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

Risk Assessment and Risk Management Plan for **DIR 101**

Limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance

Applicant: Monsanto Australia Limited

July 2010

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application DIR 101 from Monsanto Australia Ltd (Monsanto). The licence authorises dealings involving the limited and controlled release of two genetically modified (GM) cottons into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the requirements of the legislation. RARMP's apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public[1].

The application

Monsanto has applied for a licence for dealings involving the intentional release of two GM cottons (Bollgard III and Bollgard III/Roundup Ready Flex®) on a limited scale and under controlled conditions. The GM cottons have been genetically modified for insect resistance either alone or in combination with herbicide tolerance. The trial is proposed to take place at up to 50 sites per year in up to 34 New South Wales (NSW),Queensland (Qld) and Western Australian (WA) LGAs. The trial may occur on a maximum total area of 1150 ha, between October 2010 and October 2014.

The GM cottons are produced by conventionally crossing GM VIP3A insect resistant cotton with the commercially released GM cottons Bollgard II® cotton (insect resistant) and Bollgard II®/Roundup Ready Flex® cotton (insect resistant/herbicide tolerant). In addition to the insect resistance gene and herbicide tolerance gene, the GM cottons contain marker genes, including antibiotic resistance genes that were used to identify transformed plants during initial development of the GM plants in the laboratory. All of the introduced genes were originally derived from common bacteria.

The purpose of the trial is to generate data for future submissions to regulatory agencies, to breed and develop varieties using elite germplasm suitable for use under Australian conditions, and for seed increase. Material from the GM cotton will not be used in human food or animal feed during the release.

Monsanto proposed a number of controls to restrict the spread and persistence of the GM cottons and the introduced genetic materials in the environment that were considered during the evaluation of the application.

[1] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), previous approvals and relevant scientific/technical knowledge. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP has also been considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated. This included consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM cottons; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify risks that required further assessment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM cottons into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

The licence conditions require Monsanto to **limit** the release to a total area of 1150 ha between October 2010 and October 2014. Each year, no more than 50 sites are permitted for planting. The **control** measures include containment provisions at the trial sites, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with

Regulator's transportation guidelines; and conducting post-harvest monitoring at all trial sites to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of the two GM cottons (Bollgard III and Bollgard III/Roundup Ready Flex®) on a maximum total cumulative area of 1150 ha in up to 34 LGAs in Qld, NSW and WA between October 2010 and October 2014, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Abbreviations

the Act	*Gene Technology Act 2000*
aad	3"(9)-O-aminoglycoside adenyltransferase
Act2	Actin2
aph4	hygromycin B phosphotransferase
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Cry	Crystal protein
CSD	Cotton Seed Distributors
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTP	Chloroplast transit peptide
DIR	Dealings Involving intentional Release
DNA	Deoxyribonucleic Acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FMV	Figwort mosaic virus
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
GUS	β-glucuronidase protein
ha	Hectare
HGT	Horizontal gene transfer
LGA	Local government area
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nptII	Neomycin phosphotransferase II
OGTR	Office of the Gene Technology Regulator
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RMP	Resistance management plan
TGA	Therapeutic Goods Administration
Ubi3	Ubiquitin3
uidA	*β-glucuronidase* gene
Vip	Vegetative insecticidal protein

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 101 from Monsanto Australia Limited (Monsanto). The licence authorises dealings involving the limited and controlled release of cotton genetically modified (GM) for insect resistance and herbicide tolerance.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the Risk Analysis Framework and are finalised following consultation with a wide range of experts, agencies and authorities, and the public[2].

The application

Monsanto has applied for a licence for dealings involving the intentional release of two GM cottons (Bollgard III and Bollgard III/Roundup Ready Flex®) on a limited scale and under controlled conditions. The GM cottons have been genetically modified for insect resistance either alone or in combination with herbicide tolerance. The trial may take place at up to 50 sites per year in up to 34 LGAs in Qld, NSW and WA, on a maximum total area of 1150 ha, between October 2010 and October 2014.

The applicant intends to release two GM cottons. The first, to be known as Bollgard III cotton, contains three insect resistance genes. It is generated by conventionally crossing the commercially released GM Bollgard II® cotton (containing the insect resistance genes *cry1Ac* and *cry2Ab*) with another GM cotton, VIP3A cotton (containing the insect resistance gene *vip3A*). In the second GM cotton, insect resistance is combined with herbicide tolerance by conventionally crossing the GM VIP3A cotton with commercially released GM Bollgard II®/Roundup Ready Flex® cotton (containing the insect resistance genes *cry1Ac* and *cry2Ab* as well as two copies of the herbicide tolerance gene *cp4 esps*). The second GM cotton is to be known as Bollgard III/Roundup Ready Flex® cotton.

In addition to the genes conferring insect resistance and herbicide tolerance, the GM cottons contain antibiotic resistance genes and a reporter gene. The GM cottons proposed for release contain the antibiotic resistance selectable marker genes neomycin phosphotransferase II (*nptII*), 3"(9)-O-aminoglycoside adenyltransferase (*aad*) and hygromycin B phosphotransferase (*aph4*). These genes were originally derived from the common gut bacterium *Escherichia coli*. The *nptII* gene confers resistance to antibiotics such as kanamycin and geneticin, and the *aph4* gene confers resistance to the antibiotic hygromycin. These genes were used only as selective markers during early stages of development of the GM plants in the laboratory. The *aad* gene, which confers resistance to the antibiotics

[2] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

spectinomycin and streptomycin, is linked to a bacterial promoter that does not function in plants so the gene is not expected to be expressed in the GM cotton plants. The GM cottons also contain the β-glucuronidase (*uidA*) gene from *E. coli*, which encodes an enzyme enabling visual identification of plant tissues in which this gene is being expressed.

In addition, the GM cottons contain short regulatory elements used to control expression of the genes. These sequences are derived from plants (including thale cress, pea, petunia and soybean), a soil bacterium (*Agrobacterium tumefaciens*) and plant viruses (Cauliflower mosaic virus and Figwort mosaic virus).

The purpose of the trial is to generate data for future submissions to regulatory agencies, to breed and develop varieties using elite germplasm suitable for use under Australian conditions, and for seed increase. Material from the GM cotton will not be used in human food or animal feed.

Monsanto proposed a number of controls to restrict the spread and persistence of the GM cottons and the introduced genetic materials in the environment that were considered during the evaluation of the application.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), previous approvals and relevant scientific/technical knowledge. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP has also been considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

A reference document, *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)*, was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au>.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated. This included consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM cottons; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify risks that required further assessment. The principal reasons for this include:

- limits on the size, locations and duration of the release proposed by Monsanto
- suitability of controls proposed by Monsanto to restrict the spread and persistence of the GM cotton plants and their genetic material

- widespread presence of the same genes or sequences in the environment
- toxicity of the proteins encoded by the introduced insect resistance genes is expected to be limited to certain insects in the order Lepidoptera
- the GMOs are produced by conventional breeding of other GM cottons which have previously been assessed as posing negligible risks
- limited ability and opportunity for the GM cotton plants to transfer the introduced genes to commercial cotton crops or other cotton plants
- none of the GM plant materials or products would be used in human food or animal feed as part of the release.

Risks to the health and safety of people, or the environment, from the proposed release of the GM cotton into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through proposed licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

Licence conditions

The Regulator has imposed a number of licence conditions, including requirements to:

- limit the release to a cumulative total area of 1150 ha between October 2010 and October 2014 in up to 34 LGAs in Qld, NSW and WA
- locate the field trial sites at least 50 m away from natural waterways
- restrict gene flow via pollen from field trial sites by surrounding the trial site with:
  - a 100 m monitoring zone and a 3 km isolation distance between the site and other cotton crops, or
  - a 20 m pollen trap of non-GM cotton or commercially approved GM cotton
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering
- restrict gene flow via pollen from the glasshouses by:
  - implementing an insect control program within the glasshouses, or
  - maintaining a distance of at least 3 km from the nearest cotton crop
- harvest and gin all cotton from the trial separately from other cotton

- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for research or further planting
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- after cleaning of sites, monitor for and destroy any GM cotton that may grow for at least 12 months, and until no volunteers are observed for a continuous 6 month period
- transport the GM plant materials in accordance with Regulator's transportation guidelines, unless otherwise specified for particular circumstances
- restrict access to the trial sites to authorised personnel only
- not permit the use of GM plant material or products to be used for human food or animal feed.

The sale of lint is permitted.

Other regulatory considerations

Australia's gene technology regulatory system operates as an integrated legislative framework involving the Regulator and other regulatory agencies that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)[3].

APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cottons proposed for release meet the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to their production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. The applicant intends to apply herbicide to the GM cottons during the trial, which is also subject to regulation by the APVMA.

FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from the GM parent cottons, ie Bollgard II®, Roundup Ready Flex® and VIP3 GM cottons. However, the applicant does not intend to use materials from the GM cottons generated in the proposed release in human food.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM cottons, or to justify a reduction in containment conditions. This would include:

- additional data on the potential toxicity of Vip3A in combination with the proteins encoded by the other introduced insect resistance genes to non-target invertebrates

[3] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR).
Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

- phenotypic characterisation of the GM cottons, in particular of traits which may contribute to weediness, persistence, and ability to disperse in the environment
- data on the effects on non-target insects and weediness potential of Bollgard III or Bollgard III/Roundup Ready Flex® combined with insect resistant WideStrike™ cotton.

Suitability of the applicant

The Regulator is satisfied that Monsanto is suitable to hold a DIR licence as no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has the capacity to meet the conditions of the licence.

Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of the two GM cottons (Bollgard III and Bollgard III/Roundup Ready Flex®) on a maximum total cumulative area of 1150 ha in up to 34 LGAs in Qld, NSW and WA between October 2010 and October 2014, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed (Figure 1).

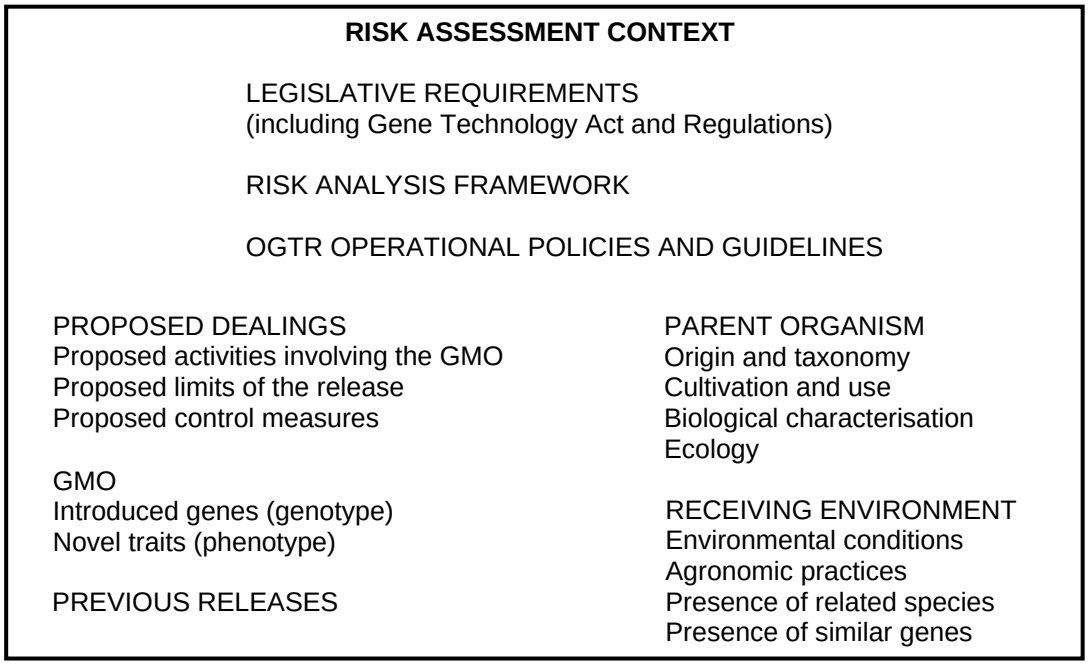

Figure 1. Parameters used to establish the risk assessment context

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* (the Act) and Gene Technology Regulations 2001 (the Regulations, Section 2), the *Risk Analysis Framework*, and operational policies and guidelines <http://www.ogtr.gov.au>.

3. In addition, establishing the risk assessment context for this application includes consideration of:

- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the GMOs and the nature and effect of the genetic modification (Section 6)
- the receiving environment (Section 7)
- previous releases of these or other GMOs relevant to this application (Section 8).

Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size,

locations and duration of the release and controls have been proposed by the applicant to restrict the spread and persistence of the GMO and its genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). The decision is provided in Section 3 of Chapter 2.

Section 3 The proposed dealings

8. Monsanto Australia Limited (Monsanto) proposes to release two GM cottons which have been genetically modified (GM) for insect resistance and herbicide tolerance into the environment under limited and controlled conditions.

9. The dealings involved in the proposed intentional release would include:

- conducting experiments with the GMOs
- breeding the GMOs
- propagating, growing, raising or culturing the GMOs
- transporting the GMOs
- disposing of the GMOs
- possession, supply or use of the GMOs for the purposes of any of the above.

These dealings are detailed further throughout the remainder of the current Chapter.

3.1 The proposed activities

10. Monsanto states that the aim of the proposed release is to develop and assess two GM cottons, to be known as Bollgard III and Bollgard III/Roundup Ready Flex® cotton. These will be generated by conventionally crossing commercially released GM Bollgard II® (insect resistant) and Bollgard II®/Roundup Ready Flex® (insect resistant/herbicide tolerant) cotton with another insect resistant GM cotton, VIP3A[4] cotton, for use in Australia. GM VIP3A cotton has previously been released in Australia in limited and controlled field trials (see section 8.1 for details). The applicant proposes to use non-PC2 glasshouses and field testing in the process of developing the GM cottons.

11. The proposed four year trial program is comprised of:

- trials for generating data for future submissions to regulatory agencies

[4] VIP3A cotton is also known as Vip and COT102 cotton by other agencies/in other jurisdictions.

- breeding and variety development trials using elite germplasm suitable for use under Australian conditions
- seed increase plantings.

12. The proposed activities would be overseen by Monsanto, with aspects conducted by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Cotton Seed Distributors (CSD).

13. Subject to approval by the Regulator, the applicant proposes to sell lint harvested from the GM Bollgard III and Bollgard III/Roundup Ready Flex® cotton seed.

3.2 The proposed limits of the dealings (size, locations and duration)

3.2.1 The proposed limits of the dealings in the field

14. The applicant proposes to limit the release to 50 sites per year between October 2010 and October 2014, including summer plantings in current cotton growing regions of Queensland and New South Wales, and winter plantings in prospective cotton growing areas in north west of Western Australia. The maximum combined area of plantings would be 50 ha in the first year, 100 ha in the second year, and 500 ha per year in the third and fourth years. The applicant proposes that the area planted to the GM cottons would not exceed 100 ha per individual planting.

