTII and HPH) which are expressed in the GM cottons;
- the potential exposure to the GUS, NPTII and HPH proteins from other sources in the environment;
- the potential toxicity and allergenicity of the new proteins expressed in the GM cottons; and
- the potential toxicity and allergenicity of the GM cottons.

Section 2.1 Toxicity and allergenicity of non-GM cotton

122. 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 2002b) 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/ pubform/riskassessments.htm.* Information on non-GM cotton is included here to establish baseline data for comparison with the GM cottons being considered in this risk assessment.

123. 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 (e.g. dihydrosterculic, sterculic and malvalic acids).

124. 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 cottonseed 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.

125. 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 pesticides that could be found in the cotton trash and stubble.

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 2002b), 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 cottons

128. The applicant proposes to destroy the GM cottonseed produced in the trial, apart from some seed which will be retained for research, the second season trial under the proposed release or for possible future trials (subject to further approvals). Since it is not intended that any product of the proposed release would 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, the applicant would need to obtain approval from FSANZ.

129. The applicant does not intend to sell the lint produced from the release. As a result, there will be no opportunity for humans to be exposed to the GM cottons through cotton lint in clothing or other household products. Therefore, potential risks to humans as a result of such exposure to GM cotton products will not be discussed.

130. Potential exposure of people to the GM cottons will be by means of:

- working with cotton (e.g. on cotton farms, in cotton processing facilities); and
- living in or near the areas where GM cottons are grown (general environmental exposure, e.g. people breathing cotton pollen).

2.2.2 Exposure to GM cotton through working with cotton and living near cotton plantations

131. 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 introduced proteins expressed in the GM cottons), or to other cellular components of the cotton plants, will only occur if plant cells are ruptured.

132. Even if the cells rupture, exposure to the introduced proteins expressed in the GM cottons will be low as these proteins are expected to be only present at relatively low levels in the GM cotton tissues. For instance, the amount of NPTII protein expressed in GM plants is generally low, ranging for example from 0.00005 to 0.001% of the fresh weight of cotton seed, potato tuber or tomato fruit (Miki & McHugh 2004). The level of expression of GUS protein in the GM cottons is not known, but the applicant proposes to collect data on the level of GUS enzyme[4] activity, both in the RbcS-GUS and the 35S-GUS lines, as part of the proposed field trial (see Appendix 1 for details).

133. Even if exposure to the introduced proteins occurs, these proteins are not toxic or allergenic to humans (see Section 2.7 of this Appendix). For example, expression of the GUS and NPTII proteins controlled by the *35S* promoter in Bollgard II® cotton, which is being grown

[4] Enzymes are proteins which catalyse specific biochemical reactions.

commercially (OGTR 2002a) and approved for the use in human food and animal feed (FSANZ 2002), has not caused any adverse effects.

134. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002b), therefore limiting possible exposure to cotton pollen as a potential airborne allergen. Dermal exposure of workers to cotton pollen is possible, but the amounts to which workers might be exposed is expected to be very low.

135. The primary processing of seed cotton at cotton gins, and the bulk handling of cottonseed and cotton fibre, can create and stir up fine dust and lint particles. Use of personal protective equipment by exposed workers is commonplace in such facilities to prevent respiratory irritations. Since cotton lint contains no DNA or protein and the fibre characteristics are expected to be equivalent to non-GM varieties, cotton lint is no 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 (subject to further approvals).

136. Specific licence conditions have been imposed to prevent cottonseed escaping into the environment after harvest and during transportation to research facilities. The GM cottonseed will be required to be transported within a primary, sealed container that is packed in a secondary unbreakable container and stored in a locked facility. Seeds and plant material not required for research or possible future trials (subject to further approvals) will be destroyed.

Section 2.3 Exposure of livestock and wildlife, including mammals, birds and fish, to the GM cottons

137. None of the cotton plants from the proposed release or their by-products will be used as stockfeed. As discussed in Section 2.1 of this Appendix, most mammals avoid feeding on cotton, non-GM or GM, due to its production of toxic, anti-nutrient substances and due to its plant morphology. The applicant proposes to destroy all materials produced in the release, apart from some cottonseed for use in research, the second season trial under the proposed release or in possible future trials (subject to further approvals). As noted in Chapter 1 and Appendix 1, the cotton lines proposed for release have been generated by transformation of the Coker 315 cultivar developed as a part of a CSIRO research and development program and are not suitable for commercial development in Australia.

138. In the field, seed cotton is present as large lint-covered seeds that are unattractive to avian species (OGTR 2002b), which means that birds are not likely to be exposed to the introduced proteins expressed in the seeds of the GM cottons.

139. Cottonseed or pollen does not enter aquatic habitats in any significant quantity (OGTR 2002b), and therefore the level of exposure of aquatic species to the GM cottons will be very low. In addition, licence conditions require that the GM cottons must not be grown within 50 metres of a natural waterway.

140. The small scale (0.1 hectares) and limited duration (two summer growing seasons) of the proposed release will further limit the potential for exposure of stock and wildlife to the GM cottons.

141. Specific licence conditions have been imposed to prevent cottonseed escaping into the environment. The GM cottonseed will be required to be transported within a primary, sealed container that is packed in a secondary unbreakable container and is stored in a locked facility.

Seeds not required for research or possible future trials (subject to further approvals) will be destroyed.

Section 2.4 Exposure of invertebrates, including beneficial insects, to the GM cottons

142. 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 that feed on the plants. Relative exposure will be greatest for herbivorous species feeding on the cotton plants. Pollinator species and various adult insects that feed on 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.

143. However, the small size and limited duration of the proposed release will limit the potential for exposure of invertebrates to the GM cottons.

Section 2.5 Exposure of microorganisms, particularly soil microorganisms, to the GM cottons

144. 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 the soil.

145. After the cotton is harvested, the remaining plant material will be incorporated into the soil. A study monitoring the persistence of the *nptII* gene in decaying tobacco and potato plants in the field shows that genomic DNA may persist in the field for several months (Widmer et al. 1997). A study assessing the persistence of DNA in decomposing leaves of GM poplar trees shows that fragments of the DNA encoding the NPTII protein are not detectable in the field after four months (Hay et al. 2002). Data on the persistence of the introduced proteins in soil are not available.

146. The proposed release is small in size and limited in duration, which will limit exposure of microorganisms to the GM cottons.

Section 2.6 Other sources of GUS, NPTII and HPH proteins in the environment

147. The GUS and NPTII proteins are widespread in the environment since they are derived from, and naturally produced by, the common gut bacterium *Escherichia coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment. The gene encoding the HPH protein has also been isolated from strains of *E. coli*, but data on the occurrence of this protein in the environment are not available.

148. *E. coli* lives in the digestive tract of vertebrates, including humans (Jefferson et al. 1986), and utilises the GUS enzyme in its carbohydrate and energy metabolism. GUS activity, serving the same function, is also found in a wide number of other bacteria, including other microorganisms of the digestive tract and many soil bacteria (Gilissen et al. 1998).

149. GUS activity is very common in almost all tissues of vertebrates. It plays a major role in the degradation of glycosamino-glucuronides and in the release of active hormones from steroid

hormone-glucuronides. Tissues with high GUS activity include the preputial gland, kidney, liver and spleen (Gilissen et al. 1998).

150. GUS activity is also present in invertebrates such as molluscs, nematodes and insects. GUS is abundantly present in many fresh food products such as raw beef and oysters (Gilissen et al. 1998). GUS activity has been detected in over 50 different plant species including a number of human food sources such as carrot, parsley and celery (Hu et al. 1990). However, if endogenous GUS activity is found in plants, the activity is very low and its functions are unknown (Gilissen et al. 1998).

