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

Risk Assessment and Risk Management Plan for **DIR 091**

Commercial release of cotton genetically modified for insect resistance (WideStrike™ Insect Protection Cotton)

Applicant: Dow AgroSciences Australia Ltd

November 2009

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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 091) from Dow AgroSciences Australia Ltd (Dow) for a commercial release of genetically modified (GM) cotton.

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 on whether or not to issue a licence to deal with a 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[1].

The application

Dow has applied for a licence for dealings involving the intentional release of GM WideStrike™ Insect Protection (WideStrike™) cotton. The applicant proposed that the commercial release would allow WideStrike™ cotton to be grown in all cotton growing areas of Australia south of latitude 22º South, and that plant material from the GM cotton be used in the same manner as plant material from non-GM cotton and other commercially approved GM cotton(s), and enter general commerce.

WideStrike™ cotton has been genetically modified for resistance to insects. The GM cotton contains two genes derived from a common soil bacterium. These genes confer resistance to a range of major lepidopteran caterpillar pests of cotton.

In addition to the genes for insect resistance, the GM cotton contains a selectable marker gene from a common soil bacterium. This gene confers tolerance to the herbicide glufosinate ammonium. During development of the GM cotton, this marker gene enabled identification and selection of plant tissues in which this herbicide tolerance gene was also present. Short regulatory sequences that control expression of the genes are also present in the GM cotton.

WideStrike™ cotton has been previously approved for field trials in Australia under licences DIR 040/2003 and DIR 044/2003 issued to Dow. There have been no reports of adverse effects on human health and safety or the environment resulting from these releases.

The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances. Therefore, WideStrike™ GM cotton is also subject to regulation by the Australian Pesticide and Veterinary Medicines Authority (APVMA). The APVMA is currently assessing an application from Dow for WideStrike™ cotton. The applicant does not intend glufosinate ammonium to be used as an herbicide in the field and therefore does not intend to seek approval from APVMA for the use of this herbicide on WideStrike™ cotton.

[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), and in the Regulator's Risk Analysis Framework (OGTR 2007)

The oil and cotton linters derived from this GM cotton have been approved by Food Standards Australia New Zealand (FSANZ) for use in human food[2].

Confidential Commercial Information

Some details, including the gene and protein sequences of the introduced synthetic genes and molecular characterisation of WideStrike™ cotton, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP and on the consultation RARMP.

A **hazard** identification process was used in the first instance to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Fourteen events were identified whereby the proposed dealings might give rise to harm to people or the environment. The risk assessment 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.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events 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 fourteen events in relation to both the magnitude and probability of harm, in the context of the large scale of the release proposed by the applicant, gave rise to three identified risks that required further assessment to determine their level of risk to people or the environment. The potential adverse outcomes to the environment associated with these events were toxicity to non-target invertebrates and weediness. The remaining eleven events were not assessed further as they were considered not to give rise to an identified risk to human health and safety or the environment (refer to Chapter 2 for more information).

Risk of toxicity to non-target invertebrates

One event was considered that might cause toxicity to non-target invertebrates as a result of the release of the GM cotton line via direct or indirect ingestion of the insect resistance proteins by non-target invertebrates (Event 2, Identified Risk 1).

The risk assessment considered the consequence and likelihood of harm that might result from the above event. The estimate of the level of risk for this event is **low**.

[2] Insect-protected, glufosinate ammonium-tolerant cotton line MXB-13, Dow AgroSciences, FSANZ Application A518.

Risk of weediness

Two events were considered that might result in the GM WideStrike™ cotton exhibiting greater weediness than the non-GM cotton or other GM cotton lines previously approved for commercial release.

- Expression of the introduced genes for insect resistance improving the survival of the GM cotton plants and leading to increased spread and persistence north of latitude 22º South (Event 7, Identified Risk 2).
- Expression of the introduced *cry* genes in other insect resistant GM cotton plants as a result of gene transfer leading to increased spread and persistence (Event 10, Identified Risk 3).

The risk assessment considered the consequence and likelihood of harm that might result from each of the above events. The estimate of the level of risk for Event 7 (Identified Risk 2) is **low** and Event 10 (Identified Risk 3) is **negligible**.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment.