15. Monsanto proposes to conduct field plantings in all current and two prospective cotton growing areas, within the local government areas (LGAs) listed in Table 1.

Table 1 Local government areas proposed for field planting of the GMOs proposed for release

Queensland	New South Wales	Western Australia
Balonne Shire	Balranald Shire	Shire of Broome
Banana Shire	Bogan Shire	Shire of Wyndham-East Kimberley
Burdekin Shire	Bourke Shire	
Central Highlands Regional	Carrathool Shire	
Western Downs Regional	Central Darling Shire	
Goondiwindi Regional	Coonamble Shire	
Isaac Regional	Gunnedah Shire	
Lockyer Valley Regional	Gwydir Shire	
Paroo Shire	Hay Shire	
Roma Regional	Inverell Shire	
Somerset Regional	Lachlan Shire	
South Burnett Regional	Liverpool Plains Shire	
Toowoomba Regional	Moree Plains Shire	
	Narrabri Shire	
	Narromine Shire	
	Walgett Shire	
	Warren Shire	
	Warrumbungle Shire	

16. Only trained and authorised staff would be permitted access to the proposed field locations.

3.2.2 The proposed limits of the dealings in glasshouses

17. The applicant proposes to grow the GMOs in two glasshouses in the Qld LGAs of Toowoomba Regional and Brisbane City, for the duration of the release. These glasshouses are not certified by the Regulator as physical containment level 2 facilities. Therefore, dealings conducted within the glasshouses are considered dealings involving intentional release. The glasshouses would be surrounded by a fence with locked gates to limit access to trained and authorised staff.

3.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment

18. The applicant has proposed a number of controls to restrict the spread and persistence of the GM cottons and the introduced genetic material in the environment, including:

- locate the field trial sites at least 50 m away from natural waterways
- restrict gene flow via pollen using one of the following measures:
    - surround the trial site with a 100 m monitoring zone and maintain a 3 km isolation distance between the site and intentionally planted cotton crops, or
    - surround the trial site by a 20 m pollen trap of non-GM (conventional) cotton or commercially approved GM cotton leaving a 2.5 m wide path unplanted for access to the GMOs
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering
- ensure the pollen trap plants are grown in such a way as to ensure flowering at the same time and for the same period of time as the GM cotton
- locate the glasshouses at least 20 km from the nearest cotton crop
- implement an insect control program within the glasshouses
- harvest and gin all cotton plant materials (GM and non-GM) separately from other cotton
- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for future research or release
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- after cleaning of sites, monitor for and destroy any GM cotton that may grow for at least 12 months, and until no volunteers are observed for a continuous 6 month period
- transport and store the GM plant materials in accordance with Regulator's guidelines
- restrict access to the site to authorised personnel only
- not permit the use of GM plant material or products to be used for human food or to be fed to animals as part of this release.

19. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this Chapter). Their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.2.1.

Section 4 The parent organism

20. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia but is grown as an agricultural crop in NSW and southern and central Qld. Further detailed information about the parent organism is contained in a reference document, *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008) that was produced to inform the risk assessment process for licence applications involving GM cotton plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au>.

21. Bollgard III and Bollgard III/Roundup Ready Flex® have been bred into cotton varieties suitable for Australian conditions.

Section 5 The GM parental cottons

22. The GMOs proposed for release are the result of conventional crossing different GM cottons: three GM cottons are used as parents for breeding the two GMOs proposed for release. They are Bollgard II®, Bollgard II®/Roundup Ready Flex® and VIP3A cotton. In addition to information on non-GM cotton, information on these GM cottons will be used as baselines against which the new GM cottons will be compared throughout the RARMP. The genetic modifications of the GM parental cottons and other information relevant to the risk assessment are described in Section 6.

Section 6 The GMOs, nature and effect of the genetic modification

6.1 Introduction to the GMOs

23. The applicant proposes to release two GM cottons. The first, to be known as Bollgard III cotton, will contain three insect resistance genes. It will be generated by conventionally crossing the commercially released insect resistant GM Bollgard II® cotton with another insect resistant GM cotton, VIP3A cotton. The second GM cotton is to be known as Bollgard III/Roundup Ready Flex® cotton. In this GM cotton, insect resistance will be combined with herbicide tolerance by conventionally crossing the GM VIP3A cotton with commercially released GM Bollgard II®/Roundup Ready Flex® cotton and so it will be insect resistant and tolerant to the herbicide glyphosate. In addition to the genes conferring insect resistance and herbicide tolerance, the GM cottons contain introduced antibiotic resistance genes, a reporter gene and regulatory elements (Table 2 and Table 3). Monsanto proposes to conduct breeding during the course of the release, and as a result all possible combinations of the four genetic modifications present in the parental GM cottons could occur in GM cotton plants (Bollgard II® cotton contains two genetic modifications, see Table 2).

Table 2 Genetic modifications present in the GM parental cottons from which the GMOs proposed for release will be generated

GM cotton	Plasmid name	Promoter	Gene	Termi-nator	Additional genetic elements	Function
VIP3A (COT102)	pCOT1	actin2 promoter	*vip3A*	nos	First exon and intron of actin-2	insect resistance
		ubiquitin3 promoter	*aph4*	nos	ubi3 intron	antibiotic resistance
Bollgard II® (MON15985) [1,3]	PV-GHBK04[2]	35S	*cry1Ac*	7S 3'		insect resistance
		35S	*nptII*	nos		antibiotic resistance
		Tn7	*aad*	none		antibiotic resistance
	PF-GHBK11	35S	*cry2Ab*	nos	PetHSP70, Ctp2	insect resistance
		35S	*uidA*	nos		reporter
Roundup Ready Flex® [1] (MON 88913)	PV-GHGT35	P-FMV/ TSF2	*cp4 epsps*	rbcS-E9	Ctp2	herbicide tolerance
		P-35S/ ACT8	*cp4 epsps*	rbcS-E9	Ctp2	herbicide tolerance

[1] These cottons have been approved for commercial release, individually and in combination, under licence DIR 066/2006.

[2] The cotton genetically modified using this plasmid was approved for commercial release as INGARD® cotton (MON531).

[3] Bollgard II® cotton (MON15985) was produced by transforming the GM cotton known as INGARD® cotton (MON531 containing the *cry1Ac, nptII* and *aad* genes) with a second construct (containing the *cry2Ab* and *uidA* genes).

Table 3 Detail of genes and regulatory elements present in the GM cottons

Genetic element	Full name	Source organism / further description
Gene sequences		
vip3A	vegetative insecticidal protein 3A	Modified synthetic version of v*ip3Aa1* gene from *Bacillus thuringiensis* strain AB88
aph4	hygromycin B phosphotransferase	*Escherichia coli* strain K-12
cry1Ac	crystal protein 1Ac	Modified synthetic fusion protein with amino acids 1-466 from *cry1Ab* and amino acids 467-1178 from *cry1Ac*, both from *B. thuringiensis* subsp. *kurstaki*
nptII	neomycin phosphotransferase type II	*E. coli* Tn5 transposon
aad	3"(9)-O-aminoglycoside adenyltransferase	*E. coli*. This gene is under the control of its native (bacterial) promoter. Therefore, the gene will not be expressed in the GM cottons.
cry2Ab	crystal protein 2Ab2	Modified synthetic version of *cry2Ab2* gene from *B. thuringiensis* subsp. *kurstaki*
uidA	beta-glucuronidase	*E. coli*
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase	*Agrobacterium* sp. strain CP4
Promoters		
Act2	*actin2* gene promoter, including untranslated leader sequence with the first exon and intron of the *actin2* gene.	*Arabidopsis thaliana* (thale cress)
Ubi3	*ubiquitin3* gene promoter	*A. thaliana*
35S	CaMV 35S promoter	Cauliflower mosaic virus (CaMV), a pararetrovirus that infects a wide range of cruciferous plant species.
Tn7	Transposon 7 promoter	*E. coli*
P-FMV/TSF2	EF1alpha promoter (including non-translated leader with intron sequences) with FMV 35S enhancer sequence	*A. thaliana* , Figwort mosaic virus (FMV)
P-35S/ACT8	*actin8* promoter (including non-translated leader with intron sequences) with CaMV 35S enhancer sequence	*A. thaliana*, CaMV
Terminators		
nos	3' non-translated sequence of the nopaline synthase gene	*Agrobacterium tumefaciens*
7S 3'	3' non-translated sequence of the beta-conglycinin alpha-subunit gene	*Glycine max* (soybean)
rbcS-E9	3' non-translated sequence of the ribulose-1,5-bisphosphate carboxylase small subunit E9 gene	*Pisum sativum* (pea)
Other elements		
PetHSP70	5' untranslated leader of the heat shock protein 70 gene	*Petunia* x *hybrida* (petunia)
Ctp2	Chloroplast targeting peptide from the *epsps* gene	*A. thaliana* (thale cress)

24. VIP3A cotton contains a synthetic copy of the *vip3Aa1* gene from the bacterium *Bacillus thuringiensis* strain AB88, which confers resistance to various lepidopteran insects including significant pests of cotton.

25. Bollgard II® cotton contains the *cry1Ac* and *cry2Ab* genes derived from *B. thuringiensis* subsp. *kurstaki.* The *cry1Ac* and *cry2Ab* genes encode insecticidal proteins which are specifically toxic to caterpillar larvae of certain species of lepidopteran insects including significant pests of cotton.

26. Roundup Ready Flex® cotton contains two copies of the *5-enolpyruvylshikimate-3-phosphate synthase* (*cp4 epsps*) gene from the soil bacterium *Agrobacterium* sp. strain CP4 (Barry et al. 1992). Unlike plant EPSPS enzymes, the CP4 EPSPS enzyme can function in the presence of glyphosate, the active constituent of a number of herbicides including Roundup Ready® herbicide. Expression of *cp4 epsps* in Roundup Ready Flex® GM cotton confers tolerance to glyphosate (Barry et al. 1992).

27. The GM cottons proposed for release contain the antibiotic resistance selectable marker genes *neomycin phosphotransferase II* (*nptII*), *3"(9)-O-aminoglycoside adenyltransferase* (*aad*) and *hygromycin B phosphotransferase* (*aph4*). These genes were originally derived from the common gut bacterium *E. coli*. The *nptII* gene confers resistance to antibiotics such as kanamycin and geneticin, and the *aph4* gene confers resistance to the antibiotic hygromycin. These genes were used only as selective markers during early stages of development of the GM plants in the laboratory. The *aad* gene, which confers resistance to the antibiotics spectinomycin and streptomycin, is linked to a bacterial promoter that does not function in plants so the gene is not expected to be expressed in the GM cotton plants. The GM cottons also contain the *β-glucuronidase* (*uidA*) gene from *E. coli*, which encodes an enzyme enabling visual identification of plant tissues in which this gene is being expressed.

28. In addition, the GM cottons contain short regulatory elements used to control expression of the genes (Table 2 and Table 3). These sequences are derived from plants (including thale cress, pea, petunia and soybean), a soil bacterium (*A. tumefaciens*) and plant viruses (CaMV and FMV).

29. The VIP3A GM cotton has been described and assessed in the RARMPs prepared for previous limited and controlled DIR licence applications (refer to DIRs 017/2002, 025/2002, 034/2003, 036/2003, 058/2005, 065/2006 and 073/2007[5]). Bollgard II® and Roundup Ready Flex® GM cottons have previously been described and assessed for commercial release (refer to RARMPs for DIRs 012/2002, 059/2005 and 066/2006), in addition to other applications for limited and controlled release. Assessments of Bollgard II® and Roundup Ready Flex® GM cottons in the context of commercial release throughout Australia concluded that they pose negligible risks to human health and safety and the environment (refer to RARMPs for DIR 059/2005 and DIR 066/2006). Consequently, the focus of the current assessment is VIP3A cotton, individually and in combination with the other genetic modifications.

6.2 The introduced genes, their encoded proteins and their associated effects

6.2.1 The vip3A *gene and the encoded protein*

30. The *vip3A* gene is a modified synthetic copy of a *vip3A* gene from the bacterium *Bacillus thuringiensis* (Bt) strain AB88, which was isolated from sour milk (Estruch et al. 1996). Bt produces a range of insecticidal proteins, including the crystal (Cry) proteins (also

5 Previous RARMPs are available at <http://www.ogtr.gov.au> or by contacting the OGTR.

known as δ-endotoxins) and vegetative insecticidal proteins (Vips). Vips are secreted by various *Bacillus* species during vegetative growth stages and sporulation, whereas the Cry proteins are expressed by Bt only during sporulation and form crystalline inclusions in spores (reviewed by Estruch et al. 1997).

31. Vips isolated from Bt and *B. cereus* have shown activity against a range of lepidopteran species and several coleopteran species (Warren 1997), and the activity of Vip3A has been shown to contribute significantly to the toxicity of Bt spores to insects (Donovan et al. 2001). To date 45 *vip3A*-class genes have been cloned (Crickmore et al. 2010), and closely related genes have been detected in approximately 50% of the Bt strains surveyed (Liu et al. 2007; Hernandez-Rodriguez et al. 2009). Hernandez-Rodriguez et al. (2009) surveyed 507 strains of Bt and found that 91.5% of those with *vip3* genes also contained *cry1A* and *cry2* genes, and speculated that these genes are encoded by the same plasmids.

32. The *vip3Aa19* gene (Entrez Accession number DQ539887) was modified to accommodate the preferred codon usage in plants (Murray et al. 1989). In Vip3Aa19, glutamine is present at position 284, whereas Vip3Aa1 has lysine at the same position. The other amino acid residues are identical in both proteins. The applicant states that the amino acid substitution does not appear to have changed the insecticidal activity of the protein. However, comparison of the reported activity of Vip3A (Hill et al. 2003) with VIP, a closely related Vip protein described by Selvapandiyan et al. (2001), raises the possibility that the amino acid substitution in Vip3A could contribute to altered specificity. The related VIP varies by two amino acids from Vip3A (one of which is the lysine to glutamine substitution at residue 284) and has been shown to be toxic to *Plutella xylostella* (diamondback moth), whereas Vip3A is not.

33. The Bt variety *kurstarki* HD1 strain containing a similar *vip3A* gene is used in commercial Bt sprays in Australia (http://www.apvma.gov.au).