151. In summary, GUS enzyme activity has been detected in numerous microbial, plant and animal species, including species used as raw food (Gilissen et al. 1998).

152. Humans continually ingest kanamycin-resistant microorganisms, some containing the NPTII protein. The diet, especially raw salad, is the major source. Estimated conservatively, each human ingests 1.2×10^6 kanamycin-resistant microorganisms daily (Flavell et al. 1992). Large numbers of kanamycin- or neomycin-resistant bacteria already inhabit the human digestive system (Levy et al. 1998), with Flavell et al. (1992) estimating about 10^{12} per person. Kanamycin resistant bacteria have been isolated from soil, river water and sewage (Smalla et al. 1993).

153. The HPH protein confers resistance to the antibiotic hygromycin B and is produced by strains of the bacterium *E. coli* (Rao et al. 1983). Resistance to hygromycin B is also found naturally in the hygromycin B producing organism *Streptomyces hygroscopicus* (Leboul & Davies 1982). Hygromycin resistant bacteria have been isolated from human clinical sources in Europe (Salauze et al. 1990) and from bovine and porcine faeces in Japan (Ohmae et al. 1979).

Section 2.7 Toxicity and allergenicity of the introduced proteins

154. There will be no opportunity for people to consume food products (oil and linters) of the GM cottons, nor will cottonseed be fed to livestock. It is also worth noting that two of the proteins (GUS and NPTII) present in the GM cottons are the same as those present in commercially released Bollgard II® cotton. Oil and linters derived from Bollgard II® cotton have been approved for use in human food (ANZFA 2002).

2.7.1 Toxicity

155. Studies using the purified forms of the introduced proteins have been conducted, as the generally 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.

THE GUS PROTEIN

156. Acute oral toxicity studies in mice with purified GUS protein at doses of up to 100 mg/kg did not show any adverse effects (Naylor 1992). Studies feeding humans and animals with 10^{10} GUS-containing *E. coli* bacteria per ingestion also did not show any toxic or pathogenic reactions (Gilissen et al. 1998).

157. FSANZ has concluded that food derived from glyphosate-tolerant sugarbeet and from Bollgard II® cotton, both expressing the GUS protein, is safe for human consumption (ANZFA

2001a; ANZFA 2002). The US EPA does not consider GUS to be toxic for mammals and has approved its exemption from the requirement to establish tolerance levels (EPA 2001).

158. The GUS protein from *E. coli* is rapidly (<15 seconds) degraded in simulated gastric fluid and loses its activity by heating/cooking (Fuchs & Astwood 1996).

159. The *uidA* gene, encoding GUS, that was introduced into the GM cottons proposed for release has been modified to include a plant intron (Ohata et al. 1990) in order to prevent the synthesis of a functional GUS protein in prokaryotes. The functional protein can only be produced once the gene construct is integrated into the plant genome (for details see Appendix 1).

160. When the sequence of the GUS protein expressed in the GM cottons was compared to all protein sequences in publicly available databases, it only shared sequence similarities to homologous *E. coli* and other glucuronidase proteins, as expected (information provided by the applicant). These proteins have not been described as toxins to humans. Metabolites of *E. coli* GUS activity are non-toxic (Gilissen et al. 1998).

THE NPTII PROTEIN

161. The antibiotic resistance gene *nptII* has been inserted in a wide range of GMOs. None of the organisms modified by inserting the *nptII* gene have shown any adverse effects that could relate to the *nptII* gene product (Flavell et al. 1992). The *nptII* gene was introduced into mammalian cell lines with no effects on viability or growth. During gene therapy experiments, mammalian cells expressing the NPTII protein have been infused into cancer patients. Again, no adverse effects have been observed (Flavell et al. 1992).

162. NPTII protein produced in GM tomatoes fed to rodents was rapidly inactivated and degraded (Calgene 1990). An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight, did not show any adverse effects (Fuchs et al. 1993b).

163. Other regulatory agencies, in Australia and in other countries, have previously assessed the use of the *nptII* gene as a selectable marker in food crops and approved the gene and the encoded protein for use in human food and animal feed.

164. The US FDA has concluded that NPTII does not possess any properties that would distinguish it toxicologically from other phosphorylating enzymes in the food supply, and which are present in all plants and animals. NPTII is approved as an additive in food for human consumption in the USA (FDA 1994). The US EPA has also established an exemption for NPTII from the requirement for a residue tolerance limit when used as a plant pesticide inert ingredient (EPA 1994).

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

166. Currently, both kanamycin and neomycin are not in clinical use. Therefore, the potential for inactivation of an oral dose of these antibiotics in humans, by consuming plant material containing the antibiotic resistance genes, is not a hazard.

167. Human food, especially raw vegetables, is a major source of kanamycin/neomycin resistance genes (Flavell et al. 1992). Since the introduction of antibiotics, humans have been

exposed to kanamycin/neomycin genes and their products with no adverse effects (Flavell et al. 1992).

THE HPH PROTEIN

168. Hygromycin B is, after kanamycin, the second most frequently used antibiotic for selection in GM plants. In 2001 and 2002, the HPH protein was the second most prevalent antibiotic resistance marker listed in the US field trial database (Miki & McHugh 2004).

169. The current range of marker genes including the hygromycin B resistance gene, *hph*, do not code for any protein with known toxicity and are therefore considered safe (Malik & Saroha 1999).

170. The HPH protein is currently undergoing assessment in the USA for use in cotton and corn. The US EPA has established an exemption for HPH from the requirement for a residue tolerance limit when used as a plant pesticide inert ingredient in cotton (US EPA 2004). This regulation eliminates the need to establish a maximum permissible level for residues of HPH when used as a plant-incorporated pesticide inert ingredient. Based on the extremely low expression levels noted in GM cotton tissues, acute oral exposure studies in mice and rapid degradation of the HPH protein upon exposure to simulated gastric intestinal fluid, the EPA has determined that HPH incorporated in GM cotton will not pose toxicity to humans or animals.

171. An application for the use, in human food, of material from a GM cotton line (COT102) containing the *hph* gene is currently being assessed by FSANZ. The draft assessment report prepared by FSANZ recommends giving approval to the sale and use of oil and linters in food products from this GM cotton line (FSANZ 2004).

172. Currently, hygromycin is not in clinical use, and there is no cross-resistance with other antibiotics used for human therapy (EFSA 2004). Therefore, the potential for inactivation of an oral dose of this antibiotic in humans, by consuming plant material containing the antibiotic resistance gene, is not a hazard.

173. Hygromycin is used in veterinary medicine for the treatment of swine and poultry, as an anti-worming agent, (Karenlampi 1996; EFSA 2004), but its use is limited and declining. There are no current registered uses of hygromycin B listed with the Australian Pesticides and Veterinary Medicines Authority (APVMA).

174. The Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority proposes a classification of antibiotic resistance genes according to their biological distribution and based on the present state of therapeutic importance of the relevant antibiotics. According to this classification, the *hph* gene belongs to the antibiotic resistance genes whose use in GM plants, either for field trials or commercial release, is regarded to be safe and does not need to be restricted (EFSA 2004).

2.7.2 Allergenicity

175. 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 (Metcalfe et al. 1996; Taylor & Lehrer 1996).

176. 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; Taylor 1995; Fuchs &

Astwood 1996; Metcalfe et al. 1996; Kimber et al. 1999; ANZFA 2001b), 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.

177. Most allergens are present as a major protein component in the specific food representing from 2% up to 80% of total protein (Fuchs & Astwood 1996).

178. Double-blind placebo-controlled food challenges showed 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). The same author also suggests that when a protein is present in food at levels well below 1 mg per serving, the hazard for allergic 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 cottons in food.