The Regulator's *Risk Analysis Framework* (OGTR 2007) defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. The level of risk to human health and safety and the environment for twelve of the fourteen events assessed was estimated as negligible. For these events, no specific risk treatment measures are imposed.

The risk estimate for the two remaining events was low. A low risk is defined as a risk that is minimal but may evoke actions for mitigation beyond normal practices[3]. The Regulator has imposed specific licence conditions to treat the risk of spread and persistence of the GM cotton line in northern Australia. These include transport conditions and restrictions on where the seed from WideStrike™ cotton can be fed to animals.

The Regulator has also imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and included provisions to require collection of information to verify the findings of the RARMP.

The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

Conclusions of the RARMP

The risk assessment concludes that this commercial release of WideStrike™ cotton to be grown in areas south of latitude 22º South, and the entry of products derived from the GM cotton into general commerce Australia wide, poses **negligible** risks to the health and safety of people, and **negligible** to **low** risks to the environment as a result of gene technology.

The risk management plan concludes that one of the low risks requires specific risk treatment measures. General licence conditions are imposed to ensure that there is ongoing oversight of the release.

[3] The risk assessment methodology used by the Regulator is outlined in more detail at the OGTR website.

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Abbreviations

Abbreviation	Definition
the Act	*Gene Technology Act 2000*
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
bar	Bialaphos resistance gene
Bt	*Bacillus thuringiensis*
CCI	Confidential Commercial Information
Cry	Crystalline toxin from *Bacillus thuringiensis*
cv	Cultivar
DAP	Days after planting
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme linked immunosorbent assay
FSANZ	Food Standards Australia New Zealand
GI_{80}	Growth inhibition 80; the concentration of a test substance which inhibits the growth of 80% of the individuals tested
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
L	Litre
LC_{50}	Lethal concentration 50; the concentration of a test substance which kills 50% of the individuals tested
m	Metre
m^2	Square metre
mas	mannopine synthase
mM	Millimolar
NAG	N-acetyl-L-glufosinate ammonium
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OCS	octopine synthase
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin acetyl transferase
PC2	Physical Containment Level 2
RARMP	Risk Assessment and Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RMP	Resistance Management Plan
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
Ti	Tumour inducing
TIMS	Transgenic and Insect Management Strategy
TGA	Therapeutic Goods Administration
Ubi	Ubiquitin
USDA	United States Department of Agriculture
UTR	Untranslated region

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 091) from Dow AgroSciences Australia Ltd (Dow) for a commercial release of genetically modified (GM) cotton.

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 on whether or not to issue a licence to deal with a 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[4].

The application

Dow has applied for a licence for dealings involving the intentional release of GM WideStrike™ Insect Protection (WideStrike™) cotton. The applicant proposes that the commercial release would allow WideStrike™ cotton to be grown in all cotton growing areas of Australia south of latitude 22º South and that plant material from the GM cotton be used in the same manner as plant material from non-GM cotton and other commercially approved GM cotton(s), and enter general commerce.

WideStrike™ GM cotton has been genetically modified for resistance to insects. The GM cotton contains the synthetic genes *cry1Ac(synpro)* and *cry1F(synpro)*, the sequences of which were originally derived from the common soil bacterium *Bacillus thuringiensis* (Bt). These genes confer resistance to a range of major lepidopteran caterpillar pests of cotton. The two *cry* genes are synthetic genes: the *cry1Ac(synpro)* gene is composed of part of the *cry1Ac*, *cry1Ca3* and *cry1Ab1* genes from Bt; and *cry1F(synpro)* is composed of parts of the *cry1Fa*, *cry1Ca3* and *cry1Ab1* genes. These genes encode the protein toxins Cry1Ac(synpro) and Cry1F(synpro).

In addition to the *cry1Ac(synpro)* and *cry1F(synpro)* genes, the GM cotton contains a selectable marker gene (*pat*) from the common soil bacterium *Streptomyces viridochromogenes*. The *pat* gene confers tolerance to the herbicide glufosinate ammonium. During development of the GM cotton, this marker gene enabled identification and selection of transformed plant tissues. The applicant does not intend glufosinate ammonium to be used on the GM cotton.