6.2.2 Toxicity/allergenicity of the end products associated with the introduced vip3A *gene*

Toxicity and allergenicity of Vip3A to humans

34. The World Health Organisation's International Programme on Chemical Safety evaluated the environmental safety of use of Bt as a pest control agent and concluded that, because of the specificity of the mode of action of Bt toxins, Bt products are unlikely to pose any hazard to humans, other vertebrates, or the great majority of non-target invertebrates (International Programme on Chemical Safety 1999). In this report it was noted that Bt has not been reported to cause adverse effects on human health when present in drinking water or food. Two human studies found no observable health effect of an oral dose of 1000 mg of Bt spores per day for 3 or 5 days (McClintock et al. 1995; reviewed by Betz et al. 2000).

35. Inhalation and ingestion of Bt is not known cause allergic reactions (International Programme on Chemical Safety 1999). There have been rare reports of occupational allergies associated with the use of Bt insecticidal products containing Bt.

36. A formal survey of farm workers who picked or packed vegetables that had been repetitively treated with Bt sprays was undertaken by Bernstein in 1996. Prior to this study only one documented and three other questionable cases of overt human disease associated with Bt pesticide had been reported (Bernstein et al. 1999). Bernstein's survey indicated that exposure to Bt products could lead to allergic skin sensitisation and induction of IgE and IgG antibodies. However there were no reports of occupationally related clinical allergic disease in any of the workers, or of antibodies to the endotoxin proteins of the Bt sprays.

37. The US EPA has since determined that the dermal allergic reactions reported by Bernstein et al. (1999) were not due to Bt itself or any of the Cry toxins. The reported reactions were determined to be due to non-Cry proteins produced during fermentation or to added formulation ingredients (EPA 2001).

38. Searches of the FARRP Allergen Database, performed according to CODEX guidelines (Codex Alimentarius Commission 2003) have shown no matches of the Vip3A protein to known allergens (information supplied by applicant).

39. Food Standards Australia New Zealand (FSANZ) assessed the safety of human food derived from linters and cotton seed oil from VIP3A cotton, and concluded that studies to determine potential toxicity of Vip3A demonstrate that it is non-toxic to mammals (FSANZ 2004). Also, the Vip3A protein has been demonstrated to be heat labile (Estruch et al. 1996), which would lead to a decreased exposure in processed products. The studies assessed by FSANZ are summarised in Hill et al. (2003).

Toxicity to target lepidopteran insects

40. Following ingestion by insects, Vip3Aa1 protein is activated by proteolytic processing in the midgut (Yu et al. 1997; Lee et al. 2003a). In susceptible insect species, activated Vip3Aa1 binds to midgut epithelium cells and the insects show gut paralysis, lysis of midgut epithelium cells and death (Yu et al. 1997). Vip3Aa1 forms channels in midgut epithelium cell membranes (Lee et al. 2003a) which are thought to mediate its effects on the midgut. Binding of Vip3 proteins to the midgut requires the presence of specific receptor proteins on the midgut epithelium surface, and is thought that this is the mechanism by which Vip3 proteins have a high degree of target insect specificity.

41. The mode of action of Vip3A proteins is similar to that of Cry proteins, however Vip3A proteins bind to different insect midgut proteins than Cry proteins (Lee et al. 2006; Sena et al. 2009), and form membrane channels with different biophysical properties (Lee et al. 2003c). The different biochemistries underlying the activity of these toxins correlates with reports that insects resistant to the Cry1Ac protein remain susceptible to Vip3A (Jackson et al. 2007). There is no sequence similarity between Vip3A proteins and Cry proteins (Estruch et al. 1996).

42. The VIP3A GM cotton has been shown in field trials to have activity against a range of lepidopteran insects: *Helicoverpa armigera* (cotton bollworm), *H. punctigera* (native budworm), *H. zea* (corn earworm), *Heliothis virescens* (tobacco budworm), *Spodoptera exigua* (beet armyworm), *Pectinophora gossyipiella* (pink bollworm), *Pseudoplusia includens* (soybean looper), *Trichoplusia ni* (cabbage looper) and *Bucculatrix thurberiela* (cotton leaf perforator) (unpublished studies summarised by Hill et al. 2003; Whitehouse et al. 2007; Llewellyn et al. 2007a). Laboratory assays using various preparations of Vip3Aa1 protein have also shown activity against some of these species (Estruch et al. 1996; Liao et al. 2002) and also *S. frugiperda* (fall armyworm), *Manduca sexta* (tobacco hornworm) and *Agrotis ipsilon* (black cutworm) (Lee et al. 2003a; Cotton Catchment communities CRC 2007).

43. *H. armigera*, *H. punctigera*, *S. exigua* and *P. gossypiella* are cotton pests in Australia. Species related to *Agrotis ipsilon*, *B. thurberiela*, *S. exigua* and *S. frugiperda* are also Australian cotton pests.

Toxicity to non-target organisms

44. Vip3A proteins are not expected to be toxic to organisms which lack the receptors to which Vip3A binds, such as those found on the brush border membrane vesicles in the midguts of some lepidopteran larvae (Lee et al. 2003b). Selvapandiyan et al. (2001)

proposed differential modes of action against different pests, based on results with deletion mutants of their newly described Vip3 protein on a number of Lepidopteran pests. Their Vip3 protein is closely related to the Vip3A protein in VIP3A GM cotton.

45. The applicant has conducted studies to test for toxicity of Vip3A to a range of non-target organisms, which are summarised in Hill (2003), a document submitted to regulatory authorities in the USA. These studies were considered in detail in the RARMP prepared in respect of application DIR 058/2005. In summary, laboratory experiments indicated that Vip3A protein expressed by *E. coli* or GM maize is not toxic to mice, Bobwhite quail, channel catfish, a range of non-lepidopteran insect species (including representative Coleoptera, Hymenoptera, Isotomidae and Neuroptera) and several other invertebrates (including water fleas and earthworms). The exposure levels reached in these studies were estimated to be substantially greater than levels expected in the field, based upon expression levels of Vip3A in VIP3A GM cotton.

46. Whitehouse et al. (2007) studied the effects of VIP3A cotton on the invertebrate community at two Australian field sites, in comparison to non-GM cotton. The authors found that there were no major differences in species richness or diversity of beneficial and non-target communities. Several indirect effects were detected, which were thought to result from factors such as the increased numbers of bolls on VIP3A plants and the reduced abundance of the target *Helicoverpa* spp. larvae (which are prey for several species).

6.2.3 The plant antibiotic resistance marker gene aph4 *and its encoded protein*

47. The *aph4* antibiotic resistance marker gene is present in the GM Bollgard III and Bollgard III/Roundup Ready Flex® cotton proposed for release. The *aph4* gene encodes a hygromycin phosphotransferase enzyme which inactivates the antibiotic hygromycin B. The APH4 protein is in common use as a selectable marker in the production of GM plants (Miki & McHugh 2004), and was used in the initial laboratory stages of development of the GM cotton plants, to enable selection of cells containing the desired genetic modification.

48. APH4 protein purified from *E. coli* has been shown to have very low acute oral toxicity for mice and be rapidly inactivated in simulated mammalian gastric juice (reviewed by FSANZ 2004). The APH4 protein falls within the typical relative molecular weight range of allergenic proteins but shows no sequence homology to known allergens in the SBI Allergen database (reviewed by Vlachos 2002). Searches of the FARRP Allergen Database, performed according to CODEX guidelines (Codex Alimentarius Commission 2003) have shown no matches of the Vip3A protein to known allergens (information supplied by applicant).

6.2.4 The introduced genes in Bollgard II® and Roundup Ready Flex® cottons

49. The introduced genes in Bollgard II® and Roundup Ready Flex® cottons are listed in Table 2 and Table 3. Those have been described in detail in the RARMPs for the commercial release applications DIR 059/2005 and DIR 066/2006 (http://www.ogtr.gov.au), and will not be described further here. As indicated in Chapter 1, Section 6.1, this assessment will focus on the modifications in the VIP3A cotton in combination with those in Bollgard II® and Roundup Ready Flex® cotton, which have not been assessed for commercial release.

6.3 The regulatory sequences

50. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants are mRNA terminators, including a poly-adenylation signal. Other regulatory sequences, such as enhancers, may contribute to the expression pattern of a given gene. The regulatory sequences used in the GM cottons are listed in Table 2 and Table 3.

6.3.1 Regulatory sequences for expression of the vip3A gene

51. The expression of the *vip3A* gene is controlled by the *actin-2* promoter from *A. thaliana*, including the first intron and exon in the 5' untranslated region (An et al. 1996). The *actin-2* promoter is a strong, constitutive promoter that is expected to lead to expression of the Vip3A protein throughout the growing season in most plant tissues.

52. The termination signals for the *vip3A* gene are provided by the mRNA termination region of the *A. tumefaciens* nopaline synthase gene, *nos* (Bevan et al. 1983). *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants.

6.3.2 Regulatory sequences for the expression of the aph4 gene

53. The expression of the *aph4* gene in the VIP3A cotton is controlled by the *ubiquitin 3* (*ubi3*) promoter from *A. thaliana*. This promoter has been shown to lead to constitutive expression in all plant parts (Norris et al. 1993).

54. It also contains the *nos* terminator from *A. tumefaciens* (see above).

6.3.3 Regulatory sequences for the expression of the introduced genes in Bollgard II® and Roundup Ready Flex® cotton

55. The introduced regulatory sequences in Bollgard II® and Roundup Ready Flex® cottons are listed in Table 2 and Table 3. Those have been described in detail in the RARMPs for the commercial release applications DIR 059/2005 and DIR 066/2006 (http://www.ogtr.gov.au), and will not be described further here. As indicated in Chapter 1, Section 6.1, this assessment will focus on the modifications in the VIP3A cotton in combination with those in Bollgard II® and Roundup Ready Flex® cotton, which have not been assessed for commercial release.

6.4 Method of genetic modification

56. The insect resistant GM cotton proposed for release (Bollgard III) has been derived from selected conventional crosses of GM VIP3A cotton with GM Bollgard II® cotton. The insect resistant/herbicide tolerant GM cotton (Bollgard III/Roundup Ready Flex®) will be produced by conventionally crossing GM VIP3A cotton with GM Bollgard II®/Roundup Ready Flex® cotton.

57. The methods by which the parent GM cottons were produced have been described in detail in previous RARMPs (DIR 017/2002 for VIP3A, DIR 012/2002 for Bollgard II® and DIR 035/2003 for Roundup Ready Flex® cotton) and are summarised here.

58. VIP3A and Roundup Ready Flex® GM cottons were generated by *Agrobacterium*-mediated transformation with the disarmed binary vector plasmids described in Table 2. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants (Van Larebeke et al. 1974). Plants can be genetically modified by the transfer of DNA (located between specific border sequences on a plasmid) from *A. tumefaciens*, mediated by genes from the virulence region of tumour-inducing plasmids.

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

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

60. The VIP3A GM cotton was produced using protocols similar to those described by Murray et al (1999), and Roundup Ready Flex® GM cotton was produced using protocols described by Zambryski (1992). The cotton variety used for transformation was Coker 312 for both VIP3A and Roundup Ready Flex® cotton. Transformed cotton cells were selected through their ability to grow in the presence of appropriate selective agents: hygromycin for VIP3A cotton (the *aph4* gene encodes resistance to this antibiotic); and glyphosate for Roundup Ready Flex® cotton (the *cp4 epsps* gene encodes tolerance to this herbicide, for *in-vitro* cell cultures as well as whole plants). GM cotton plants were regenerated from the selected cells.

61. For each of these genetic modifications, GM plants containing one specific transformation event are proposed for release. Each event has been bred into cotton varieties suited to Australian conditions.

62. Bollgard II® cotton was then created by adding new genes (on plasmid PF-GHB K04, see Table 2) into the cotton meristematic cells of INGARD® cotton by microprojectile bombardment (McCabe & Martinell 1993). The *uidA* gene was used as a marker to identify transformed plantlets in tissue culture. INGARD® is itself a GM cotton, which was created using *Agrobacterium*-mediated transformation to genetically modify variety DP50B using protocols as described by Zambryski (1992).

6.5 Characterisation of the GMOs

63. No stability, molecular and phenotypic characterisation data is currently available for Bollgard III or Bollgard III/Roundup Ready Flex® GM cotton, however all parental GM cottons are well characterised:

- The stability, molecular and phenotypic characterisation of Bollgard II® and Roundup Ready Flex® cotton has previously been considered in the RARMPs prepared in respect of applications DIR 012/2002, DIR 035/2003 and DIR 055/2004, DIR 059/2005 and DIR 066/2006. To summarise, these transformation events have been subject to detailed molecular and phenotypic characterisation and shown to be stably inherited. Because they have previously been approved for commercial release, Bollgard II® and Roundup Ready Flex® GM cotton will not be discussed further in this section.
- The stability, molecular and phenotypic characterisation of VIP3A cotton and expression of the *vip3A* gene have been described in the RARMPs prepared in respect of applications DIR 058/2005, DIR 065/2006 and DIR 073/2007, and are summarised below.

6.5.1 Stability and molecular characterisation of VIP3A cotton

64. The applicant has provided data showing Mendelian inheritance of the *vip3A* transgene through five generations of back-crossing and selfing. Southern blot analysis has demonstrated that a single copy of the *vip3A* gene and the *aph4* gene is inserted in VIP3A cotton and no vector backbone sequences are present.

6.5.2 Phenotype of VIP3A cotton

65. The introduced *vip3A* gene in the VIP3A GM cotton has been shown to provide resistance against herbivory by some lepidopteran insects, including two major Australian cotton pests, *H. armigera* and *H. punctigera*. Australian field trials of VIP3A cotton have shown that VIP3A cotton sustains less pest damage than unsprayed non-GM plants, and exhibits an improved retention of fruiting structures compared to non-GM plants

(Whitehouse et al. 2007; Llewellyn et al. 2007a). The GM plants are expected to remain susceptible to attack by other insects such as thrips, mites and aphids, as well as fungal and bacterial pathogens, which occur in Australian cotton growing regions.

66. The applicant stated that under glasshouse and field trial conditions, agronomic characteristics such as fertility and growth rates appear to be the same in the GM cotton compared to the non-GM parent cultivar Coker 312. Field trials indicated that in Narrabri, NSW there was no difference in final height between VIP3A cotton (in cultivar Coker 312) and non-GM cotton plants (cultivar Sicala 40), whereas the VIP3A cotton plants were significantly taller in trials in Kununurra, WA. Boll production was higher for the VIP3A cotton plants in both trials, due to the reduced pest pressure in these plants (Whitehouse et al. 2007).

67. Characterisation of the expression level of Vip3A protein in VIP3A GM cotton showed that Vip3A is present at the highest level in leaves, and is also present in seeds, roots, flower buds, bolls and pollen. Vip3A was not detectable in nectar or cotton fibre (for further detail, see the RARMPs prepared for DIR 058/2005 and DIR 065/2006). The expression levels of Vip3A in field grown plants remained relatively constant throughout the cotton growing season (Llewellyn et al. 2007a).