179. None of the introduced proteins in the GM cottons are derived from known sources of allergens, nor are they expected to be present as major components of the GM cotton plants (see Appendix 1). Furthermore, as noted in Section 2.2 of this Appendix, GM cotton seed produced in the proposed field trial will not be used in human food or animal feed.

THE GUS PROTEIN

180. The GUS protein is unlikely to be a major allergen and does not display the characteristics common to known allergens or homology to known allergens. The GUS protein sequence does not share any structurally significant sequence similarity to sequences within an allergen database (information provided by the applicant).

181. The widespread occurrence of GUS and the constant exposure of consumers to the protein further reduce the chances of GUS being a putative allergen (Gilissen et al. 1998). In addition, GUS protein, if ingested by humans, will be readily degraded in the digestive tract since exposure of GUS to simulated mammalian digestive systems results in rapid denaturation (Fuchs & Astwood 1996; ANZFA 2002).

THE NPTII PROTEIN

182. The NPTII protein does not display characteristics common to known food allergen proteins (Fuchs et al. 1993b; FDA 1994; FDA 1998; ANZFA 2001c). The NPTII protein is heat labile and degrades rapidly in simulated human gastric fluid. No NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot and enzyme activity (Fuchs et al. 1993b).

183. Other regulatory agencies, in Australia and in other countries, have previously assessed the use of the *nptII* gene in food crops. The FDA has evaluated data submitted for deliberate releases of GMOs expressing NPTII protein and concluded that NPTII does not have any of the characteristics (Taylor et al. 1987) associated with allergenic proteins (US FDA 1998). In addition, proteins and DNA sequence comparison using sequences from four separate databases

(Genbank, EMBL, PIR29, Swiss-Prot) indicated that NPTII does not have significant homology to any proteins listed as food allergens or toxins in these databases (Fuchs & Astwood 1996).

184. A number of genetically modified food crops containing the *nptII* gene have been approved for commercial release by international regulatory agencies. No adverse effects on humans, animals or the environment have been reported from these releases (US FDA 1998; Flavell et al. 1992; EFB 2001).

THE HPH PROTEIN

185. The antibiotic resistance protein HPH is not known to be allergenic. The HPH protein is produced naturally by strains of the bacterium *E. coli*. *E. coli* is widespread in human and animal digestive systems and in the environment. Therefore, the HPH protein is not derived from a source known to produce allergens.

186. In addition, the HPH protein does not share protein sequence similarity to proteins known to be mammalian toxins or human allergens (US EPA 2004).

Section 2.8 Toxicity and allergenicity assessment of the GM cottons

187. The GUS gene and the antibiotic resistance genes operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the three genes (*uidA*, *nptII* and *hph*), their products or their metabolic pathways, and no reason to expect that this is likely to occur. Therefore, no hazards are envisaged from the combined expression of the introduced proteins.

SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY

188. It is considered that the risk of the GM cottons being toxic or allergenic for humans or other organisms is negligible because:

- the proposed release is small in scale (0.1 hectares) and limited in duration (two summer growing seasons);
- the introduced proteins are naturally produced in strains of the common gut bacterium *E. coli* and are therefore already present in the environment;
- very similar proteins to GUS and NPTII are produced by a number of other organisms that are widespread in the environment and present in human food;
- none of the GM cotton materials from the release will be used in human food or animal feed;
- exposure to the introduced proteins through working with the GM cotton plants is very low;
- processing of the GM seed cotton will only occur on a small scale (for preparation of seed for possible future trials) and lint from GM cottons is not more likely to induce adverse responses in workers than is lint from non-GM cotton;
- the introduced proteins are not known to be allergenic, nor do they have properties characteristic of known allergenic proteins;
- cotton pollen is not wind-dispersed and therefore not likely to be an air-borne allergen;

- feeding studies indicate that the introduced proteins (GUS, HPH and NPTII) are not toxic; and
- no toxic or allergic effects have been reported from similar GM cottons expressing the same proteins that have been extensively field trialed or commercially released in Australia.

189. The licence holder is required to report any adverse effects on human health and safety (e.g. allergic reactions as a result of occupational exposure to the cottons) or to the environment to the Regulator.

APPENDIX 3 WEEDINESS

190. 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 (RARMP). In this Appendix, risks posed by the proposed dealings to the environment are considered in relation to the potential for the GMOs to become problematic weeds.

SECTION 1 NATURE OF THE WEEDINESS HAZARD

191. 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.

192. 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.

193. However, because environmental conditions have a big influence on these attributes, and other factors such as plant community composition and availability of key resources (e.g. space, water, light and nutrients) influence the potential of a plant species to invade, weedy characteristics alone are not enough to determine if a plant will become a problematic weed. Therefore, the most successful predictors of weediness remain taxonomic affinity to other weedy species and the history of a given species' weediness elsewhere in the world (Panetta 1993; Pheloung et al. 1999).

194. The 60 GM cotton lines differ from conventional non-GM cotton in the expression of either two or three additional proteins. The β-glucuronidase (GUS) and the neomycin phosphotransferase (NPT II) enzymes[5] are present in 30 of the GM cotton lines while another 30 lines, in addition, contain the hygromycin phosphotransferase enzyme (HPH), all derived from the common gut bacterium *Escherichia coli* (see Appendix 1 for details of protein expression in the GMOs).

[5] Enzymes are proteins which catalyse specific biochemical reactions.

195. 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 introduced genes or as a result of unintended effects of the genetic modification.

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

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

197. 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;
- the potential weediness of the GM cotton lines; and
- the potential for spread and persistence of the GM cottons beyond the release site.

Section 2.1 Inherent weediness of conventional non-GM cotton

198. 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 2002b) 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/ pubform/riskassessments.htm.* 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 assessed.

199. 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).

200. As mentioned above, another important element in predicting 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* (Fryxell 1992; Craven et al. 1994) of which only one *(G. tomentosum)* is listed as a weed in the USA (Holm et al. 1997).

Section 2.2 Potential selective advantage conferred by the introduced proteins

2.2.1 GUS enzyme

201. In the proposed field trial, the *uidA* gene encoding the GUS enzyme enables visual identification of plant tissues in which this gene is being expressed. GUS activity is naturally found in microorganisms, vertebrates and invertebrates (Gilissen et al. 1998) but there is very

little activity in plants. The GUS enzyme is involved in bacterial carbohydrate and energy metabolism and is very unlikely to confer any selective advantage to GM cotton that might result in weediness (Gilissen et al. 1998).

2.2.2 NPTII enzyme

202. The NPTII enzyme could confer a selective advantage to GM cotton plants in the presence of a high concentration of the antibiotics neomycin or kanamycin. Since antibiotics are not applied to cotton crops, and are not likely to be present in any environment where cotton grows, the expression of NPTII is highly unlikely to confer any selective advantages on the GM cottons. In addition, there is no evidence nor any reason to expect that expression of the protein would alter any of the characteristic attributes of cotton that would be important for weediness.

2.2.3 HPH enzyme

203. The HPH enzyme could confer a selective advantage to GM cotton plants in the presence of a high concentration of the antibiotic hygromycin B. Since antibiotics are not applied to cotton crops, and are not likely to be present in any environment where cotton grows, the expression of HPH is highly unlikely to confer any selective advantages on the GM cottons. In addition, there is no evidence nor any reason to expect that expression of the protein would alter any of the characteristic attributes of cotton that would be important for weediness.

Section 2.3 Potential weediness of the GM cottons

204. 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.