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

The *cry1Ac(synpro)* and *cry1F(synpro)* genes were introduced separately into cotton plant tissue (American cotton cultivar GC510) to generate transformation events 281-24-236 and 3006-210-23, respectively. Each insecticidal gene was introduced in combination with the

[4] 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 the OGTR website), and in the Regulator's *Risk Analysis Framework* (OGTR 2007)

selectable marker gene, *pat*, providing a means of selection of plant cells expressing the desired modifications. The gene constructs were introduced into the original events by *Agrobacterium*-mediated transformation. This method has been widely used in Australia and overseas for introducing new genes into plants.

The two cotton events expressing the insecticidal genes were combined by conventional breeding to generate the GM cotton proposed for release (WideStrike™ cotton). This GM cotton contains both the *cry1Ac(synpro)* and *cry1F(synpro)* genes and two complete copies of the *pat* gene; as well as a small additional fragment of the *pat* gene.

WideStrike™ cotton has been previously approved for field trials in Australia under licences DIR 040/2003 and DIR 044/2003 issued to Dow. There have been no reports of adverse effects on human health and safety or the environment resulting from these releases.

The oil and cotton linters derived from this GM cotton have been approved by Food Standards Australia New Zealand (FSANZ) for use in human food[5].

Confidential Commercial Information

Some details, including the gene and protein sequences of the introduced synthetic genes and molecular characterisation of WideStrike™ cotton, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment takes into account information in the application, relevant previous approvals, current scientific knowledge, and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP and on the consultation RARMP.

A reference document, *The Biology of* Gossypium hirsutum *and* Gossypium barbadense *(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.

The risk assessment begins with a hazard identification process, to consider what harm to the health and safety of people or the environment could arise during this release and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment. The receiving environment includes commercially approved GM cotton lines currently grown in Australia. In taking into account a potential risk, the Regulator must consider the probability and potential impact of an adverse outcome over the foreseeable future.

Fourteen events were identified whereby the proposed dealings might give rise to harm to people or the environment. The risk assessment 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.

A risk is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent

[5] Insect-protected, glufosinate ammonium-tolerant cotton line MXB-13, Dow AgroSciences, FSANZ Application A518.

an identified risk and do not advance any further in the risk assessment process. The events that are considered to have the potential to lead to adverse outcomes are assessed further to determine the seriousness of harm (consequence) that could result and how likely it is that the harm would occur. The level of risk is then estimated using the Risk Estimate Matrix (see below and Chapter 2).

		RISK ESTIMATE			
LIKELIHOOD	Highly likely	**Low**	**Moderate**	**High**	**High**
	Likely	**Negligible**	**Low**	**High**	**High**
	Unlikely	**Negligible**	**Low**	**Moderate**	**High**
	Highly unlikely	**Negligible**	**Negligible**	**Low**	**Moderate**
		Marginal	Minor	Intermediate	Major
		CONSEQUENCES			

Figure 1The OGTR Risk Estimate Matrix (OGTR 2007)

Risk Estimate Matrix: A negligible risk is considered to be insubstantial with no present need to invoke actions for mitigation. A low risk is considered to be minimal but may invoke actions for mitigation beyond normal practices. A moderate risk is considered to be of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. A high risk is considered to be unacceptable unless actions for mitigation are highly feasible and effective.

The characterisation of the fourteen events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, gave rise to three identified risks that required further assessment to determine their level of harm to people or the environment.

The consequence and likelihood assessments used to derive risk estimates for the three Identified Risks are summarised in Table 1 (the detailed risk assessments are in Chapters 3 and 4 of the RARMP). More information on the remaining events that were considered not to give rise to an identified risk is provided in Chapter 2. If a risk is estimated to be higher than negligible, risk treatment measures may be required to protect the health and safety of people or the environment.