68. Compositional analysis of VIP3A and (non-GM) Coker 312 cotton seed from plants grown in the US was reviewed by Hill et al. (2003). Measurements for 47 components were made for both VIP3A and Coker 312 cotton seed grown at a number of locations and in two growing seasons. Few significant differences were obtained between VIP3A and Coker 312 cotton seed and these measurements for the components concerned were not found significant over other years and locations.

69. Australian field studies on cotton plants expressing the *vip3A* gene indicate resistance to *Helicoverpa* species (Whitehouse et al. 2007; Llewellyn et al. 2007a). A preliminary field survey conducted as part of DIR 017/2002 indicated that non-lepidopteran insect numbers were not affected by the Vip3A protein. No significant difference in insect abundance was found between the non-GM and VIP3A GM cottons, except that a greater number of mirids were found on VIP3A GM cotton.

70. Field trials were conducted by Whitehouse et al. (2007) to compare the diversity of the arthropod community on unsprayed non-GM (Sicala 40) and VIP3A (Coker 312) cotton. VIP3A cotton appeared to have little effect on the diversity of the arthropod community except on *Helicoverpa* species. There were no major differences in either species richness or diversity of the beneficial and non-target communities between the VIP3A and non-GM cotton, although cotton cultivar accounted for 2-7% of the variance of arthropod communities. There was no detrimental effect of VIP3A cotton on eggs of parasitoids. The number of predatory beetles and the pest mirid (*Creotiades dilutus*) was higher in the VIP3A cotton, although this was probably due to the higher boll counts in this crop. Higher boll counts would mean that more food is available to cotton pests. Whitefly numbers were higher in a small VIP3A plot, but this may have been a result of differences in leaf hair between cultivars. VIP3A cotton controlled *Helicoverpa* larvae leading to higher boll counts.

71. Studies conducted in the USA by Privalle (2002) indicate that Vip3A protein residues that may be incorporated into agricultural soils from VIP3A plants (eg via post harvest tillage) are not likely to persist or accumulate, but rather degrade rapidly. Vip3A protein was extracted from GM maize leaf samples (corresponding to concentrations of approximately 58 and 14 µg/g dry weight) and incorporated into five soil types for 29 days. The loss of protein bioactivity in the soil samples was defined using the decreased mortality of the target lepidopteran larval pest of maize, the black cutworm (*A. ipsilon*), to estimate DT_{50} (time to dissipation of 50% of the initial bioactivity). All soils had DT_{50} values of five days or less.

Section 7 The receiving environment

72. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes: any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agricultural practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009).

7.1 Relevant abiotic factors

73. The size, locations and duration of the proposed limited and controlled release are outlined in Section 3.2. The proposed dealings involve planting cotton at up to 50 sites in current and potential cotton growing areas in 13 Qld LGAs, 18 NSW LGAs and two WA LGAs and growing GM cotton in glasshouse facilities in Brisbane and Toowoomba (see Section 3.2).

74. The abiotic factors relevant to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). To summarise, factors restricting where cotton can be grown in Australia are water availability (ie irrigation or rainfall), soil suitability and, most importantly, temperature. Cotton seedlings may be killed by frost and a minimum of 180 frost-free days of uniformly high temperatures (averaging 21-22°C) are required for crop growth (Duke 1983). Growth and development of cotton plants below 12°C is minimal and a long, hot growing season is crucial for achieving good yields (Constable & Shaw 1988).

75. The LGAs within which the release is proposed (see Table 1) encompass a broad range of climate types (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). Proposed planting areas in traditional cotton-growing regions of NSW and Qld predominantly have subtropical and grassland climate types, with small areas of temperate climate. The Qld LGA of Burdekin includes regions classified as tropical and subtropical. Proposed planting areas in WA include regions with tropical and grassland climates. Rainfall and temperature statistics representative of the geographical areas within which the release is proposed are given in Table 4.

Table 4 Temperature and rainfall data representative of proposed release sites*

LGA	Weather station	Mean temperature (ºC)				Mean monthly rainfall (mm)	
		Summer max.	Summer min.	Winter max.	Winter min.	Summer	Winter
Shire of Broome	Broome PO	33.6	26.1	28.7	14.9	126.2	10.1
Shire of Wyndham-East Kimberley	Kimberley Research Station	36.3	24.6	31.5	15.0	184.9	2.8
Burdekin Shire	Ayr DPI Research Station	31.7	22.5	25.5	12.3	190.1	18.4
Central Highlands Regional	Emerald PO	34.1	21.0	23.3	7.8	98.0	27.8
Roma Regional	Surat	33.9	20.1	20.5	5.1	71.9	34.3
Goondiwindi Regional	Goondiwindi PO	33.6	19.4	18.9	5.6	72.4	38.4
Toowoomba Regional	Toowoomba	27.2	16.3	17.0	5.9	124.4	49.4
Paroo Shire	Cunnamulla PO	35.3	21.5	19.8	6.5	45.6	22.0
Moree Plains Shire	Moree PO	34.4	18.7	19.5	4.3	64.1	39.2
Bourke Shire	Bourke PO	35.6	20.3	19.0	5.6	38.8	23.6
Central Darling Shire	Wilcannia	34.7	19.1	18.1	5.1	25.3	19.4

Narrabri Shire	Narrabri West PO	33.3	18.7	18.8	4.5	73.7	45.6
Narromine Shire	Trangie Research Station	32.4	17.8	16.2	3.9	48.2	34.1
Hay Shire	Hay	32.3	16.0	16.1	4.2	27.0	33.0

*Data were taken from the Australian Bureau of Meteorology website <http://www.bom.gov.au/climate/averages/>. Temperature and rainfall data are an average of at least 33 years of records. Summer entries are averages of monthly data from December to February, and winter entries are averages of monthly data from June to August.

7.2 Relevant biotic factors

76. The biotic factors pertaining to the growth and distribution of commercial cotton in Australia are discussed in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). In addition, the following points are of particular relevance to this release:

- The majority of the proposed release sites are in commercial cotton growing areas.
- GM cottons constitute the majority of Australian cotton crops (see Chapter 1, Section 7.4)

77. Invertebrates, vertebrates and microorganisms are expected to be exposed to the introduced genes, their encoded proteins and end products.

7.3 Relevant agricultural practices

78. The limits and controls of the proposed release are outlined in Section 3.2 of this Chapter. With regard to the agricultural practices, the GMOs proposed for field release would be planted with a small plot cone seeder or with commercial planting equipment. The rows would be irrigated by channel, drip or pivot irrigation and managed similar to commercial GM cotton crops with the exception that no insect resistance management (IRM) plan is required by the APVMA for a field trial.

7.4 Presence of related plants in the receiving environment

79. Cotton cultivation is widespread and established in the majority of Qld and NSW LGAs in which the release is proposed. Experimental cotton crops have been grown for over a decade in WA (in the Ord River Irrigation Area and in areas near Broome) and northern Qld (in the Burdekin Bowen Basin area), and although some commercial cultivation has occurred it is not yet widespread or well established in these regions.

80. Data on the cultivation of commercial cotton in Australia are discussed in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* document (OGTR 2008). *G. hirsutum* is the most common species of cotton commercially grown in Australia. In contrast, *G. barbadense* varieties comprised very little in the 2008/2009 season (pers. comm. Cotton Australia, 2009).

81. Herbicide tolerant and/or insect resistant GM cotton plants (*G. hirsutum*) are used widely in commercial cotton production, comprising about 95% of commercially grown cotton crops in the 2008/2009 growing season (pers. comm. Cotton Australia, 2009). In contrast, non-GM cotton comprised approximately 5% of commercially grown cotton. For a list of relevant approvals for commercial releases of GM cottons in Australia, see Section 8.1.

82. In southern Australia, ephemeral populations of cotton may be present outside of cultivation. Cultivated cotton can persist as a perennial plant in tropical areas and small populations of naturalised cotton (*G. hirsutum* and *G. barbadense*) exist in northern Australia, particularly in areas associated with a prolonged supply of fresh water (Hnatiuk 1990). The majority of naturalised *G. hirsutum* populations occur in the Northern Territory

(NT), while naturalised *G. barbadense* occurs mainly along the eastern regions of Qld (data from Australian Virtual Herbarium, <http://www.anbg.gov.au/avh/>).

83. There are 17 native species of *Gossypium* in Australia, most of which can be found in the NT and the north of WA (OGTR 2008). *G. australe* is the most widely distributed species throughout northern Australia, occurring from the east to west coast and predominantly north of the Tropic of Capricorn (Australian Virtual Herbarium, <http://www.anbg.gov.au/avh/>). The native *Gossypium* species prefer well-drained sandy loams and are rarely found on heavy clay soils favoured by cultivated cotton (OGTR 2008). Generally, they are found in native vegetation and not in disturbed/modified habitats such as agricultural areas (Groves et al. 2002).

84. Well established genetic incompatibility prevents crossing of native cotton species with cultivated cotton in the natural environment (discussed in OGTR 2008).

7.5 Presence of the introduced genes or similar genes and encoded proteins in the environment

85. All of the introduced genes were originally derived from common bacteria.

86. The introduced *cry* genes as well as the *vip3A* gene were originally isolated from Bt. Bt is an aerobic gram-positive endospore-forming bacterium that is ubiquitous in the environment. Bt is found in soil and plant communities worldwide and strains have been isolated from habitats including soil, insects, stored-product dust and deciduous and coniferous leaves (Schnepf et al. 1998 and references therein). Naturally occurring Bt strains typically contain *cry* genes (Masson et al. 1998). They may also contain *vip3* genes, eg Estruch et al. (1996) reported that approximately 15% of the *Bacillus* strains they analysed had *vip3* homologues.

87. In the past several decades, the use of Bt in products for insect control has resulted in occupational exposures of agricultural workers (eg inhalation of sprays) and dietary exposure through consumption of Bt-treated fruit and vegetables. Commercial GM cotton plants containing *cry* genes, such as the parental GM cotton Bollgard II®, also contribute to the presence of *cry* genes in agricultural areas of Australia. The genes and their encoded proteins are thus widespread in the soil environment and have also been found associated with plant products and insects (Schnepf et al. 1998). The Bt variety kurstaki strain HD1 used in Bt sprays in the US was demonstrated to contain a protein that reacts with anti-Vip3A antibodies (reviewed in Hill et al. 2003, p. 160ff). Bt variety kurstaki HD1 is also used in commercial Bt sprays in Australia (http://www.apvma.gov.au). On this basis, people and other organisms have a long history of exposure to Bt toxins, including Cry and Vip3A proteins.

88. The *cp4 epsps* gene was isolated from *A. tumefaciens* strain CP4 (reviewed by Padgette et al. 1996). This bacterium can also be found on plants and fresh plant produce. Similar EPSPS proteins are present in plants, bacteria and fungi, eg as described in Charles et al. (1986), Duncan et al. (1984) and Gasser et al. (1988). Commercially grown GM plants containing *cp4 epsps* genes, such as the parental GM cotton Bollgard II®/Roundup Ready Flex®, and Roundup Ready® GM cotton and canola also contribute to the presence of *cp4 epsps* genes in agricultural areas of Australia. On this basis, people and other organisms have a long history of exposure to CP4 EPSPS proteins.

89. The marker genes *nptII*, *aad*, *aph4* and *uidA* are derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997), and as such it is expected to be routinely encountered by humans.

90. Short regulatory sequences are derived from plants (including thale cress, pea, soybean and petunia), a soil bacterium (*A. tumefaciens*) and plant viruses (CaMV and FMV) that are also commonly encountered by humans and other organisms.

Section 8 Australian and international approvals

8.1 Australian approvals of GM cotton

8.1.1 Previous releases approved by the Regulator or the Genetic Manipulation Advisory Committee

91. Bollgard III and Bollgard III/Roundup Ready Flex® cotton have not been previously approved for release in Australia. However, all parental GM cottons, ie Bollgard II®, Bollgard II®/Roundup Ready Flex® and VIP3A cotton have individually been approved by the Regulator for release in Australia.

92. Previous approvals for the limited and controlled release of VIP3A cotton are:

- DIR 017/2002 (planted at Moree Plains and Narrabri), DIR 025/2002 (planted at Wyndham-East Kimberley), DIR 036/2003 (planted at Narrabri) issued to CSIRO
- DIR 034/2003 issued to Syngenta, under which no planting occurred
- DIR 058/2005 and DIR 065/2006 (both planted at Narrabri) and DIR 073/2007 (under which no planting occurred) issued to Deltapine, later transferred to Monsanto. The latter DIR included a GM VIP3A/Roundup Ready Flex® cotton.

93. Approvals for commercial releases of other relevant GM cottons in Australia are:

- insect resistant INGARD® *G. hirsutum* (DIR 022/2002; withdrawn from the market in 2004 in favour of Bollgard II® *G. hirsutum*)
- glyphosate tolerant Roundup Ready® *G. hirsutum* (DIR 023/2002 and DIR 066/2006; withdrawn from the market in 2009 in favour of Roundup Ready Flex® *G. hirsutum*)
- glyphosate tolerant / insect resistant Roundup Ready®/INGARD® *G. hirsutum* (DIR 023/2002; withdrawn from the market in favour of Bollgard II®/Roundup Ready® *G. hirsutum*)
- insect resistant Bollgard II® *G. hirsutum* (DIR 012/2002 and DIR 066/2006)
- insect resistant / glyphosate tolerant Bollgard II®/Roundup Ready® *G. hirsutum* (DIR 012/2002 and DIR 066/2006; withdrawn from the market in 2009 in favour of Bollgard II®/Roundup Ready Flex® *G. hirsutum*)
- glyphosate tolerant Roundup Ready Flex® *G. hirsutum* (DIR 059/2005 and DIR 066/2006)
- glyphosate tolerant / insect resistant Roundup Ready Flex®/Bollgard II® *G. hirsutum* (DIR 059/2005 and DIR 066/2006)
- glufosinate ammonium tolerant LibertyLink® *G. hirsutum* (DIR 062/2005)
- glufosinate ammonium tolerant/insect resistant LibertyLink®/Bollgard II® *G. hirsutum* (DIR 062/2005)
- insect resistant WideStrike™ cotton (DIR 091).

94. In addition, a number of limited and controlled releases of similar GMOs have been approved, including releases of GM *G. hirsutum* cottons containing other *cry* insect resistance genes, and release of the Bollgard II®/Roundup Ready Flex® traits in *G. barbadense* cotton. To date, the Regulator has not received any reports of adverse effects caused by these authorised releases.

8.1.2 Approvals by other Australian government agencies

96. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS), Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

97. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from INGARD®, Bollgard II®, Roundup Ready Flex® and VIP3A GM cottons (under applications A341, A436, A553 and A509, respectively, assessments are available at <www.foodstandards.gov.au/foodstandards/applications/>). As all parental GM cottons are approved, an additional approval for the GM Bollgard III and Bollgard III/Roundup Ready Flex® cottons would not be necessary. However, the applicant does not intend to use materials from the GM cottons generated in the proposed release in human food.

98. APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cottons proposed for release meet the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to their production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. The applicant intends to apply herbicide to the GM cottons during the trial, which is also subject to regulation by the APVMA.

8.2 International approvals of GM cotton

99. To date, there have been no international approvals for the release of Bollgard III or Bollgard III/Roundup Ready Flex® cottons.

100. International approvals have been given for the environmental release of the parent GM cottons which are to be combined in the proposed release (Table 5). In addition to the commercial release approvals listed below, approved field trials of VIP3A cotton have been conducted in Argentina (2001-2002), Burkina Faso (2004-2006), China (2001-2003), Costa Rica (2002, 2007-2009), India (2002-2006), Republic of South Africa (2002-2005), the USA (2000-2009), Vietnam (2002-2003) and Zimbabwe (2003-2004). In 2005, the United States Department of Agriculture Animal and Plant Health Inspection Service determined non-regulated status of VIP3A cotton (USDA-APHIS 2005), allowing its unconfined cultivation and agricultural use. There have also been approvals for the import of VIP3A cotton for food and feed use in Australia and New Zealand (2005) and Mexico (2010).

Table 5 Years in which international approvals were granted for the widespread environmental release of Bollgard II®, Roundup Ready Flex® and VIP3 GM cottons

Country	Bollgard II®	Roundup Ready Flex®	VIP3
Australia	2002	2006	
Brazil	2009		
Burkina Faso	2008		
India	2006		
South Africa	2003	2007	
United States	2002	2004	2005

Chapter 2 Risk assessment

Section 1 Introduction

101. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

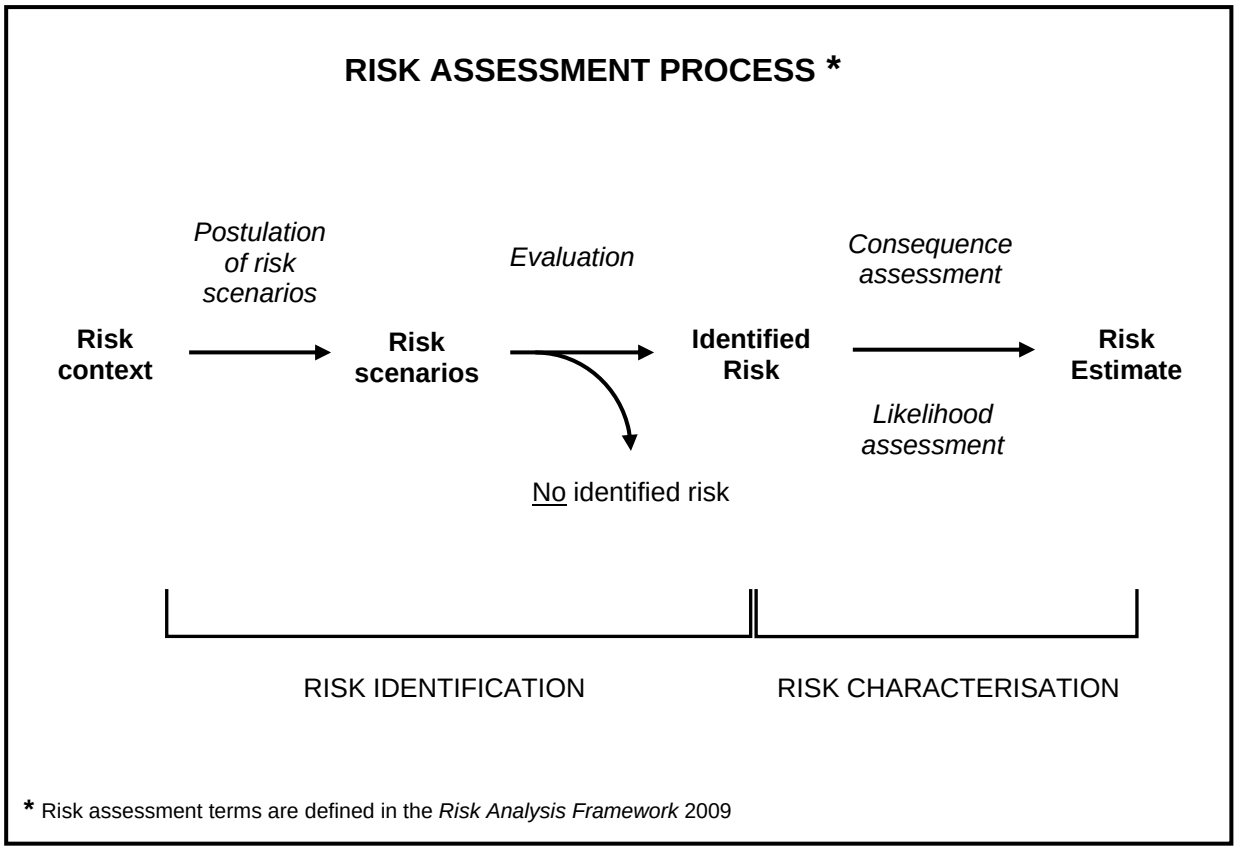

Figure 2. The risk assessment process.

102. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

103. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

104. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios

postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

105. Identified risks (ie those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk identification

106. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the parent organism(s)
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs.

107. Eight risk scenarios were identified and evaluated. These are summarised in Table 6, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment. More detail of the evaluation of these scenarios is provided later in this section.

108. Bollgard II® and Roundup Ready Flex® GM cottons have previously been described and assessed for commercial release (refer to RARMPs for DIRs 012/2002, 059/2005 and 066/2006), in addition to other applications and assessments for limited and controlled release. Previous assessments concluded that the commercial release of Bollgard II® and Roundup Ready Flex® GM cottons throughout Australia poses negligible risks to human health and safety and the environment (refer to RARMPs for DIR 059/2005 and DIR 066/2006). Those RARMPs assessed the potential risks associated with both traits individually and in combination. Consequently, the focus of the current assessment is the genetic modifications in VIP3A cotton in combination with the other genetic modifications. Thus, the genes present in Bollgard II® and Roundup Ready Flex® cotton (*cry1Ac, cry2Ab, nptII, aad*, *uidA* and *cp4 epsps*) will only be considered further in combination with *vip3A*.

109. As discussed in Chapter 1, Section 6, some of the proposed GM cotton plants would contain the antibiotic resistance selectable marker gene *aph4*. The prevalence of the *aph4* gene in the environment and the lack of evidence of toxicity and allergenicity of the protein was reviewed in Chapter 1, Section 6.2. The *aph4* gene was considered in previous assessments for DIRs 017/2002, 025/2002, 058/2005 and 065/2006 (limited and controlled releases). Since this gene has not been found to pose risks to either people or the environment (EFSA 2004), its potential effects will not be further assessed for this application.

110. All of the introduced gene regulatory sequences (gene promoters, gene terminators and untranslated leader sequences with exons and introns) operate in the same manner as do regulatory elements endogenous to cotton plants, and are sourced from organisms to which humans and other organisms are normally exposed. Any potential for adverse impacts from the introduced regulatory elements are considered equivalent to and no greater than those from endogenous regulatory elements of cotton.

111. As previously mentioned (Chapter 1, Section 7.4), GM insect resistant and/or herbicide tolerant cotton dominate the Australian cotton industry. In the 2008/09 growing season, 95% of cotton planted was GM cotton, including Bollgard II®, Roundup Ready®, Roundup Ready Flex®, Bollgard II®/Roundup Ready®, Bollgard II®/Roundup Ready Flex® and a small amount of Liberty Link® cotton. Bollgard II® insect resistant GM cotton and combinations with Bollgard II® comprised up to 83% of cotton grown (pers comm. Cotton Australia, 2009). Considering the abundant presence of these GM cottons, they will form part of the baseline in addition to non-GM cotton for the risk assessment process.

Table 6 Summary of risk scenarios from dealings with GM Bollgard III cotton and Bollgard III / Roundup Ready Flex® cotton.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	Exposure of people, other vertebrates and micro-organisms to GM plant material containing the proteins encoded by the introduced genes.	Allergic reactions in people or toxicity in people and other vertebrates or micro-organisms	No	• The introduced genes, or homologues, and their encoded proteins occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other vertebrates and micro-organisms. • None of the GM cotton material would be used in human food or animal feed. • The limited scale, and other proposed limits and controls, further reduce exposure of people and other vertebrates and micro-organisms to products of the introduced genes.
	Exposure of invertebrates to GM plant material containing the proteins encoded by the introduced genes.	Toxicity to non-target invertebrates	No	• The *cp4 epsps* gene and encoded protein is widespread in the environment and is not known to be toxic to invertebrates. • The insect resistance genes, or homologues, and their encoded proteins are widespread in the environment. • The toxicity of the proteins encoded by the individual insect resistance genes and any combination effects are expected to be limited to lepidopteran insects. • The limited scale, and other proposed limits and controls, reduces exposure of invertebrates to the products of the introduced genes.
Section 2.2 Spread and persistence of the GM cotton plants in the environment	Expression of the introduced genes improving the survival of the GM cotton plants.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Cultivated cotton is not considered to be weedy and the genetic modifications are not expected to change the weediness characteristic of the GMOs. • Resistance to Lepidoptera and tolerance to herbicide is unlikely to increase weediness as abiotic factors limit the spread and persistence of cotton in Australia. • The limits and controls proposed for the

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
				release would minimise persistence.
	Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Cotton seeds have limited dispersal characteristics, which are not expected to be changed in the GMOs. • The proposed limits and controls would minimise dispersal, such as locating the field trial sites at least 50 m from natural waterways and transporting material according to the Regulator's guidelines.
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	Expression of the introduced genes in other cotton plants.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Cotton is predominately self-pollinating and outcrossing is limited. • The applicant proposed a number of controls, including a 20 m pollen trap or 3 km isolation distance which would restrict gene flow via pollen. • Risk scenarios 1 – 3 did not constitute identified risks for people or the environment.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	Expression of the introduced genetic material in other organisms as a result of horizontal gene transfer.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Horizontal gene transfer from a GM plant is an extremely rare event. • The introduced gene sequences and regulatory sequences or components thereof are already present in the environment and are available for transfer via demonstrated natural mechanisms. • Risk scenarios 1 – 4 associated with expression of the introduced genes did not constitute identified risks for people or the environment.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	Changes to biochemistry, physiology or ecology of the GM cotton plants resulting from expression, or random insertion, of the introduced genes.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Unintended, adverse effects, if any, would be minimised by the proposed limits and controls. • Obvious unexpected alterations in the parental GM cottons were not observed, and the introduced genes and their encoded proteins are not expected to interact in a way which would affect the biochemistry, physiology and ecology of the GM cottons.
Section 2.6 Unauthorised activities	Use of the GMOs outside the proposed licence conditions (non-compliance).	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

112. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

113. Allergenicity is the potential of a substance, including proteins, to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

114. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes for insect resistance and herbicide tolerance. Workers cultivating the GM cotton would be exposed to all plant parts. Organisms may be exposed directly to the proteins encoded by the introduced genes through biotic interactions with GM cotton plants (vertebrates, invertebrates, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM cotton plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Risk scenario 1 Exposure of people, other vertebrates and micro-organisms to GM plant materials containing the proteins encoded by the introduced genes

115. The proteins expressed from the introduced genes for insect resistance and herbicide tolerance could be toxic or allergenic for people, or toxic for other organisms. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these people, other vertebrates or micro-organisms.

116. Although no toxicity studies have been performed on the GM cotton plant material, the introduced genes were isolated from naturally occurring bacteria that are already widespread and prevalent in the environment (see Chapter 1, Section 6, particularly Section 6.2.2, and Section 7.5). People and animals are exposed to proteins similar to those encoded by these genes through their diet and the environment. No information was found to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people, other vertebrates and micro-organisms (Chapter 1, Section 6.2.2).

117. FSANZ has approved food derived from the three GM parent cottons (Bollgard II®, VIP3A and Roundup Ready Flex®). Since Bollgard III and Bollgard III/Roundup Ready Flex® have been produced by conventional breeding, and food derived from each of the GM parents has been approved, no further approval by FSANZ would be required for the use of products from Bollgard III or Bollgard III/Roundup Ready Flex® in human food. There is no indication or anticipation that the combination of the multiple proteins encoded by the various introduced genes would lead to an increase in the potential for toxicity or allergenicity to humans and other organisms that were unaffected by the individual proteins. Unintended effects of the proposed release are discussed in risk scenario 7.

118. The applicant proposes to sell lint from the GM cottons. A study of the accumulation of mineral nutrients in *G. hirsutum* fruit found no detectable nitrogen in fibre fractions (Leffler & Tubertini 1976). Using more sensitive methods, specific proteins were detected at very low levels in raw, but not processed, linters and lint (Sims et al. 1996). Therefore, the safety of wearing cotton clothing or using other products made from cotton is not expected to be affected by the genetic make up of the cotton plants from which these components have been derived, that is, whether or not it is derived from GM or non-GM cotton plants.

119. Cotton pollen may be an allergen (Chakraborty et al. 2001), although allergic responses to the commercial cultivars of cotton have not been reported in Australia. Due to the limited quantities of pollen released by cotton, it is expected that people would be exposed to small quantities, if any, of pollen. As discussed above, the encoded proteins in the GM cottons are not considered to be toxic or allergenic and the GM cotton plants are unlikely to be any more toxic or allergenic than commercially released GM or non-GM cotton.

120. The proposed limits and controls of the trial (Chapter 1, Section 3.2 and 3.3) would minimise the likelihood of exposure of people, other vertebrates and micro-organisms to GM plant materials. Human contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. There is little potential for exposure of the public to GM plant material via ingestion, as no GM plant material would be used for human food as part of this release. Similarly, livestock would not be intentionally exposed as the GM plant material would not be used as animal feed.

121. Researchers and technical staff conducting the trials would be exposed to the GM plant materials during all phases of the trial. Workers may come into contact with the proteins encoded by the introduced genes when the plant cells have been damaged, or via pollen. Cotton plants possess leaves with sharp edges and irritating hairs, therefore workers typically wear protective clothing which reduces dermal contact. Exposure to the GM cotton is unlikely to lead to an adverse outcome as the GM cotton plants are unlikely to be any more toxic or allergenic than commercially released GM or non-GM cotton.

122. After harvest the applicant proposes to destroy GM cotton materials produced, apart from lint and some plant materials for research purposes and new plantings. These measures would minimise exposure to the GM plant material.

123. ***Conclusion***: The potential for allergic reactions in people, or toxicity in people, other vertebrates and micro-organisms as a result of exposure to GM plant materials containing the proteins encoded by the introduced genes is **not** identified as a risk that warrants further assessment.