205. Growth characteristics of the RbcS-GUS and 35S-GUS plants are the same as those of non-GM cotton when grown in the greenhouse (information provided by the applicant). GM cotton lines containing the *uidA* reporter gene controlled by the *35S* promoter have been grown previously in the field under PR100 and PR100X. Various GM cottons expressing the GUS, NPTII and/or HPH proteins have been previously approved for release (for details see Table 1, Chapter 1). No deleterious effects were reported from these releases. This suggests that the genetic modifications in these GM cotton lines are unlikely to lead to any unintended effects on characteristics typically associated with weediness.

206. Extensive agronomic assessment does not seem to be necessary at this early stage of comparing the efficacy of the promoters since the 60 GM cotton lines are not intended for any commercial release, the field trial is of a small scale and the cotton variety that has been used is not suitable for commercial development.

207. There is no evidence, nor any reasons to believe, that expression of the *uidA* gene encoding GUS or the antibiotic resistance genes in the GM cottons would alter any of the characteristic attributes of cotton important for weediness (OGTR 2002b). The antibiotic resistance genes would only confer a selective advantage in the presence of the antibiotics kanmycin/neomycin or hygromycin B, but these antibiotics are not used in agriculture.

Section 2.4 Spread of GM cottons beyond the release site

208. Cotton is primarily a self-pollinating plant, with pollen that is unlikely to be dispersed by wind (OGTR 2002b). However, insect vectors may transfer pollen to other cotton plants. Pollen dispersal studies have shown that outcrossing is localised around the pollen source and

decreases significantly with distance (Umbeck et al. 1991; Llewellyn & Fitt 1996). Pollen flow is also discussed in Appendix 4, Section 1 in relation to gene transfer. As proposed by the applicant, licence conditions have been imposed to require surrounding the GM cotton with a non-GM cotton pollen trap, to limit outcrossing to non-GM cotton.

209. The proposed dealings will include retention of some cottonseed for research and/or possible future trials (subject to further approvals) or for planting in the subsequent season field trial authorised by the same licence. To prevent seed dispersal, licence conditions have been imposed to require that GM cottonseed be stored or transported within a primary, sealed container that is packed in a secondary unbreakable container.

210. GM cottonseed could be dispersed beyond the limits of the trial sites on equipment during and after planting and harvesting. Licence conditions have been imposed to require cleaning of equipment used in connection with the release, so as to prevent the GM cottonseed escaping into the environment.

Section 2.5 Persistence of the GM cottons at the release site

211. Following harvest of the seed cotton from the release site, 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. The applicant proposes to destroy any cotton plants that emerge.

212. Twelve months of post-harvest monitoring of the release site after post-harvest cleaning is 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

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

- the proposed release is small in scale (0.1 hectares) and limited in duration (two summer growing seasons);
- cotton has a low potential for dispersal by natural means;
- 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 or to confer a selective environmental advantage;
- other GM cottons containing the same proteins, grown commercially or under limited and controlled conditions in Australia, have not become problematic weeds; and
- major constraints on weediness of both GM and non-GM cottons are water availability, nutrient availability, plant competition, herbivory, frost and fire.

214. It is considered that the risks of the GM cotton lines establishing as a weed is very low and will be managed by applying various strategies to limit the spread and persistence of the GM cottons from the release site. Licence conditions have been imposed to manage this risk, including a requirement to conduct post-harvest inspections of the release site to ensure

volunteer plants are destroyed before flowering and GM cottons are unable to persist in the environment (see Chapter 2 and Appendix 5 for details).

APPENDIX 4 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

215. 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 (RARMP). This Appendix considers potential hazards that may be posed through the transfer of the introduced genetic materials from the GM cottons to other organisms.

216. Gene transfer is the movement of genetic material between individuals. Within a species genetic material is 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 produces triticale. In animals, fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile, meaning hybridisation may or may not lead to the introgression of new genetic material into a population.

217. Without the application of gene technology, gene transfer is not readily observed between distantly related species, except for between bacteria and between viruses. However transfer of genetic material 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. However, there seems to have been only very limited transfer of genetic materials from plants to other types of organisms.

218. The likelihood of hazards arising from gene transfer is dependent on a number of factors that must form a necessary 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 genetic material 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.

219. 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 genetic materials from the GM cottons to other plants, including other cotton plants;
- **Section 2** details the nature and likelihood of a hazard arising through transfer of the introduced genetic materials from the GM cottons to microorganisms;
- **Section 3** details the nature and likelihood of a hazard arising through transfer of the introduced genetic materials from the GM cottons to animals, including humans; and
- **Section 4** draws together the conclusions from these sections.

220. A detailed assessment of the likelihood of gene transfer from GM cottons to other organisms is 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, 040/2003, 044/2003 and 048/2003.

SECTION 1 GENE TRANSFER FROM THE GM COTTONS TO OTHER PLANTS

Section 1.1 Nature of the gene transfer hazard

221. Transfer of the introduced genes (*uidA, nptII* and *hph*) or regulatory sequences to other cultivated (including volunteer), naturalised (feral) or native cotton plants would present the same hazards and have the same potential impacts as their presence in the GM cottons (see Appendices 2-3 for details). However, if such a transfer occurred, it would increase the possibility that these genetic materials would further spread and persist in the environment.

222. If gene transfer to other plant species were to occur, any resulting hazards to the environment could be highly varied, broadly depending upon the nature of the genetic materials 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 and their progeny gained a selective advantage, such as enhanced survival or reproductive capacity.

Section 1.2 Potential hazards from the introduced genetic materials

1.2.1 The *uidA* (reporter) gene

223. Plants expressing this gene could produce the GUS protein. GUS expression is unlikely to be toxic or allergenic to humans and other organisms (see Appendix 2 for details) and is unlikely to affect weediness (see Appendix 3 for details), therefore this would not be expected to confer any selective advantage on the plant.

1.2.2 The *nptII* and *hph* (antibiotic resistance) genes

224. Plants expressing these genes could become resistant to the antibiotics kanamycin and neomycin (if expressing *nptII*) and/or hygromycin B (if expressing *hph*). This would only have an impact on plant survival if these antibiotics were used on the plants, or otherwise present in the environment of the plants, and were limiting their growth. Antibiotics are not generally applied to crops and would not limit their growth except at very high concentrations not found in the natural or agricultural environment. Expression of the *nptII* marker gene enabled selection of plant cells containing the genetic modification in the laboratory. The *hph* gene was not used as a selectable marker (see Appendix 1 for details).

225. It has been claimed that the *hph* gene may also confer some tolerance to the herbicide glyphosate (Penaloza-Vazquez et al. 1995a; Penaloza-Vazquez et al. 1995b) although this has not been substantiated (see Appendix 1 for details). The applicant does not intend to use this potential trait. Additionally, normal cultural practices of herbicide rotation would limit the persistence of this potential trait in other plants.

1.2.3 Promoters and other regulatory sequences

226. If these sequences were to be transferred to other plants without the associated introduced 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.

227. Some of the introduced regulatory sequences are derived from plants (*Gossypium hirsutum* L. (cultivated cotton) and *Ricinus communis* (castor bean)) and others are derived from plant pathogens (Cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*). However the regulatory sequences from CaMV and *A. tumefaciens* are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

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

Section 1.3 Likelihood of a hazard arising through transfer of the introduced genetic materials to other plants

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

1.3.1 Transfer to other cultivated and volunteer cotton

230. For a detailed consideration of the likelihood of gene transfer occurring, including an overview of the pollination biology of cotton, see the document “The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia” (OGTR 2002b). This document is available at *www.ogtr.gov.au/pubform/riskassessments.htm* and was produced in order to inform the risk assessment processes for licence applications involving GM cottons.