Table 1 Summary table for the risk assessment

Potential adverse outcome	Event that may give rise to the adverse outcome	Consequence assessment	Likelihood assessment	Risk estimate	Risk treatment required?
Toxicity to non-target invertebrates (see Chapter 3)	**Identified Risk 1** Direct or indirect ingestion of the introduced Cry1Ac(synpro) and Cry1F(synpro) proteins by non-target invertebrates.	**Minor** • Non-target dietary toxicity studies suggest Cry1Ac(synpro) and Cry1F(synpro) proteins are toxic or growth inhibitory only to a limited range of insects, including the specified target insects. • A field study suggests that growing WideStrike™ cotton plants has no significant effect on non-target invertebrate populations when compared to unsprayed non-GM cotton. • Non-GM cotton is sprayed with insecticides which impact on non-target insects.	**Unlikely** • Exposure to the GM cotton lines and the Cry proteins would occur mostly to those non-target invertebrates directly/indirectly consuming the GM cotton within the cotton field. • Non-target invertebrates appear insensitive to the levels of Cry1Ac(synpro) and Cry1F(synpro) proteins expressed in the WideStrike™ plants.	**Low**	**No**, however PRR conditions are imposed.
Weediness (see Chapter 4)	**Identified risk 2** Expression of the introduced genes for insect resistance improving the survival of GM cotton plants and leading to increased spread and persistence north of latitude 22º South.	**Minor** • The expressed genes for insect resistance are not expected to impact on health of humans, other vertebrates or microorganisms. • The expression of *cry* genes will not extend the range of GM cotton compared to non-GM cotton.	**Unlikely** • WideStrike™ cotton will not be grown north of latitude 22º South. • WideStrike™ cotton volunteers can be effectively controlled by mechanical means, or if still at the seedling stage by the use of herbicides. • The chance of GM volunteer plants arising from unintended seed dispersal finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton. • The expressed genes for insect resistance would only confer a selective advantage in areas where insect predation limits cotton.	**Low**	**Yes** PRR conditions are imposed.
	Identified risk 3 Expression of the introduced *cry* genes in other insect resistant GM cotton plants as a result of	**Minor** • The expressed genes for insect resistance are not expected to impact on health of humans, other vertebrates or microorganisms. • The expression of *cry* genes will not extend the range of GM cotton compared to non-GM cotton. • Although the effects of combining the *cry* genes from WideStrike™ and Bollgard® cotton	**Highly unlikely** • Cotton is primarily self-pollinating and gene transfer to other insect resistant GM cotton plants would only occur over short distances and at low frequencies. • The GM cotton will not be grown north of latitude 22º South. • The chance of GM volunteer plants arising from seed dispersal finding suitable conditions to establish as weeds may be no	**Negligible**	**No**

Potential adverse outcome	Event that may give rise to the adverse outcome	Consequence assessment	Likelihood assessment	Risk estimate	Risk treatment required?
	gene transfer leading to increased spread and persistence.	could provide unexpected protection from herbivory, if GM cotton were to spread and persist it is expected to have a limited impact on native vegetation and this would only occur in areas with suitable environmental conditions.	greater than for non-GM cotton plants. • Although reduced lepidopteran insect herbivory may offer a small competitive advantage, abiotic and biotic factors are likely to be more important in limiting the spread and persistence of cotton, especially in southern Australia. • Insect resistant cotton volunteers can be effectively controlled by mechanical means, or if still at the seedling stage by the use of herbicides.		

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment.

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. Low risks are defined as minimal but may invoke actions for mitigation beyond normal practices. The level of risk to human health and safety and the environment for twelve of the fourteen events assessed was estimated as negligible. Therefore, no specific risk treatment measures are imposed in relation to these. The risk estimate for the two remaining events was low. The Regulator has proposed several licence conditions that would treat the low risk of spread and persistence of the GM cotton in northern Australia. The Regulator has also imposed licence conditions under post-release review (PRR).

Licence conditions

The licence contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects. There are also general conditions to ensure ongoing oversight of the release.

The Regulator has also imposed several specific licence conditions including requirements to:

- transport whole GM cotton seed in covered vehicles in areas north of latitude 22º South
- in areas north of latitude 22º South, only feed GM cotton seed to livestock inside stockyards, feedlots or dairies
- inform people of the specific conditions referred to in the above dot points
- survey areas where livestock are fed GM cotton seed north of latitude 22º South, in order to determine the incidence of volunteer plants in these areas
- undertake confirmatory research to collect further information on potential effects on key non-target invertebrates.

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 Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine and Inspection Service (AQIS) [6]. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies.

FSANZ is responsible for human food safety assessment, including GM food. FSANZ has approved the use of linters and cotton seed oil from WideStrike™ cotton for use in human food.