Risk scenario 2 Exposure of invertebrates to GM plant material containing the proteins encoded by the introduced genes

124. The proteins expressed from the introduced genes for insect resistance and herbicide tolerance are toxic for certain invertebrates. If non-target invertebrates were exposed to the resulting compounds through direct or indirect ingestion of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these non-target invertebrates.

125. As indicated in Chapter 1, Section 7, the herbicide resistance gene *cp4 epsps* is widespread in the environment through the presence of naturally occurring bacteria as well as in Roundup Ready® and Roundup Ready Flex® cotton and other GM crops approved for commercial release. Other EPSPS enzymes are also present in plants, bacteria and fungi. There is no data to suggest that CP4 EPSPS is toxic to invertebrates. Thus, the toxicity of CP4 EPSPS proteins to invertebrates will not be considered further in this risk scenario.

126. Invertebrates in cotton growing regions of Australia are already widely exposed to Cry proteins through the commercially released Bollgard II® GM cotton. In the 2008/09 season, 95% of the commercial cotton crop was GM cotton, of which 83% was Bollgard II® cotton (pers. comm. Cotton Australia, 2009). No adverse outcomes on non-target invertebrates have been reported from these releases. Thus the toxicity of the Cry proteins to invertebrates will not be considered further in this risk scenario.

127. Bt strains which express a range of similar Cry and Vip toxins are also widespread in the agricultural and natural environments (Chapter 1, Section 7.5).

128. The primary purpose of the Vip3A protein is to provide resistance to insect herbivory. The encoded insect resistance protein is known to be toxic to a range of lepidopteran insect pests of cotton, including the major pests in Australia *H. armigera* and *H. punctigera*, as well as other target pests. Thus, the toxicity of these proteins for insect pests of cultivated cotton is not considered to be an adverse outcome but rather the intent of the genetic

modification. However, non-target invertebrate species may also be sensitive to these toxins. Non-target invertebrates (including predators of targeted pests and other beneficial insects) may ingest GM plant materials containing the insecticidal proteins, either directly through eating the GM cottons or indirectly through eating other organisms which have previously fed on the GM cotton plants. This may result in toxicity in non-target invertebrates. Relative exposure would be greatest for herbivorous species feeding on the cotton plants. Pollinator species and various adult insects that feed on pollen may also be exposed to the proteins. Sap feeders, such as aphids, would have minimal exposure, as the sap is composed mainly of sugars and mineral salts dissolved in water. In GM corn plants, no Cry1Ab protein could be detected in phloem sap (Raps et al. 2001). Exposure of invertebrates via the soil may occur when cotton tissues from the proposed release decompose or as a result of root exudation.

129. Laboratory studies suggest that the encoded Vip3A protein is not toxic to a range of invertebrates including Coleoptera, Neuroptera, Hymenoptera and Isotomidae (Hill et al. 2003). This has been substantiated by preliminary Australian field studies which showed no differences between GM and non-GM cotton fields in terms of invertebrate species richness and diversity (Whitehouse et al. 2007) (Chapter 1, Section 6.2.2).

Synergistic, additive or antagonistic effects of the insecticidal proteins

130. The addition of the *vip3A* gene to the *cry* genes already present in the Bollgard II® or Bollgard II®/Roundup Ready Flex® cotton could result in an interaction between the Cry and Vip3A proteins produced. If an interaction occurs between these proteins the combined effect could either be greater than (synergistic effect), equal to (additive effect), or less than (antagonistic effect) the sum of the effects of the individual proteins.

131. Synergistic or additive effects could be expected to occur between toxins isolated from the same or different strains of bacteria, particularly where different receptor molecules are involved (Schnepf et al. 1998). Antagonistic reactions are not expected to occur between toxins present in the same strain of bacteria as these toxins would have evolved together. Antagonistic effects are more likely to occur between toxins isolated from different strains of bacteria (del Rincon-Castro et al. 1999), particularly where the same receptor molecule is targeted. Such effects could act to decrease the efficacy of the insecticidal toxins.

132. Evidence suggests that the Vip3A and Cry proteins bind to different receptors in the insect midgut epithelium (see Chapter 1, Section 6.2.2), so it is possible that an additive or synergistic effect between the three insecticidal proteins in combination in the GM cottons may occur. This could increase the toxic effect and/or range of insects sensitive to either the Vip3A or Cry1Ac and Cry2Ab proteins alone.

133. The specificities of the Vip3A, Cry1Ac and Cry2Ab proteins appear to be restricted to overlapping subsets of lepidopteran insects. Therefore, any increase in the range of sensitive insects as a result of the expression of both insecticidal proteins is expected to be confined to lepidopteran species. It is noteworthy that the same or similar proteins are present in the microbial formulations in commercial Bt insecticide preparations (Hill et al. 2003). It is not expected that the range of sensitive insects would increase beyond those sensitive to the Bt insecticides. However, some uncertainty exists in this area due to data gaps.

134. In addition, exposure of non-target invertebrates to the Vip3A, Cry1Ac and Cry2Ab proteins is expected to be limited due to the limited size and short duration of the proposed release.

135. ***Conclusion***: The potential for toxicity to non-target invertebrates as a result of exposure to GM plant materials containing the proteins encoded by the introduced genes is **not** identified as a risk that warrants further assessment.

2.2 Spread and persistence (weediness) of the GM cotton plants in the environment

136. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM cotton plants in particular, is given in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). In summary, the document concludes that modern cultivars of non-GM cotton are not recognised as weeds in Australia where cotton occurs almost exclusively as a managed agricultural crop.

137. Cotton has been grown for centuries throughout the world without any reports that it is a serious weed, and is likewise not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2002; Groves et al. 2003). The weed status of cotton has also been considered extensively in RARMPs produced during the assessment of a variety of GM cottons, including recent commercial approvals DIR 062/2005, DIR 066/2006 and DIR 091.

138. Scenarios that could lead to increased spread and persistence of the GM cotton include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These risk scenarios could lead to increased exposure of vertebrates (including people), invertebrates and micro-organisms to the encoded proteins.

Risk scenario 3 Expression of the introduced genes improving the survival of the GM cotton plants

139. If the GM cotton plants were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials has been considered in risk scenarios 1 and 2 and was not considered an identified risk.

140. If the expression of the introduced genes for insect resistance and herbicide tolerance were to provide the GM cotton plants with a significant selective advantage over commercially released GM or non-GM cotton plants and if they were able to establish and persist in favourable non-agricultural environments, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM cotton plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM or commercially released GM cotton.

141. Bollgard III/Roundup Ready Flex® cotton contains two copies of the *cp4 epsps* gene. Roundup Ready Flex® GM cottons have previously been described and assessed for commercial release (refer to Chapter 1, Section 8.1.1), in addition to other applications for limited and controlled release. Previous assessments concluded that the commercial release of Roundup Ready Flex® GM cottons throughout Australia pose negligible risks to human health and safety and the environment (refer to RARMPs for DIR 059/2005 and DIR 066/2006). Consequently, the expression of the introduced *cp4 epsps* genes improving the survival of the GM cotton plants will not be considered further in this risk scenario.

142. The CP4 EPSPS protein that confers tolerance to glyphosate and the Cry1Ac, Cry2Ab and Vip3A insecticidal proteins operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the herbicide tolerance and insecticidal proteins or their metabolic pathways, and no reason to expect that this is likely to occur. There is no evidence or reasonable expectation that synergistic effects are likely to occur from the combination of the two traits, or that they would result in new or increased risks relating to human health and safety or the environment.

143. Bollgard III cotton expresses three genes encoding insecticidal toxins. This could confer a selective advantage on the GM plants in regions where lepidopteran insect predation limits one or more of the key life stages of cotton and lead to weediness. This would particularly be the case if additive or synergistic effects between the expressed Cry and Vip3A proteins led to increased toxicity of the GM plants to a wider range of susceptible pest species. As discussed in risk scenario 2, there is some uncertainty about the range of lepidopteran species susceptible to the combination of proteins. However, the range is unlikely to be expanded beyond lepidopteran insects.

144. Some of the proposed trial sites are in the LGAs of Burdekin, Isaac, Broome and Wyndham-East Kimberley, parts of which are north of latitude 22° South. Experience from growing cotton previously in northern Australia has suggested that insect pressure is higher in tropical regions during the wet season compared to the current southern cotton growing regions. *Spodoptera litura* and *Pectinophora gossypiella* are thought to be important pests of cotton in northern Australia, in addition to *H. armigera* and *H. punctigera* (Strickland et al. 2000; Strickland et al. 2003; Cotton Catchment communities CRC 2006). Vip3A is stated to be active against *P. gossypiella* and the related army worms, *S. frugiperda*, and *S. exigua*. Additionally, the related VIP protein has activity against *S. litura* (Selvapandiyan et al. 2001), whereas insecticides may be needed to control *S. litura* on Bollgard II® cotton (Strickland et al. 2003).

145. Grasshoppers (order Orthoptera) are considered to be the most important insect herbivores in tropical savannah ecosystems (Andersen & Lonsdale 1990) and are thought to be important in controlling cotton volunteers in areas north of latitude 22º South. No information is available on the effect of Bollgard III cotton on grasshopper populations, although it is acknowledged that Cry1 and Vip3Aproteins are generally specific to insects within the order Lepidoptera.

146. If the pests that limit the spread and persistence of cotton in northern Australia are sensitive to Vip3A, then the genes present in Bollgard III and Bollgard III/Roundup Ready Flex® cotton may offer a selective advantage.

147. However, the spread and persistence of cotton plants is limited by a number of abiotic factors including water and nutrient availability, temperature and soil type (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008). The importance of these may vary between northern or southern Australia. A modelling study has indicated that cold stress is the most significant factor affecting persistence of cotton plants in southern Australia and dry stress is most significant in northern Australia (Rogers et al. 2007). The germination and survival of any GM cotton seedlings is likely to remain limited by these environmental factors rather than lepidopteran herbivory. However, some uncertainty remains in this area due to data gaps.

148. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM cotton plants proposed for release. The release would be limited in size and number of locations. The applicant proposes a number of control measures, including post harvest monitoring of the release sites for at least twelve months or until the site has been clear of volunteers for six months; and to destroy any volunteers found prior to flowering.

149. ***Conclusion:*** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes improving the survival of the GM cotton plants is **not** identified as a risk that warrants further assessment.

Risk scenario 4 Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions

150. If the GM cotton plants were to be dispersed from the release sites they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM cotton plants have been assessed in risk scenario 1 and 2 and were not an identified risk. The potential for the introduced genes to result in improved survival of the GM cotton plants in the environment was assessed in risk scenario 3 and was not an identified risk.

151. In a natural situation cotton does not reproduce vegetatively (Sheelavantar et al. 1975; OGTR 2008), and therefore dispersal of GM cotton materials other than seed would be highly unlikely to result in the establishment of the GM cotton plants in the environment. Seed production, dispersal and digestibility characteristics are not expected to be altered in the Bollgard III or Bollgard III/Roundup Ready® cotton compared to non-GM or commercially released GM cotton.

152. Dispersal of reproductive GM plant materials, for example viable seed, could occur through a variety of ways including endozoochory (dispersal through ingestion by animals), the activity of animals such as herbivores or through extremes of weather such as flooding or high winds, or via human activity.

153. In the field, seed cotton is present as large lint-covered bolls. Mammals, including rodents, generally avoid feeding on cotton plants, in particular finding the seed unpalatable because of its high gossypol content. They are therefore unlikely to carry bolls any great distance from the cotton fields. Similarly, there is no evidence of avian species transporting cotton seed (OGTR 2008). Dispersal by authorised people entering the proposed trial sites would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

154. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal (Chapter 1, Section 3.2 and 3.3), for example, the proposed release sites will be located at least 50 m away from natural waterways in areas not prone to flooding (information provided by applicant) and the applicant proposes regular monitoring for volunteers in the field in which the cotton is planted, as well as any connecting irrigation channels.

155. The applicant proposes not to feed whole cotton seed to animals. Therefore, there would be no deliberate spread of whole cotton seed outside the proposed locations for stockfeed. In addition, the applicant proposes to transport any plant material according to the Regulator's transport guidelines and any spillage of seed during transport to and from the release sites would be rare. Any incident involving spillage of GM seed is expected to be readily controlled through cleaning and monitoring of the site of the spill. In addition, the opportunity for an adverse outcome from any such rare occurrence is further diminished by the need for appropriate environmental conditions for germination, survival and persistence of any escaped seeds.

156. Furthermore, the applicant proposes to thoroughly clean pickers and module builders after the GM cotton material has been harvested to prevent dispersal of seed to other locations. The applicant proposes to destroy all plant materials other than lint and some materials collected for future research or planting. The ginning and cleaning process are designed to remove as much trash (small particles of leaf and seed) from the lint as possible in order to create a saleable product. These processes would reduce the potential for inadvertent dispersal of seed from the GMO after ginning.

157. ***Conclusion:*** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials through various means including animals and extreme weather conditions is **not** identified as a risk that warrants further assessment.

2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

158. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hegde 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and nearby cotton plants, related weeds or native plants (Glover 2002).

159. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. For an increased potential for adverse effects to arise as a result of gene flow of the introduced genetic elements from Bollgard III and Bollgard III/Roundup Ready Flex® cotton to sexually compatible plants, both of the following steps must occur:

- transfer of the introduced genetic elements to sexually compatible plants
- increased potential for adverse effects, such as toxicity or spread and persistence of the recipient plants, due to expression of the introduced gene(s).

160. Baseline information on vertical gene transfer associated with non-GM cotton plants is provided in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). In summary, cotton is predominantly self-pollinating and outcrossing is rare, although cross-pollination can occur at low levels over short distances. The only sexually compatible species present in Australia that could receive genes from the GM cotton are *G. hirsutum* and *G. barbadense* (including both cultivated GM and non-GM cotton, and naturalised cotton).

161. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distances from cultivated cotton fields. Furthermore, there is well established genetic incompatibility between native *Gossypium* species and cultivated cotton; the likelihood of fertile hybrids occurring between cultivated cotton and native *Gossypium* species is very low (summarised in OGTR 2008). Therefore, these species are not considered further.

Risk scenario 5 Expression of the introduced genes in other cotton plants

162. Transfer and expression of the introduced genes for insect resistance and herbicide tolerance to other cotton plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

163. As discussed in risk scenario 1, the proteins encoded by the introduced genes are unlikely to be allergenic to people or toxic to people or organisms other than certain invertebrates. As discussed in risk scenario 2, the GM cottons are expected to be toxic to target invertebrates and there is expected to be limited toxicity to non-target invertebrates. This will be the same if the introduced genes are expressed in other cotton plants.