231. Cotton is primarily self-pollinating, and as cotton pollen is large and heavy, it is not easily dispersed by wind (Jenkins 1992). In a cropping situation, however, a low level of pollen transfer, by insect pollinators, to nearby cotton plants would be likely (OGTR 2002b). Cotton pollen dispersal studies consistently show that when out-crossing occurs, it is localised around the pollen source and decreases significantly with distance (OGTR 2002b and references therein).

232. Studies carried out near the proposed trial site at Narrabri in New South Wales, Australia, demonstrated that out-crossing events are generally rare, with out-crossing rates declining from 0.4% at 1 m to below 0.03% at 16 m and not-detected at 20 m from GM cotton plants (Llewellyn & Fitt 1996). These out-crossing frequencies are relatively low compared to those seen in other countries, possibly due to differences in pollinator species, particularly the absence of bumble bees in Australia (Llewellyn & Fitt 1996). In the out-crossing experiments conducted at Narrabri, no bees were detected, and although small numbers of wasps and flies were recorded, it was suggested that hibiscus beetles were likely to be the major cross-pollinators in these trials (Llewellyn & Fitt 1996).

233. *G. hirsutum* (Upland cotton) is the most widely planted commercially cultivated cotton in Australia. *G. barbedense* (pima cotton) is also used for commercial cotton production, but only to a very minor extent in Australia (OGTR 2002b). *G. hirsutum* and *G. barbedense* are closely related and hybridisation between the two species can occur, yielding fertile progeny (Brubaker

et al. 1999). 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.

234. Transfer of the introduced genes or regulatory sequences to other cultivated cotton would present the same hazards as their presence in the GM cottons proposed for release (see Appendices 2-3 for details). However, if such a transfer occurred, it would increase the possibility that these genetic materials could persist and further spread in the environment.

235. The likelihood of a hazard arising through transfer of the introduced genetic materials to other cultivated cotton will be further minimised due to the small scale (0.1 hectares) and limited duration (two summer growing seasons) of the proposed release. Licence conditions have been imposed to limit the likelihood of it occurring by including a requirement that the GM cottons be isolated from other cotton crops by a 20 m 'pollen trap' of non-GM cotton, to limit cross-pollination to plants outside the release site (see Chapter 2 and Appendix 5 for details).

1.3.2 Transfer to naturalised (feral) cotton

236. Transfer of the introduced genes or regulatory sequences to naturalised cotton could also increase the likelihood that these genetic materials 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 (OGTR 2002b). The remnants of these populations are geographically isolated from the proposed trial site in New South Wales.

237. Transfer of the introduced genes or regulatory sequences to naturalised cotton plants would present the same hazards as their presence in the GM cottons proposed for release (see Appendices 2-3 for details). However, if such a transfer occurred, it would increase the possibility that these genetic materials could persist and further spread in the environment.

238. The likelihood of a hazard arising through transfer of the introduced genetic materials to naturalised cotton is minimised given the geographic separation of naturalised cotton populations from the proposed release site of the GM cottons, and due to the small scale (0.1 hectares) and limited duration (two summer growing seasons) of the proposed release.

239. Licence conditions have been imposed to limit cross-pollination to plants outside the release site (see Section 1.3.1 above or Chapter 2 and Appendix 5 for details).

1.3.3 Transfer to native cottons and other plant species

240. Australian flora contains 17 native *Gossypium* species, all of which 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 (OGTR 2002b). These hybrids were sterile, however, effectively eliminating any potential for introgression of *G. hirsutum* genetic materials into *G. sturtianum* populations.

241. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from cultivated cotton fields. Thus gene transfer from

the GM cottons to native cottons is prevented not only by genetic incompatibility but also by geographic constraints to cross-pollination (OGTR 2002b).

242. The failure of cross-pollination due to well established genetic incompatibility also prevents gene transfer from the GM cottons to other plant species.

SECTION 2 GENE TRANSFER FROM THE GM COTTONS TO MICROORGANISMS

Section 2.1 Nature of the gene transfer hazard

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

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

Section 2.2 Potential hazards from the introduced genetic materials

2.2.1 The *uidA* (reporter) gene

245. The *uidA* gene present in the GM cottons has been modified for optimal expression in plants by the introduction of an intron and consequently a functional GUS enzyme[6] would not be expressed from this gene in bacteria (see Appendix 1 for details). Even if a functional GUS enzyme was expressed in bacteria, this would not have a significant effect on microbial communities as GUS is involved in bacterial carbohydrate and energy metabolism and is widespread among bacterial species (see Appendix 1 for details).

2.2.2 The *nptII* and *hph* (antibiotic resistance) genes

246. Microorganisms could become resistant to the antibiotics kanamycin and neomycin (if expressing *nptII*) and/or hygromycin B (if expressing *hph*). The consequences of this for human health and safety and the environment would depend on other characteristics of the microorganism (e.g. pathogenicity), the use and significance of the antibiotics in clinical and/or veterinary practice and whether these antibiotics limit growth or survival of the microorganism in other circumstances.

247. Some microorganisms may be limited by antibiotics, either due to the use of antibiotic medicines or in some limited environmental situations where competing microorganisms produce antibiotics.

248. It has been claimed that the *hph* gene may also confer some tolerance to the herbicide glyphosate (Penaloza-Vazquez et al. 1995a; Penaloza-Vazquez et al. 1995b) although this has not been substantiated (see Appendix 1 for details). Microorganisms expressing the *hph* gene

[6] Enzymes are proteins which catalyse specific biochemical reactions.

could potentially develop some tolerance to glyphosate, however since microorganisms are not controlled by herbicides, no selective advantage would result.

2.2.3 Promoters and other regulatory sequences

249. If these sequences were to be transferred to microorganisms without the associated introduced genes found in 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 any resulting phenotypic change.

250. Some of the introduced regulatory sequences are derived from plants (*G. hirsutum* (cultivated cotton) and *R. communis* (castor bean)) and others are derived from plant pathogens (CaMV and *A. tumefaciens*). However the regulatory sequences from CaMV and *A. tumefaciens* are not pathogenic by themselves.

251. There is a possibility that introduced viral sequences could recombine with the genome of another virus infecting GM plants to create a novel virus. The likelihood of this occurring and creating a hazard to human health and the environment is addressed in Section 2.4.2 of this Appendix.

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

Section 2.3 Other sources of the introduced genetic materials in the environment

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

254. All of the introduced genes in the GM cottons are already present in the environment, being derived from common gut bacteria (*Escherichia coli*). The regulatory sequences are derived from common soil bacteria (*A. tumefaciens*), a virus (CaMV) and two plants (*G. hirsutum* (cultivated cotton) and *R. communis* (castor bean)).

2.3.1 The *uidA* (reporter) gene

255. The *uidA* gene is derived from the common gut bacterium *E. coli*, which is widespread in the environment and is also present in the digestive tract of vertebrates including humans (Jefferson et al. 1986; Gilissen et al. 1998). Endogenous GUS enzyme activity is found in many other bacterial species, in virtually all tissues of vertebrates, and also in invertebrates (Jefferson 1987; Gilissen et al. 1998; see also Appendix 2).

256. Degradation of GM cotton plant material and incorporation into soil also potentially exposes soil dwelling microorganisms to the introduced *uidA* gene.

2.3.2 The *nptII and hph* (antibiotic resistance) genes

257. The *nptII* gene was originally isolated from a mobile genetic element (transposon) found in the plasmids and chromosomes of *E. coli*. *E. coli* is widespread in human and animal digestive systems as well as in the environment. Transposons are readily transferable between

bacterial species in nature. The *nptII* gene is associated with transposon Tn5 (Beck et al. 1982) and is observed in gram negative bacteria and *Pseudomonas* sp. (e.g. Smalla et al. 1993). While it is widely dispersed in the environment, other genes that also confer resistance to kanamycin and neomycin are more common, and also readily transferable among bacterial species (Belgian Biosafety Server 1999 and references therein).