The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances. Therefore, these plants are also subject to regulation by the APVMA. The APVMA is currently assessing an application from Dow for WideStrike™ cotton.

Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend this herbicide to be used on the cotton and therefore is not seeking approval for this from the APVMA.

The Regulator is liaising closely with the APVMA during the assessment of the application pertaining to this commercial release of GM WideStrike™ cotton.

An AQIS permit has been granted to allow the importation of seed.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for reduced containment measures for the commercial release of WideStrike™ cotton north of latitude 22º South. This would include:

- characteristics, type and abundance of beneficial/non-target invertebrates in crops of the GM cotton grown north of latitude 22º South
- information on the potential for WideStrike™ cotton to have increased survival in the natural environment compared to other commercial GM and non-GM cottons as a result of the introduced genes for insect resistance
- information on any potential synergistic effects of the introduced genetic material when stacked with Bollgard II® cotton [either as individual genes or in combination].

The applicant would be encouraged to work with the Regulator in the design of experiments to address these issues, and would require an additional authorisation from the Regulator to undertake plantings of GM WideStrike™ cotton north of latitude 22º South.

[6] More information on Australia's integrated regulatory framework for gene technology is contained in the Risk Analysis Framework available from the OGTR (free call 1800 181 030 or at the OGTR website).

Conclusions of the RARMP

The risk assessment concludes that this commercial release of WideStrike™ cotton to be grown in areas south of latitude 22º South, and the entry of products derived from the GM cotton into general commerce Australia wide, poses **negligible** risks to the health and safety of people, and **negligible** to **low** risks to the environment as a result of gene technology.

The risk management plan concludes that one of the low risks requires specific risk treatment measures. General licence conditions are imposed to ensure that there is ongoing oversight of the release.

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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people or the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation[7] , details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 2.

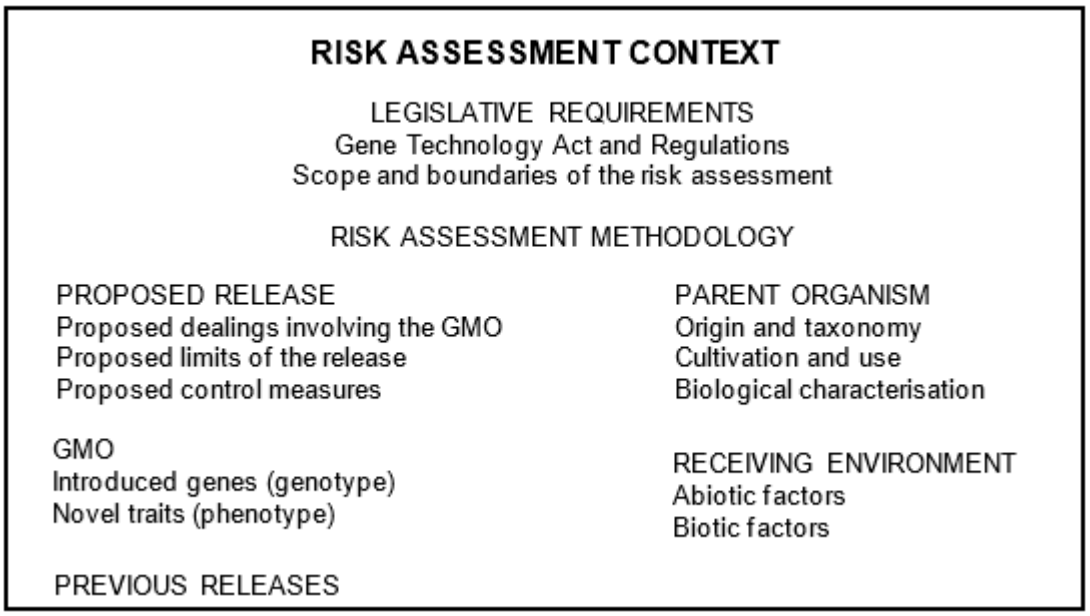

Figure 2Components of the context considered during the preparation of the risk assessment

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

- the legislative requirements (Section 2)
- the risk assessment methodology[8]
- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the GMO, nature and effect of the genetic modification (Section 5)
- the receiving environment (Section 6)
- previous releases of this or other GMOs relevant to this application (Section 7).