164. It should be noted that Bollgard III and Bollgard III/Roundup Ready Flex® cotton contain three or four independent genetic modifications, respectively, so the introduced genes have inserted into different regions of the cotton genome and segregate independently of one another. This means that after any initial outcrossing of Bollgard III or Bollgard III/Roundup Ready Flex® cotton to other cotton, any subsequent generations of

cotton volunteers may contain either all of the *cry, vip3A* and *cp4 epsps* genes, any subset of these genes, or none of the introduced genes. Any GM cotton produced from outcrossing containing either fewer or no genes encoding insecticidal proteins or herbicide tolerance will have equivalent or less insecticidal efficacy or herbicide tolerance than a GM cotton volunteer plant containing all the introduced genes. Therefore, segregation of the *cry*, *vip3a* and *cp4 epsps* genes will not impact on the assessment and will not be considered further.

165. Roundup Ready Flex® GM cotton has previously been described and assessed for commercial release (refer to Chapter 1, Section 8.1.1), in addition to other applications for limited and controlled release. Previous assessments concluded that the commercial release of Roundup Ready Flex® GM cotton throughout Australia pose negligible risks to human health and safety and the environment (refer to RARMPs for DIR 059/2005 and DIR 066/2006). Consequently, the expression of the introduced *cp4 epsps* genes in other sexually compatible plant species, including all other commercially approved GM cottons, as a result of gene transfer will not be considered further in this risk scenario.

166. In the event that the genes for insect resistance were transferred to feral or cultivated cotton, the plants would have a survival advantage only in regions where lepidopteran insect pests may limit their growth or regulate their populations. However, as discussed in risk scenario 3, the distribution of cotton is primarily determined by soil type, soil moisture and temperature, rather than by insect pressure (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008).

167. Therefore, as is the case for the GM cotton plants proposed for release, expression of the introduced genes in other *G. hirsutum* would also result in plants limited by these factors. The expression of the introduced genes in the sexually compatible species *G. barbadense* is also unlikely to give these plants a significant selective advantage. The conditions that limit the spread and persistence of any hybrids between non-GM *G. hirsutum* and *G. barbadense* (OGTR 2008) would be expected to limit the spread and persistence of any hybrids between Bollgard III and Bollgard III/Roundup Ready Flex® GM cotton and *G. barbadense*.

168. A number of insect resistant GM cottons are currently approved for commercial release in Australia and may be grown in the areas proposed for this release. These include the GM parent cotton, Bollgard II® (most recently assessed in the RARMP for DIR 066/2006), which was present in 83% of cotton grown in the 2008/09 season (pers. comm. Cotton Australia, 2009). Should gene transfer occur to Bollgard II® GM cotton the resulting plants would be highly similar to the GMO proposed for release. Therefore, any adverse outcomes expected for those offspring would be comparable to Bollgard III GM cotton (see previous risk scenarios for details).

169. WideStrike™ GM cotton, containing *cry1Ac(synpro)* and *cry1F(synpro)*, assessed and licensed for commercial release south of latitude 22º South in the RARMP for DIR 091, may also be planted in the vicinity of the trial sites. If crossing occurred between these GM plants and Bollgard III plants it would result in cotton plants containing a new combination of insect resistance genes [*cry1Ac(synpro), cry1Ac, cry2Ab, cry1F(synpro)* and *vip3A*]. The risk of unintentional stacking between the *cry* genes in Bollgard II® and WideStrike™ cotton leading to increased spread and persistence of GM cotton plants was assessed the RARMP for DIR 091 and estimated to be negligible. However, as discussed in risk scenario 2 there may be interactions between the Cry and Vip insect resistance proteins which may give rise to combination effects. This could potentially increase the toxic effect and/or range of susceptible insects relative to either of the parental GM plants and is an area of uncertainty due to data gaps.

170. Nonetheless, expression of three introduced genes for insect resistance in the GM cotton is not expected to increase their spread and persistence as they would still be limited by the abiotic factors discussed above.

171. Dispersal characteristics, as well as allergenicity to people and toxicity to people and organisms other than certain invertebrates are not expected to be changed in the GM cotton plants by the introduced genes (see risk scenarios 1, 2 and 4). As discussed in the *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008) cotton is predominantly self-pollinating, with pollen that is large, sticky and heavy and not easily dispersed by wind. Cotton gene flow studies consistently show that outcrossing is localised around the pollen source and decreases rapidly with distance. Furthermore, as discussed above, outcrossing will only be successful between the GM cotton plants and other *G. hirsutum* or *G. barbadense* plants due to genetic incompatibility with other *Gossypium* species. It is unlikely that expression of the introduced genes in the GM cotton will alter pollen characteristics and/or genetic compatibility relative to non-GM or commercially released GM cotton plants.

172. The applicant has proposed a number of measures to restrict the potential for pollen flow and gene transfer to sexually compatible plants (Chapter 1, Section 3.2 and 3.3). These include surrounding the trial sites with either a 20 m pollen trap (with an access path) or a 3 km exclusion zone (within which intentional planting of cotton is not allowed). The applicant also proposes to perform post harvest monitoring of each site for twelve months or until the sites have been clear of volunteers for six months; and to destroy any volunteers found prior to flowering. These proposed controls would reduce the already low likelihood of gene flow from the GMOs to other cotton resulting in expression of the introduced genes.

173. ***Conclusion:*** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes in other cotton plants as a result of gene transfer is **not** identified as a risk that warrants further assessment.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

174. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). Data is accumulating to show that HGT occurs more frequently than previously thought and can occur between different plants, as well as between plants and less complex organisms (Bock 2010). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but could be part of a scenario potentially leading to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or by altering the expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

175. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <http://www.ogtr.gov.au> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

176. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 7.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Risk scenario 6 Expression of the introduced genetic material in other organisms as a result of horizontal gene transfer

177. Assessment of possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of potential recipient organisms and the nature of the introduced genetic material. Risks that might arise through HGT from a GMO to another organism have been recently reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <http://www.ogtr.gov.au> or by contacting the Office.

178. HGT could result in the presence of the introduced genes for insect resistance and herbicide tolerance in bacteria, plants, animals or other eukaryotes. However, the introduced sequences were isolated from plants and bacteria, which are already widespread in the environment (See Chapter 1, Section 7.5), and are thus already available for transfer from those sources via demonstrated natural mechanisms.

179. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced genes or their end products are not associated with any risk then even in the unlikely event of HGT occurring, it should not pose any risk to humans, animals or the environment. Conclusions reached for risk scenarios 1 - 5 associated with the expression of the introduced sequences did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

180. ***Conclusion:*** The potential for an adverse outcome as a result of horizontal gene transfer is **not** identified as a risk that warrants further assessment.

2.5 Unintended changes in biochemistry, physiology or ecology

181. All methods of plant breeding can induce unanticipated changes in plants, including through pleiotropy[6] (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such unintended effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of an introduced gene changing chromatin structure, affecting methylation patterns, or modulating signal transduction and transcription
- increased metabolic burden associated with high level expression of an introduced gene
- novel traits arising from interactions of the protein encoded by an introduced gene product with endogenous non-target molecules

[6] Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

- secondary effects arising from altered substrate or product levels in a biochemical pathway incorporating the protein encoded by an introduced gene.

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

Risk scenario 7 Changes to biochemistry, physiology or ecology of the GM cotton plants resulting from expression or random insertion of the introduced genes

183. Various biochemical pathways of the GM cotton plants could be changed by the expression of the introduced genes, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds.

184. The outcome of random insertion of introduced genes is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. Unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

185. While no data is available on the phenotype of the Bollgard III and Bollgard III/Roundup Ready Flex® GM cotton plants, the plants will be produced by conventional breeding between GM cottons and the phenotype of the parental cottons has been characterised. The parents Bollgard II® and Bollgard II®/Roundup Ready Flex® are widely grown with no reported unexpected or adverse effects. Previous releases of VIP3A GM cotton in Australia (performed under DIRs 17/2002, 025/2002, 036/2003, 058/2005, 065/2006) did not show any unintended secondary effects from sites in NSW and WA.

186. The applicant proposes to measure the agronomic performance of the GM Bollgard III and Bollgard III/Roundup Ready Flex® GM cottons during this limited and controlled release, and any unintended effects are likely be detected during the trial.

187. Monogastrics, including humans, are affected by the toxic substances (gossypol) and anti-nutrients that are present in all cotton (OGTR 2008). Humans can consume cotton oil and linters as these are refined products that do not contain those substances. There are limits on how much cottonseed meal can be used for animal feed without toxic effects (discussed in OGTR 2008).

188. FSANZ has approved food derived from the three GM parent cottons (Bollgard II®, VIP3A and Roundup Ready Flex®). Since Bollgard III and Bollgard III/Roundup Ready Flex® have been produced by conventional breeding, and food derived from each of the GM parents has been approved, no further approval by FSANZ would be required for the use of products from Bollgard III or Bollgard III/Roundup Ready Flex® in human food. There is no indication or anticipation that the combination of the multiple proteins encoded by the various introduced genes would lead to an increase in the potential for toxicity or allergenicity to humans and other organisms that were unaffected by the individual proteins.

189. The likelihood of any unintended effects causing adverse effects is also minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale of the trial would limit the potential for adverse effects. Humans and livestock would not be intentionally exposed as the GM plant material would not be used as food or animal feed as part of the release.

190. ***Conclusion:*** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not** identified as a risk that warrants further assessment.

2.6 Unauthorised activities

Risk scenario 8 Use of GMOs outside the proposed licence conditions (non-compliance)

191. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM cotton plants outside of the proposed release areas and/or increased exposure of people and other organisms to GM material. The adverse outcomes that this risk scenario could cause are the same as those discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

192. ***Conclusion:*** The potential for an adverse outcome as a result of unauthorised activities is **not** identified as a risk that warrants further assessment.

Section 3 Risk estimate process and assessment of significant risk

193. The risk assessment begins with a postulation of potential pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen in comparison to the non-GM parent organism and within the context of the receiving environment.

194. Eight risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if it occurred were also assessed.

195. A **risk** is only identified when a risk scenario is considered to have some chance of causing harm. Risk scenarios that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

196. The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- widespread presence of the same genes or sequences in the environment
- toxicity of the proteins encoded by the introduced insect resistance genes is expected to be limited to certain insects in the order Lepidoptera
- the GMOs are produced by conventional breeding of other GM cottons which have previously been assessed as posing negligible risks
- limited ability and opportunity for the GM cotton plants to transfer the introduced genes to commercial cotton crops or other cotton plants
- limits on the size, locations and duration of the release proposed by Monsanto

- suitability of controls proposed by Monsanto to restrict the spread and persistence of the GM cotton plants and their genetic material
- none of the GM plant materials or products will be used in human food or animal feed.

197. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM cotton plants into the environment are considered to be **negligible.** Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

Section 4 Uncertainty

198. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. In addition, risk assessment is based on evidence, which is also subject to uncertainty. It is recognised that both dimensions of risk (consequence and likelihood) are always uncertain to some degree.

199. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[7]. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

200. For DIR 101, uncertainty is noted in particular in relation to the characterisation of:

- Risk scenario 2, regarding potential increases in toxicity to non-target invertebrates as a result of the combination of the introduced genes
- Risk scenario 3, associated with a potential for increased survival of the GMOs
- Risk scenario 5, regarding potential increases in toxicity to non-target invertebrates and weediness as a result of expression of the introduced insect resistance genes in Widestrike™ (a commercially approved GM cotton)
- Risk scenario 7, associated with the potential for any unintended effects as a result of changes in biochemistry, physiology or ecology of the GM cotton plants.

201. Additional data, including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM Bollgard III and Bollgard III/Roundup Ready Flex® GM cottons if they are selected for further development.

202. Chapter 3, Section 5 discusses information that may be required for future release.

[7] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2009) available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1> or via Free call 1800 181 030.

Chapter 3 Risk management plan

203. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through imposed licence conditions. The risk management plan informs the Regulator's decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

204. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

205. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

206. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions imposed by the Regulator may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

207. Australia's gene technology regulatory system operates as an integrated legislative framework involving the Regulator and other regulatory agencies that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[8].

208. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR licence applications. *The Gene Technology (Consequential Amendments) Act 2000* also requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

[8] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

209. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously given approval for the use in food of cotton seed oil and linters derived from Bollgard II®, Roundup Ready Flex® and VIP3A GM cottons (under applications A341, A436, A553 and A509, respectively, assessments are available at <www.foodstandards.gov.au/foodstandards/applications/>). As all parental GM cottons are approved, an additional approval for the GM Bollgard III and Bollgard III/Roundup Ready Flex® cottons would not be necessary. However, the applicant does not intend to use materials from the GM cottons generated in the proposed release in human food.

210. APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cottons proposed for release meet the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to their production of insecticidal substances, and therefore these plants are subject to regulation by the APVMA. The applicant intends to apply herbicide to the GM cottons during the trial, which is also subject to regulation by the APVMA.

211. No other regulatory approvals are required.

Section 3 Risk treatment measures for identified risks

212. The assessment of risk scenarios listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM cotton. The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

213. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 1150 ha in up to 34 LGAs in Qld, NSW and WA between October 2010 and October 2014), the proposed containment measures (refer to Chapter 1, Section 3), and the receiving environment (refer to Chapter 1, Section 6).

Section 4 General risk management

214. Licence conditions are imposed to control the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, locations and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are summarised in this Chapter.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by Monsanto

215. Chapter 1, Section 3.2 and 3.3 provide details of the limits and controls proposed by Monsanto in their application, and discussed in the risk scenarios characterised for the release in Chapter 2. Many of these proposed control measures are considered standard GM cotton licence conditions and have been imposed by the Regulator in previous DIR licences. The appropriateness of these controls will be discussed briefly here.

216. The proposed release will be limited to up to 50 sites per year in up to 34 LGAs in NSW, Qld and WA. The trial, including planting, harvesting and post-harvest monitoring, will be carried out by staff trained by Monsanto and supervised by an appointed project supervisor. All personnel would have appropriate training in practices relevant to the handling and disposal of GMOs. These measures will minimise the potential exposure of

humans and vertebrates to the GMOs (risk scenario 1) and the potential for the GM cottons to persist or to establish outside the proposed release sites (risk scenario 3).

217. Each site must be surrounded by a 20 m wide pollen trap or a 3 km exclusion zone, the latter in combination with a 100 m monitoring zone, to restrict gene flow from the GM cottons. As discussed in the *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008), cotton is predominantly self-pollinating, with the highest level of outcrossing occurring between adjacent rows. Outcrossing is rare beyond 20 m (Llewellyn et al. 2007b), and a 20 m pollen trap of non-GM cotton or GM cotton approved for commercial release will minimise gene transfer to sexually compatible plants (risk scenario 5).