258. The HPH enzyme is produced naturally by strains of the common bacterium *E. coli* (Rao et al. 1983). Resistance to hygromycin B is also found naturally in the hygromycin B-producing organism *Streptomyces hygroscopicus* (Leboul & Davies 1982). Hygromycin B-resistant bacteria have been isolated from human clinical sources in Europe (Salauze et al. 1990) and from bovine and porcine faeces in Japan (Ohmae et al. 1979).

259. Soil dwelling microorganisms may potentially be exposed to the introduced *nptII* and *hph* antibiotic resistance marker genes following decomposition of GM cotton plant material in the soil. For example, a study monitoring the persistence of the *nptII* gene in decaying GM tobacco and potato plants in the field shows that genomic DNA may persist in the field for several months (Widmer et al. 1997). A study assessing the persistence of DNA in decomposing leaves of GM poplar trees shows that fragments of the DNA encoding the NPTII protein are not detectable in the field after four months (Hay et al. 2002).

260. Bacterial strains resistant to the antibiotics in question already occur naturally (see above). In the unlikely event that the *nptII* and *hph* genes were transferred from the GM cottons to a bacterium, this would be unlikely to have any detectable impact on the existing level of resistance in microbial populations. This is supported by recent evaluations of the risks of antibiotic resistance marker gene transfer from GM plants to bacteria (EFSA 2004; Bennett et al. 2004).

2.3.3 Promoters and other regulatory sequences

261. CaMV is already ubiquitous in the environment and in the human diet, and the *35S* promoter derived from CaMV occurs at far higher levels in naturally infected plants than in GM plants (Hull et al. 2000).

262. The Rubisco enzyme is arguably the most abundant gene product in higher plants (Dean & Leech 1982) and it also exists in algae and photosynthetic microorganisms (Parry et al. 2003 and references therein). Hence *rbcS* promoters are already ubiquitous in the environment and animal, including human, diet.

263. The small (100 bp) intron present in the *uidA* reporter gene is derived from the catalase gene of the castor bean (*R. communis*) plant (see Appendix 1 for details). Castor bean is an ornamental plant native to Africa, but it is widely cultivated (including in Australia) for its seed oil ('castor oil') which has several uses including medicinal (e.g. laxative). Thus this regulatory sequence is already present in a plant gene in the environment. The rest of the castor bean plant is not consumed by animals due to its toxic properties (the toxin is water-soluble and is therefore not present in the seed oil). Further information on the castor bean plant is available at *http://faculty.ucc.edu/biology-ombrello/POW/castor_bean.htm.*

264. The *nos* promoter and terminator, and the *ocs* terminator, regulatory sequences are derived from the nopaline synthase (*nos*) (Depicker et al. 1982; Bevan et al. 1983) and octopine synthase (*ocs*) (De Greve et al. 1982) genes from the common soil bacterium *A. tumefaciens*, and are therefore widely dispersed in the environment.

Section 2.4 Likelihood of a hazard arising through gene transfer from the GM cottons to microorganisms

265. The likelihood of gene transfer to microorganisms creating a hazard for human health and safety or the environment depends on the characteristics of the introduced genetic materials, as well as on the likelihood of the transfer itself.

266. Most gene transfers have been identified through analyses of gene sequences (Ochman et al. 2000; Worobey & Holmes 1999). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence & Ochman 1998). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). In contrast, transfers of plant genetic materials to other microorganisms such as bacteria, viruses or fungi have been exceedingly rare (see Sections 2.4.1, 2.4.2 and 2.4.3 of this Appendix).

2.4.1 Bacteria

267. Mechanisms of conjugation (gene transfer between bacteria) and transduction (gene transfer from bacterial viruses to bacteria) will not be considered here as both these mechanisms are one step removed from the only possible route of plant to bacteria DNA transfer – natural transformation in the environment.

268. 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 of gene transfer from divergent donor organisms (Nielsen 1998).

269. 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.

270. 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 (e.g. Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory and glasshouse conditions, between homologous

sequences and under conditions of selective pressure (Nielsen et al. 1998; Mercer et al. 1999; Nielsen et al. 2000; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001); and even then only at a very low frequency.

271. A recent study reported evidence of low frequency transfer of a small fragment (180 bp) of an introduced gene derived from GM soybean to microorganisms within the small intestine of human ileostomists (i.e. individuals in which the terminal ileum is resected and digested material is diverted from the body to a colostomy bag) (Netherwood et al. 2004). However, only very low concentrations (1-3 copies per 10^6 bacteria) of the small fragment were detected in samples of microorganisms taken from the small bowel of three of seven ileostomists. Furthermore, the small fragment was only detected after two levels of amplification; (i) extensive culturing of the microorganism samples, and (ii) Polymerase Chain Reaction (PCR) analysis. The introduced gene could not be detected in faeces from human volunteers with intact digestive tracts following the consumption of a meal containing GM soybean, indicating that the introduced gene is normally completely degraded in the large intestine.

272. Introduced genetic materials acquired by bacteria are unlikely to be of significance unless they are expressed or alter the expression of host genes. There are barriers to the expression of extraneous genes in bacteria. For example:

- many plant promoters will not be active in bacteria;
- processing of the intermediate RNA may be required for protein expression (e.g. removal of introns to generate functional mRNA for translation), which will not occur in bacteria;
- coding sequences of plant genes may not be efficiently translated in bacteria due to differences in codon usage; and
- processing of an encoded 'pro-protein' may be required for production of a functional product.

273. Prokaryotes have efficient genomes and generally do not contain extraneous DNA sequences. If the genes are not useful to the organism then there will be no selective advantage in maintaining them in the genome, and they are not likely to persist. Thus the risk of gene transfer leading to harmful consequences is extremely low, and greatly exceeded by the likelihood of transfer of these genes and regulatory sequences from sources other than the GM cottons (see Section 2.3 of this Appendix).

274. The Scientific Panel on genetically modified organisms of the European Food Safety Authority (EFSA 2004) recently evaluated the risks associated with the use of antibiotic resistance marker genes in GM plants, and concluded that the frequency of horizontal gene transfer from GM plants to microorganisms is very low.

2.4.2 Viruses

275. As mentioned in Section 2.2.3 of this Appendix, there is a possibility that introduced viral sequences could recombine with the genome of another virus infecting GM plants to create a novel virus. This is most likely to occur via homologous recombination, which relies on a high level of sequence similarity. Homologous recombination occurs naturally within viral populations, and results in hybrids with essentially the same properties as the parental virus (Candresse 1997; Fraile et al. 1997; Padidam et al. 1999).

276. Homologous recombination between introduced viral genes and infecting viruses has been observed in GM plants, although at low frequencies and under conditions of selective pressure (Borja et al. 1999; Frischmuth & Stanley 1998; Greene & Allison 1996; Schoelz & Wintermantel 1993; Greene & Allison 1994; Adair & Kearney 2000; Gal et al. 2002). These cases involved restoration of an infective virus through complementation of a defective virus by viral sequences introduced into a GM plant genome. Interactions between introduced viral sequences in GM plants and infecting viruses, and an assessment of the likelihood of hazards to human health and the environment occurring from these interactions, are discussed in detail in the RARMP for licence application DIR 047/2003.

277. The GM cottons proposed for release only contain a portion of the CaMV genome, the *35S* promoter, which by itself is not pathogenic and operates in the same manner as do endogenous plant promoters. The *35S* promoter, and the introduced genes linked to it in the GM cottons, will not offer any selective advantage to a virus if they are transferred.

278. Thus the likelihood of gene transfer leading to harmful consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.3 of this Appendix).