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with

[7] The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at the OGTR website and in the *Risk Analysis Framework* (OGTR 2007).
[8] The risk assessment methodology used by the Regulator is outlined in more detail in the *Risk Analysis Framework* (OGTR 2007).

whom the Regulator must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of decisions on licence applications proposing dealings involving intentional release of a GMO into the environment.

4. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to consult with prescribed experts, agencies and authorities to seek advice on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, local council that the Regulator considered appropriate[9] and the Minister for the Environment, Water, Heritage and the Arts. A summary of issues contained in submissions received is given in Appendix B.

5. In addition, sections 50 and 51 of the Act list the matters which the Regulator must take into account in preparing the RARMPs that form the basis of his decisions on licence applications. The *Gene Technology Regulations 2001* (the Regulations) also prescribe additional matters the Regulator must consider when preparing a RARMP.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Issues contained in the submissions received, and how these were taken into account, are summarised in Appendices C and D.

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 minimum length of the second consultation period as specified in section 52(2)(d). The Regulator considered that the dealings proposed do not pose a significant risk to either people or the environment.

8. Some details of the GMO, including the gene and protein sequences of the introduced synthetic genes and molecular characterisation of WideStrike™ cotton, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. However, the applicant consented to release some of the CCI in the final RARMP and other public documents.

Section 3 The proposed release

3.1 The proposed dealings

9. Dow AgroSciences (Dow) proposed to release one cotton line, WideStrike™ Insect Protection cotton (WideStrike™ cotton), that has been genetically modified for resistance to certain lepidopteran insect pest species, into the environment. Post-harvest, the fibre would be separated from the seed. The seed may then be processed for oil, meal, hulls and linters. The applicant proposes that any cotton products, including cotton seed, would enter general commerce in Australia.

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

- conducting experiments with the GMO
- making, developing, producing or manufacturing the GMO

[9] In this instance, the Acting Regulator decided to consult with all 39 local councils in the current identified cotton growing regions of Australia south of latitude 22º South.

- breeding the GMO with Australian cotton cultivars
- propagating the GMO
- using the GMO in the course of manufacture of a thing that is not the GMO
- growing, raising or culturing the GMO
- transporting the GMO
- disposing of the GMO
- importing the GMO.

11. The dealings would also include the possession, supply or use of the GMO for the purposes of, or in the course of any of the dealings mentioned above. The above dealings are detailed further throughout the remainder of the current Chapter.

3.2 The proposed measures to limit the release

12. The applicant has stated that the principal purpose of the proposed release is to allow WideStrike™ cotton to be grown commercially in Australia, south of latitude 22º South, and for harvested plant material to enter general commerce. The applicant also proposes that no restrictions be placed on the use of the GM cotton seed, cottonseed oil and meal in animal feed or human food. Food Standards Australia New Zealand (FSANZ) has approved products from WideStrike™ cotton for use in human food (application A518).

13. The applicant has proposed to inform all personnel involved in the handling, transport or other activities with WideStrike™ cotton, or products thereof, via a notification on the seed label of relevant information.

14. The applicant has not proposed any controls to restrict the release to south of latitude 22º South, other than where the GM cotton would be grown. However, the applicant has proposed a staged introduction of WideStrike™ cotton.

Section 4 The parent organism

15. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia but is grown as an agricultural crop in New South Wales (NSW) and southern and central Queensland (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.

16. The original transformation events were generated in the cotton cultivar Acala GC510. This is a commercial cultivar released in America by Germain's Agribusiness Inc in 1984 (USDA-APHIS 2008). The transformed cotton lines were then backcrossed to the cultivar PSC-355, a cultivar grown in the USA. The applicant intends to breed the GMO with cultivars relevant to the Australian cotton growing regions.

Section 5 The GMO, nature and effect of the genetic modification

5.1 Introduction to the GMO

17. The cotton line proposed for commercial release is WideStrike™ cotton, also known as cotton line 281-24-236/3006-210-23 or MXB-13 cotton. It contains one copy of each of two synthetic versions of genes encoding δ-endotoxins Cry1Ac(synpro) and Cry1F(synpro) originally derived from the bacterium *Bacillus thuringiensis* (Table 2). The introduced genes encode proteins that are toxic to certain lepidopteran insect pests of cotton.