218. It should be noted that the applicant has proposed a cleared (sprayed) 2.5 m path or wheel tracks through the pollen trap created by vehicles accessing the site. Driving through the pollen trap is likely to damage and break some of the pollen trap plants which may reduce the number of flowers in that area. Similarly, clearing a 2.5 m wide path by spraying with herbicide would eliminate a portion of the pollen trap. However, this is unlikely to reduce the effectiveness of the pollen trap because the path makes up a relatively small portion of the total pollen trap area (risk scenario 5).

219. As an alternative to a 20 m pollen trap, a 3 km exclusion zone in combination with a 100 m monitoring zone immediately surrounding a trial site was proposed. The exclusion zone must be free of intentionally planted (GM and non-GM) cotton. The monitoring zone must be inspected every 30 days while the cotton plants are being grown at the trial site and kept free of flowering cotton plants. In the RARMP prepared for DIR 081/2007, the literature regarding outcrossing rates over heterogenous terrain was reviewed. In addition, the suitability of the combination of these controls was assessed and found acceptable to limit vertical gene transfer from GM cotton trial sites to other (GM and non-GM) cotton.

220. The applicant has also proposed to grow GM cotton in glasshouses which are not certified physical containment level 2 facilities by the Regulator. The glasshouses would be located at least 20 km from the nearest commercial cotton crop. However, it is considered that a 3 km exclusion zone as discussed in the preceding paragraph would also be adequate for the glasshouse locations. As an alternative to an exclusion zone, an insect control program within the glasshouses, as well as the physical containment of the glasshouse walls, would effectively restrict pollen dispersal by insects. The only cotton in close proximity to the glasshouses would be contained within research facilities certified to physical containment level 2 (PC2), which would further limit outcrossing (risk scenario 5). Both glasshouses are surrounded by fences and have lockable gates, and access to the glasshouses is restricted to authorised and trained persons, which will further limit contact with the general public (risk scenario 1).

221. The applicant has proposed a number of measures to restrict dispersal and persistence of the GM cotton, which are reflected in the licence conditions. The trial sites must be located more than 50 m from the nearest waterway which will minimise the chance of plant material being washed away from the site (risk scenario 4). The GM cotton must be harvested and ginned separately from other cotton crops to prevent mixing, and none of the seed or GM plant material is permitted to be used in human food or animal feed. These measures are expected to limit the potential exposure of humans and other organisms to the GMOs (risk scenario 1) and the potential for the GM cotton to be dispersed outside the proposed release site (risk scenario 4).

222. After the GM cottons have been harvested, the applicant must destroy all remaining plant materials not required for experiments or further planting, and clean the site and all

equipment used. As discussed in the *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008), cotton seeds have low dormancy levels and do not generally form a viable seed bank. However, dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture. The sites and pollen traps must be irrigated and cultivated the first spring or summer following harvest to promote cotton seed bank reduction and minimise the persistence of the GM cotton at the proposed release site. The site must also be monitored at least every two months for at least twelve months after harvest and until the site has been clear of volunteers for at least six months. These measures will limit the persistence of the GM cotton in the environment (risk scenario 3).

223. Any plant material taken off-site for experimental analysis must be transported according to the Regulator's *Guidelines for the transport of GMOs* (http://www.ogtr.gov.au/). These are standard protocols for the handling of GMOs to minimize exposure of the GMO to people and other organisms (risk scenarios 1 and 2), dispersal into the environment (risk scenario 4), and gene transfer (risk scenarios 5 and 6).

4.1.2 Summary of measures imposed by the Regulator to limit and control the proposed release

224. A number of licence conditions have been imposed to limit and control the proposed release, based on the above considerations. These include requirements to:

- limit the release to a cumulative total area of 1150 ha between October 2010 and October 2014 in up to 34 LGAs in Qld, NSW and WA
- locate the field trial sites at least 50 m away from natural waterways
- restrict gene flow via pollen from field trial sites by surrounding the trial site with:
  - a 100 m monitoring zone and a 3 km isolation distance between the site and other cotton crops, or
  - a 20 m pollen trap of non-GM cotton or commercially approved GM cotton
- remove and/or destroy any cotton plants growing in the monitoring zone prior to flowering
- restrict gene flow via pollen from the glasshouses by:
  - implementing an insect control program within the glasshouses, or
  - maintaining a distance of at least 3 km from the nearest cotton crop
- harvest and gin all cotton from the trial separately from other cotton
- remove and/or destroy all cotton plant materials from the trial site and adjacent areas (eg pollen trap, equipment cleaning areas) after harvest, except for materials required for research or further planting
- after harvest, apply measures to promote germination of any cotton seeds that may be present in the soil
- after cleaning of sites, monitor for and destroy any GM cotton that may grow for at least 12 months, and until no volunteers are observed for a continuous 6 month period
- transport the GM plant materials in accordance with Regulator's transportation guidelines, unless otherwise specified for particular circumstances
- restrict access to the trial sites to authorised personnel only
- not permit the use of GM plant material or products to be used for human food or animal feed.

225. The licence is available on the OGTR website. The sale of lint obtained from ginning of the GM seed cotton is permitted. Research with the GMOs or GM plant material may be conducted in certified physical containment facilities as a Notifiable Low Risk Dealings (NLRD) in accordance with all applicable requirements of the *Gene Technology Regulations 2001*, and therefore this activity is not covered in the licence.

4.2 Other risk management considerations

226. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures
- a requirement that the applicant allows access to the trial sites for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

227. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

228. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Monsanto suitable to hold a licence.

229. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

230. Monsanto must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Contingency plan

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

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

4.2.3 Identification of the persons or classes of persons covered by the licence

233. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting requirements

234. The licence obliges the licence holder, under section 65 of the Act, to immediately report any of the following to the Regulator:

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

235. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

236. A number of written notices are also required under the licence that assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of harvest and cleaning after harvest.

4.2.5 Monitoring for Compliance

237. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

238. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

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

Section 5 Issues to be addressed for future releases

240. Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM cottons, or to justify a reduction in containment conditions. This includes:

- additional data on the potential toxicity of Vip3A in combination with the proteins encoded by the other introduced insect resistance genes to non-target invertebrates
- phenotypic characterisation of the GM cottons, in particular of traits which may contribute to weediness, persistence, and ability to disperse in the environment
- data on the effects on non-target insects and weediness potential of Bollgard III or Bollgard III/Roundup Ready Flex® combined with insect resistant WideStrike™ cotton.

Section 6 Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of the two GM cottons (Bollgard III and Bollgard III/Roundup Ready Flex®) on a maximum total cumulative area of 1150 ha in up to 34 LGAs in Qld, NSW and WA between October 2010 and October 2014, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

241. The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities9 on the consultation RARMP for DIR 101

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence that was used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. A number of submissions received raised issues relating to risks to the health and safety of people and the environment as summarised below.

Summary of issues raised	Comments
Considers there is the potential for adverse effects of the VIP3 protein to insects other than Lepidoptera in the case of a commercial release of the GMOs. Advises the Regulator to request testing of non-target organisms that consume cotton pollen (as VIP3 is produced in pollen) as part of the research requirements. Specifically, pollen beetles, hoverflies and bees should be included for testing.	Additional data on toxicity to non-targets is not required to manage risks for this trial. However, this has been identified as a future data requirement if there is an application for reduced containment measures or commercial release.
Suggests spelling out that the release could occur in all current and future cotton cropping areas in Australia, rather than using a euphemistic expression suggesting limiting the release to 34 LGAs.	The suggestion was incorporated in the RARMP.
Suggests that the Regulator clarify which sites will have a 20 m pollen trap and which sites will have a 3 km isolation zone before issuing of a licence.	As this advice does not relate to any risks to human health and safety or to the environment, no changes were made to the RARMP or licence. However, the licence holder is required to indicate whether a pollen trap or an isolation zone will be used prior to planting the GMOs.
Notes that no products from the proposed dealing will be used for human or animal consumption and that any commercial use of lint will require the Regulator's approval.	The use of lint from the GMOs in commercial products is considered in the RARMP (risk scenario 1) and poses negligible risks. FSANZ has approved food derived from the three GM parent cottons. The licence allows the commercial use of lint.
Wants clarification why VIP3A protein used in bioactivity studies is derived from non-GM maize when Vip3 is a Bt derived protein.	The typographical error has been corrected.
Asks the Regulator to consider recent field studies investigating pest numbers in Bt cotton fields in China, as there has been an increase in mirids associated with VIP3A cotton.	GM cotton containing VIP3A alone is not being released in this field trial - it is in combination with Cry proteins. The limits and controls would minimise the likelihood of unintended effects such as alterations in pest burden. Non-target pests such as mirids may be controlled by a range of methods including insecticides.

[9] GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comments
Notes that the very extensive nature of the proposed release, including both the number of sites and the area involved, questions the definition of 'trial'.	The Regulator was satisfied that this release qualified as a limited and controlled release under the Act and, therefore the use of 'trial' is considered appropriate.
Notes that the ubiquitous and generic nature of the 'cut and paste' applications and RARMPs make it difficult to differentiate between RARMPs and does not add to the credibility of the OGTR's risk assessment process.	Applications are prepared using the application form available on the OGTR website. All RARMPs regarding dealings involving the limited and controlled release of GM plants are prepared on a case-by case basis taking into account information in the application, previous releases and relevant scientific/technical information. They are structured similarly and contain similar considerations for a number of reasons, including: ▪ the same legislation applies to all DIR licence applications ▪ all plants have a number of common characteristics ▪ the harms that could potentially arise from dealings with plant GMOs fall into a limited number of risk categories ▪ all applications under section 50A of the Act propose limits and controls that are considered in the risk assessment process. These proposed limits and controls may be highly similar or even identical for GMOs of any particular crop species, as they are informed by the available scientific/technical information, previous risk assessments and licences.
Accepts that the risks from this trial may be negligible but has some concerns that should be addressed in relation to a commercial release. Notes that changes of the pest spectrum has been observed arising from cultivation of insect resistant GM cotton. Recent fast spread of cotton pathogens in NSW and Qld may be associated with introduction of GM cotton. Potential impacts of emerging pests and pathogens, and chemical applications required to deal with these, is uncertain. Recommends research on these issues.	The limits and controls would minimise the likelihood of alterations in pest and disease burden. If an application was received for the commercial release of these GMOs, then risks to people and the environment that may arise as a result of altered pest and pathogen profiles would be considered. Pests and diseases may be controlled by a range of methods including insecticides and fungicides. The use of chemicals for pest and disease management is the responsibility of the APVMA which regulates agricultural chemical use.
Accepts that the risks from this trial may be negligible but has some concerns that should be addressed in relation to a commercial release. Notes that CSIRO studies have indicated potential adverse impacts on soil biodiversity from the release of Bt toxin from the roots of GM cotton, requiring further research on these issues both during the growing season and following harvest of GM cotton.	As discussed in Chapter 1 of the RARMP, the source organism for the *cry* and *vip* genes, *Bacillus thuringiensis*, can be found in soil samples in various environments world-wide. In addition, Bt sprays have been approved for use by the APVMA in Australian horticulture and agriculture. Bollgard II® cotton has been widely cultivated in Australia since 2003 and, although Cry proteins maybe released into the soil at very low levels, there have been no demonstrated adverse impacts on soil biodiversity. As this release will be limited in time and space, any adverse impacts on soil biodiversity would be similarly limited.
Supports the trial as Bollgard III would be an important tool for Australia's cotton industry as it would provide farmers with increased resistance management benefits as well as assisting with insect control generally.	Economic benefits, including through pest control and pest resistance management, are outside the scope of the Regulator's assessments.
Concerned about toxicity to non-target organisms. Notes OGTR's acknowledgement of uncertainty and future data requirements. Requests toxicity testing against a range of insects representative of Australian cotton growing conditions using relevant plant tissue as part of this field trial.	Additional data on toxicity to non-target organisms is not required to manage risks for this trial, and is therefore not a licence condition. However data that may be required to assess a future application for a larger scale or commercial release is noted in the RARMP and will be highlighted to the applicant.

Summary of issues raised	Comments
Notes that non-GM cotton or commercially approved GM cotton to be planted in the pollen trap surrounding a trial site and recommends that approval only be given to planting of non-GM cotton and GM cotton approved for release north or 22º South.	All plant material obtained from the pollen trap must be destroyed once the GMOs are harvested and the area where the pollen trap was grown must be monitored for volunteers, which must be destroyed. Therefore, it is unlikely that volunteer plants that combine both WideStrike GM cotton and Bollgard III cotton would occur, and hence, adverse impacts on non-target species are also unlikely. The licence has been modified to clarify that WideStrike™ cotton can only be planted in pollen traps of trial areas south of 22º South.
Currently, the licence does not consider volunteers that may occur in the monitoring or exclusion zones around the trail sites and glasshouses. Therefore, monitoring and destruction of volunteers in these zones was recommended.	The licence requires inspection of the Monitoring zone and destruction of any volunteers (condition 38). No cotton crops may be planted within the Exclusion zone but inspection for volunteers is not required to manage risk.
Recommends toxicity testing against grasshoppers (order Orthoptera) and representative leaf-rolling moths (order Lepidoptera, family Torticidae, subfamily Tortricinae; some of which are considered cotton pests while others are considered beneficial to the environment).	The potential for toxicity to non-target invertebrates has been identified as an area of uncertainty. The OGTR will discuss with the applicant data that may be required for assessment of a commercial release, which may include grasshoppers, leaf-rolling moths and other non-target insects in toxicity testing.
Recommends collection of soil persistence data for the Vip3A protein for future commercial release of the GMOs.	Preliminary data suggest that Vip3 is expressed at a level of less than 2.5 µg/g fresh root tissue (see DIR 065/2006, Table 3 on p17), and that it is not likely to persist in soil for extended periods. Should any new data indicate the possibility of harm to soil organisms, data on soil persistence may be warranted.
Recommends that the licence requires data collected during this trial to be provided as part of the annual report.	Data on soil persistence and toxicity to non-target organisms is not required to manage any risks from this release. Any data generated during the release would be assessed if a future application is submitted.
Is supportive of the application. Asks for the size of the maximum permitted planting area per site to be decreased from the current 100 ha to ensure the containment conditions can be met by the licence holder and to ensure minimising the risk of the GMOs spreading to and permeating in the environment.	In the first year of the release, the maximum combined area for planting has been set at 50 ha. A range of licence conditions have been imposed to restrict spread and persistence of the GM cotton including pollen traps/isolation zones, post-harvest monitoring of sites, and destruction of volunteers. GM cotton sites have previously been up to 100 ha and have been successfully managed with similar licence conditions to restrict spread and persistence of the GM cottons. It should be noted that all parental GM cottons are either commercially released or have been field trialled in Australia and overseas without any reports of harm occurring. Should any adverse effects occur, the licence holder is required to report these to the Regulator, who would take appropriate regulatory action, if necessary, to protect human health and safety and the environment.