2.4.3 Fungi

279. 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 B resistance 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).

280. Thus the risk of gene transfer leading to harmful consequences is extremely low, and greatly exceeded by the likelihood of transfer from other sources of these genes and regulatory sequences (see Section 2.3 of this Appendix).

SECTION 3 GENE TRANSFER FROM THE GM COTTONS TO ANIMALS, INCLUDING HUMANS

Section 3.1 Nature of the gene transfer hazard

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

Section 3.2 Potential hazards from the introduced genetic materials

3.2.1 The *uidA* (reporter) gene

282. Animal cells could express the GUS enzyme. This is not likely to lead to any significant effects, since GUS naturally occurs in animals and their intestinal fauna (Gilissen et al. 1998).

3.2.2 The *nptII and hph* (antibiotic resistance) genes

283. Animal, including human, cells could gain the ability to degrade the corresponding antibiotics. If the transfer occurred, such humans or other animals may not respond to treatment

with these antibiotics. However the gene products, the NPTII and HPH enzymes, would only be active within the transformed animal cell, where appropriate conditions and co-factors for activity exist, therefore interference with any antibiotic treatment is unlikely.

284. Kanamycin and neomycin are no longer used to any significant extent as medical, veterinary, agricultural or aquaculture treatments, because they have severe side effects and many bacteria are already resistant to them (Flavell et al. 1992) so alternative antibiotics are used. Hygromycin B is used in veterinary medicine for the treatment of swine and poultry, as an anti-worming agent (Karenlampi 1996; EFSA 2004) but its use is limited and declining. There are no current registered uses of hygromycin B listed with the Australian Pesticides and Veterinary Medicines Authority (APVMA). Animals are not controlled by antibiotics, so no selective advantage would result.

3.2.3 Promoters and other regulatory sequences

285. If these sequences were to be transferred to animals without the associated introduced 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.

286. Some of the introduced regulatory sequences are derived from plants (*G. hirsutum* (cultivated cotton) and *R. communis* (castor bean)) and others are derived from plant pathogens (CaMV and *A. tumefaciens*). However the regulatory sequences from CaMV and *A. tumefaciens* are not pathogenic by themselves.

287. Ho and colleagues (Ho et al. 2000a; Cummins et al. 2000; Ho et al. 2000b) have postulated that there are hazards to human health posed through use of the *35S* promoter from CaMV in GM plants. These hazards include (i) integration of the *35S* promoter into the genomes of species, including humans, resulting in mutations, cancer or reactivation of dormant viruses and (ii) the creation of a new human pathogen. Section 3.3 of this Appendix addresses the likelihood of such hazards occurring.

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

Section 3.3 Likelihood of a hazard arising through gene transfer from the GM cottons to animals (including humans)

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

290. 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 derived from the introduced genetic materials of GM plants in the animal diet is extremely low. For example, it has been estimated that in a diet comprising 40% GM

maize, the introduced genes would represent 0.00042% of total dietary DNA intake (Beever & Kemp 2000).

291. The fate of DNA in the digestive tract of various animals has been studied and is discussed in detail in the risk assessments for licence applications DIR 020/2002, DIR 021/2002 and DIR 022/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genetic materials in the environment (see Section 2.3 of this Appendix).

292. A recent study reported evidence of low frequency transfer of a small fragment of an introduced gene derived from GM soybean to microorganisms within the small intestine of human ileostomists (see Section 2.4.1 of this Appendix for details). However, further experiments could not detect gene transfer from intestinal microflora to mammalian intestinal epithelial cells (Netherwood et al. 2004).

293. 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 for use in food by Food Standards Australia New Zealand (FSANZ), humans would not be exposed to DNA of these GM cottons via food, excluding the possibility of gene transfer to human cells in the gastrointestinal tract.

294. 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 2002b). Thus, the likelihood of gene transfer from the GM cottons to animals, including humans, is negligible.

295. On the basis of a report on a recombinational hotspot in the *35S* promoter from CaMV (Kohli et al. 1999), the following theoretical pathway has been proposed (Ho et al. 2000a; Cummins et al. 2000; Ho et al. 2000b): (i) the *35S* promoter is unstable → (ii) high recombination frequencies → (iii) horizontal transfer to the human genome → (iv) overexpression of human genes → (v) cancer, activation of dormant viruses, toxic metabolites.

296. However, these claims have been refuted in the scientific literature (Hodgson 2000a; Hull et al. 2000; Hodgson 2000b) as several links in this uncertain chain are scientifically inaccurate, mechanistically doubtful or highly unlikely. For example:

- the reported instability of the *35S* promoter occurred only at the time of integration and was probably a factor of its unusual duplicated structure (Kohli et al. 1999);
- once integrated into the plant genome, the *35S* promoter appears to be no more prone to recombination than other plant gene sequences;
- the chance of the *35S* promoter transferring to a gastrointestinal tract bacterium, then to the human genome, and finally to spread throughout the population, is negligible. While individual gastrointestinal tract cells might take up DNA, it is difficult to envisage DNA uptake by the sex cells necessary to pass any change to offspring;
- the chance of the *35S* promoter randomly inserting into the human genome and causing overexpression of any gene is remote. For example, under optimal experimental conditions, the *35S* promoter was randomly inserted into the gene-dense genome of the plant *Arabidopsis thaliana*, and less than 1 in 1000 mutated lines gave rise to a visible change due to overexpression (Weigel et al. 2000); and

- transfer of the *35S* promoter to humans or other organisms is less likely to induce conditions such as cancer or activation of dormant endogenous viruses through genome instability than natural events such as translocation, movement of retrotransposons, or everyday exposure to mutagenic chemicals or radiation. Furthermore, it is unclear how an introduced sequence in an individual GM plant that is genetically highly unstable is able to produce stable physical forms that survive the screening and selection processes that lead to field trials of the GM plant.

297. Therefore the chance of finding an intact chain that spans the gap from the *35S* promoter in a GM plant to cancer or disease in humans is negligible.

298. Ho and colleagues (Ho et al. 2000a; Cummins et al. 2000; Ho et al. 2000b) also postulate that introduced viral sequences in GM plants can recombine with a virus and create a new human pathogen. Recombination of viruses from different hosts can lead to increased pathogenicity in humans, for example, genes from influenza strains that infect birds or pigs have recombined by natural means to strains that infect humans (Gibbs et al. 2001). However this recombination relies on the exchange of closely related (homologous) genes, and novel traits are only maintained if they are useful to the virus.

299. In theory it is possible for an introduced viral gene to recombine with either a plant or animal virus to create a novel virus that causes disease in humans. Two examples that postulate the historical transfer of plant viruses to other types of organism include geminiviral-like gene sequences in a specialised group of bacteria that are intracellular parasites of plants (phytoplasmas) (Rekab et al. 1999) and an ancestral nanovirus transferred to vertebrates (Gibbs & Weiller 1999).

300. However, this paucity of events, which possibly span many millions of years, indicates its extreme rarity. Furthermore, no human virus appears to have plant viral gene sequences present in its genome. The *35S* promoter present in the GM cottons proposed for release is not pathogenic by itself, and operates in the same manner as do endogenous plant promoters. The *35S* promoter, and the introduced genes linked to it in the GM cottons, will not offer any selective advantage to a virus if they are transferred.

SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS

Section 4.1 Conclusions regarding gene transfer to non-GM cotton and other plants

301. It is considered that risks through gene transfer from the GM cottons to non-GM cotton and other plants are very low to negligible because:

- gene transfer to other cotton crops or volunteer cotton would not pose any risks additional to the very low risks posed by the GM cottons themselves;
- genetic incompatibility prevents gene transfer to native cotton species and other plant species; and
- the proposed field trial is small (0.1 hectares) and of limited duration (two summer growing seasons).