18. The GM cotton also contains two full length copies and one partial copy of the *pat* gene, encoding the phosphinothricin acetyl transferase enzyme, that has been shown to confer tolerance to herbicides containing glufosinate ammonium. The *pat* gene was included in the GM cotton as a selectable marker gene to allow effective selection of modified plants in the laboratory. The applicant does not intend the herbicide tolerance trait to be used in the field.

Table 2 The genes introduced into the GM cotton

Gene	Protein produced	Comment	Protein function	Source
cry1Ac(synpro)	Cry toxin	Cry genes encode crystalline insecticidal proteins, highly specific to their target insects and used for insect pest control	Insect resistance	Synthetic plant codon-optimised gene from *Bacillus thuringiensis*
cry1F(synpro)	Cry toxin	Cry genes encode crystalline insecticidal proteins, highly specific to their target insects and used for insect pest control	Insect resistance	Synthetic plant codon-optimised gene from *B. thuringiensis*
pat	Phosphinothricin acetyl transferase (PAT)	Marker gene widely used in plant genetic modification; the encoded enzyme, PAT, confers tolerance to phosphinothricin herbicides	Herbicide tolerance	Plant codon-optimised gene from *Streptomyces viridochromogenes*

19. Short regulatory sequences that control expression of the introduced genes are also present in WideStrike™ cotton (Table 3). Further details of these genetic elements are given in Chapter 1, Section 5.2.

Table 3 The regulatory sequences used in the genetic modification of cotton

Regulatory Sequence	Genbank Accession No.	Description	Function	Source
(4OCS)Δmas2'	X00493 (mas) I05704 – I05712 (ocs) enhancer	Mannopine synthase promoter including 4 copies of the ocs enhancer element of the octopine synthase gene	Constitutive promoter	*Agrobacterium tumefaciens*

Regulatory Sequence	Genbank Accession No.	Description	Function	Source
ORF 25	X00493	Bidirectional polyadenylation signal of open reading frame - 25	Terminator	*Agrobacterium tumefaciens*
Ubi	138571	Constitutive promoter	Constitutive promoter	*Zea mays*

5.1.1 Target species

20. The applicant states that the target species for WideStrike™ cotton are cotton bollworm (*Helicoverpa armigera*), native budworm (*H. punctigera*), cotton bollworm (*H. zea*), tobacco budworm (*Heliothis virescens*), pink bollworm (*Pectinophora gossypiella*), beet armyworm (*Spodoptera exigua*), fall armyworm (S. frugiperda), yellowstriped armyworm (*S. ornithogalli*), other unspecified armyworms (*Spodoptera* spp., including cluster caterpillar *S. litura*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), cutworm (*Agrotis ipsilon* and other spp.), European corn borer (*Ostrinia nubilalis*) and saltmarsh caterpillar (*Estigmene acrea*).

21. A number of these species are not present in Australia, or are not major pests of commercial cotton. In the southern cotton growing areas in Australia, the major cotton lepidopteran pests are *H. armigera* and *H. punctigera*. The arthropod two-spotted spider mite (*Tetranychus urticae*) is also considered a pest in this area. Cluster caterpillar (*S. litura*) and pink bollworm (*P. gossypiella*) are lepidopteran species that are considered major additional pests in northern Australia.

5.2 The introduced genes and their encoded proteins

5.2.1 Introduction to Cry proteins

22. The Cry proteins, also referred to as δ-endotoxins or insecticidal crystal proteins, are one class of toxins produced by *Bacillus thuringiensis* (Bt). They may be defined as 'a parasporal inclusion protein from Bt that exhibits toxic effects to a target organism, or any protein that has obvious sequence similarity to a known Cry protein' (Crickmore et al. 1998).

23. During sporulation, Bt produces a parasporal crystal composed of one or more Cry proteins. The formation of the parasporal crystal distinguishes Bt from other *Bacillus* species. The Cry proteins of each Bt subspecies are often toxic to specific taxonomic classes of invertebrate. Cry proteins with toxicity to insects, including Lepidoptera (butterflies and moths), Coleoptera (beetles and weevils), Hymenoptera (wasps and bees) and Diptera (flies and mosquitoes), or to nematodes have been found (reviewed in Bravo et al. 2007).