302. Gene transfer to other cultivated, volunteer and naturalised cotton is further limited by the licence conditions, including a requirement to surround the GM cottons with a 20 m ‘pollen trap’ of non-GM cotton (see Chapter 2 and Appendix 5 for details).

Section 4.2 Conclusions regarding gene transfer to microorganisms

303. It is considered that risks through transfer of the introduced genetic materials from the GM cottons to microorganisms are negligible because:

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

Section 4.3 Conclusions regarding gene transfer to animals, including humans

304. It is considered that risks through transfer of the introduced genetic materials from the GM cottons to animals, including humans, are negligible because:

- the introduced genetic materials in the GM cottons are already present in the environment;
- most animals avoid feeding on GM or non-GM cotton plants;
- products from the GM cottons are not intended for animal feed or human food;
- FSANZ approval would be required before the GM materials could be used for human food;
- the probability of interaction, uptake and integration of intact plant genes by other organisms occurring is extremely low, especially if it involves unrelated sequences (non-homologous recombination);
- natural events of horizontal gene flow from plants to distantly related organisms are extremely rare; and
- in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely affected.

APPENDIX 5 LICENCE CONDITIONS

Gene technology regulation in Australia

The Gene Technology Act 2000 (Cth) 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 the 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.

SECTION 1 INTERPRETATION AND DEFINITIONS

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

In this licence:

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

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

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

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

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

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

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

In this licence:

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

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

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

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

'Cotton' means plants of the species *Gossypium hirsutum* L. and *G. barbadense* other than the GMOs and Pollen Trap plants;

'cotton' means plants of the species *Gossypium hirsutum* L. and *G. barbadense*;

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

(a) stalk pulling; or

(b) uprooting by ploughing; or

(c) burning; or

(d) treatment with herbicide; or

(e) hand weeding;

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

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

'GM' means genetically modified;

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

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

'Pollen Trap plant' means cotton from a Pollen Trap planted in compliance with special condition 10 of this licence;

'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 post harvest inspection conditions no longer apply in respect of that place;

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

SECTION 2 GENERAL CONDITIONS

Duration of Licence

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

Holder of Licence

2. The holder of this licence ('the licence holder') is CSIRO.

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, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings. The permitted dealings may only be undertaken for the purpose described at Attachment B.

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 grown in a Location pursuant to 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 this 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

Locations and size of trial

1. The permitted dealings with the GMOs, other than disposal of the GMOs, may only be undertaken during the summer cotton growing seasons in 2004/2005 and 2005/2006 between October 2004 and May 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, when the permitted dealings may be undertaken:

    (a) the Location 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.

Notice of planting

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

5. The notice must set out:

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

    (b) details of the Location where the GMOs are planted, including a street address and GPS coordinates for 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 GMOs are likely to be harvested (or Destroyed in lieu of harvest).

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

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.

Pollen Traps

9. A Pollen Trap must surround every Location.

10. A Pollen Trap must contain non-genetically modified plants of the species *Gossypium hirsutum* L. that is grown in such a way as to reasonably promote a dense and vigorous growth and flowering of the non-genetically modified *Gossypium hirsutum* L. at the same time as the GMOs. Pollen Trap plants must be grown in the Pollen Trap while ever the GMOs are being grown at the Location within it.

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

12. Once planted, Pollen Trap plants and Plant Material must be handled and controlled as if they are GMOs (ie. Pollen Trap plants and Plant Material are GMOs for the purposes of this licence and subject to other applicable conditions elsewhere in this licence).

13. 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.

Seed and other Plant Material may be collected

14. Parts of GMOs (including leaf tissue, flower buds, roots and stems) may be collected from a Location for the purpose of conducting experiments on it.

15. Parts of GMOs (including leaf tissue, flower buds, roots and stems) that are 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).

16. Parts of GMOs (including leaf tissue, flower buds, roots and stems) that are 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. Parts of GMOs stored in the facility must be stored in a sealed container.

17. After any experiments with the GMOs or Parts of GMOs (including leaf tissue, flower buds, roots and stems) are completed, the GMOs, or Parts of GMOs, must be Destroyed.

Crops of the GMOs must be either harvested or Destroyed

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

19. If the GMOs are harvested, they must be harvested separately from any other Cotton.

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

20. After the GMOs are harvested or Destroyed at a Location, the Location and the Pollen Trap around it must be Cleaned.

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

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

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

23. 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.

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

25. 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.)

26. 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

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

    (a) the Location;

    (b) the Pollen Trap;

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

    (d) any areas used to Clean Equipment.

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

29. 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.

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

31. Unless this licence provides otherwise, a place must be inspected at least once every 60 days until the Regulator has issued a Sign-off.

32. 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.

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

Restrictions during and after the GMOs are grown

34. Subject to condition 41 below, if the GMOs are grown at a Location, no Cotton may be grown in the Location or its Pollen Trap and that Location cannot be used in the following growing season to grow the GMOs.

35. Subject to conditions 40 and 41 below, if the GMOs are grown at a Location, no other plants may be planted at the Location and only Pollen Trap plants may be planted in the Pollen Trap.

36. Subject to condition 41 below, after the GMOs have been grown at a Location, only the following plants may be grown at the Location and its Pollen Trap:

    (a) grasses (grass pastures);

    (b) cereals (cereal crops);

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

37. Conditions 38, 39 and 40 above, do not apply in respect of a place if the licence holder has received a Sign-off in respect of that place.

Transportation of the GMOs, Pollen Trap plants and Plant Material

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

39. 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.

40. Harvested 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).

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

Contingency Plans

42. 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.

43. 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 Material; and

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

44. 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

45. 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

46. 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.

Testing methodology

47. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence (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

48. 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 6 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

305. 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).

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

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

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

309. 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.

310. 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

311. 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.

312. The application must also contain:

- additional information required for a GMO that is:
  - a plant;
  - a microorganism (not living in or on animals and not a live vaccine);
  - a microorganism 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.

313. 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.

314. 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

315. 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.

316. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people or to the environment, the Regulator must publish a notice (in national and

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

317. 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

318. 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.

319. 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.

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

- the risks posed to human health and safety or risks to the environment;
- the properties of the organism to which the dealings relate before it became a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred on the properties of the organism;
- provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
- the potential for spread or persistence of the GMO or its genetic material in the environment;
- the extent or scale of the proposed dealings; and
- any likely impacts of the proposed dealings on the health and safety of people.

321. 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

322. 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.

323. 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.

324. 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.

325. 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

326. 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.

327. 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 e.g.:

- any relevant convictions;
- any relevant revocations or suspensions of a licence or permit; and
- the capacity of the person or company to meet the conditions of the licence.

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

329. 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.

330. 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.

331. 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.

332. 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 7 SUMMARY OF PUBLIC SUBMISSIONS

Submission from: A: agricultural/industry organisation

Issues raised/consideration: **App:** appendix; **H:** human health and safety; **Res:** research.

Sub. No.	Type	Summary of issues raised	Issue	Consideration of issue
1	A	It is imperative that Australia maintains a research effort when it relates to efficacy and efficiency of introduced genes by more efficient promoters, particularly when they are derived from more widely common sources.	Res	Noted
		This will ultimately reduce the quantity of material introduced into local lines and reduce backcrossing needed to maintain current high quality traits of local cultivars.	None	Noted
		Using promoters that are in higher abundance in our environs should also minimise further any risks to human health.	H	App 2
		CSIRO and the Department of Agriculture at the intended release site have good demonstrated records of compliance with statutory requirements of such releases.	None	Noted
		Strongly supports the proposed field trial of the GM cottons.	None	Noted

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