24. Cry proteins are classified according to their degree of amino acid homology (reviewed in Hoefte & Whiteley 1989; see also Crickmore et al. 2009), which also determines their target specificity. Each Cry toxin has a defined spectrum of insecticidal activity, usually restricted to particular species within a certain taxonomic group (reviewed in Roh et al. 2007). For example, the Cry1 protein family is generally toxic to lepidopteran species.

Mode of action of Cry proteins

25. Currently, the favoured model for the mode of action of Cry proteins in lepidopteran species involves the occurrence of a series of steps before toxicity can eventuate. This model

takes into account the three-domain structure of known activated Cry proteins. The model has been reviewed (Bravo et al. 2007; Roh et al. 2007; Soberon et al. 2009) and is summarised below;

- Either one or more types of Cry proteins are present in the Bt parasporal crystals. Other proteins may also be present in those crystals.
- For solubilisation, the protoxin crystals require alkaline conditions with pH values of 10 or higher. These conditions can be found in the larval insect gut. The Cry protoxins must be partially digested by midgut proteases to release the active toxin. In the case of Cry1 proteins, 25 – 30 amino acids are cleaved off the amino-terminus and approximately half of the remaining protein off the carboxy-terminus resulting in an activated toxin with an approximate relative molecular weight of 60 – 70 kDa. The carboxy terminal portion of all known Cry proteins is highly conserved (although some Cry proteins lack this part) and it has been suggested that its function lies in aiding crystal formation of the protoxins.
- The activated toxin, which may be referred to as the truncated toxin, truncated protein or core toxin, has a three-domain structure in which the first domain is responsible for pore formation, while domains II and III are involved in receptor recognition and binding and are thus largely responsible for determining specificity (de Maagd et al. 2000; de Maagd et al. 2003).
- Specific receptors for the activated toxin are found on the brush border membrane of the midgut epithelium columnar cells (Hofmann et al. 1988; Van Rie et al. 1989; Karim et al. 2000). Currently, some candidate receptor molecules are being investigated. These receptors have not been found in mammals (Noteborn 1995, as cited in Federici 2003).
- Binding of activated Cry toxin to its receptor may lead to a change in Cry protein conformation, which facilitates insertion of at least part of domain I into the cell membrane. Upon oligomerisation of the activated, membrane-inserted toxin, pores are formed in the cell membrane with the proposed involvement of domain III. Upon pore formation, osmotic cell lysis results in leakage of intracellular contents into the gut lumen and the insect eventually dies.

26. By interchanging the individual domains from different members of the Cry protein family, chimeric proteins with altered specificity or toxicity can be produced (de Maagd et al. 1996). In addition, different modifications performed on the toxin gene, such as site-directed mutagenesis, introduction of cleavage sites in specific regions of the protein or deletion of small fragments from the amino-terminal region, can lead to improved toxicity or overcome resistance in insects (Pardo-Lopez et al. 2009).

27. A study by Hernández and Ferré (2005) indicates that Cry1Fa competes for the same binding site as Cry1Ac in the brush border membrane vesicles of *H. armigera*, *H. zea* and *S. exigua*. Binding of the Cry1Ac protein appeared stronger than that of the Cry1F protein. No indication of an additional binding site for Cry1Fa was found. It is thought that this may be the case for a wide range of lepidopteran species. For example, resistance to Cry proteins in *Plutella xylostella* is due to an autosomal recessive gene which provides resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa and Cry1Ja, implying that there is a common binding site (Tabashnik et al. 1997).

5.2.2 The introduced synthetic insecticidal crystal protein genes, cry1Ac(synpro) and cry1F(synpro), and the encoded proteins

The introduced cry1Ac(synpro) gene and its encoded protein

28. The *cry1Ac(synpro)* gene in the GM cotton event is a synthetic gene, combining parts of three different *cry* genes isolated from *Bacillus thuringiensis* (Bt) (see Figure 3). The part of the synthetic *cry1Ac(synpro)* gene which corresponds to the active core (functional) toxin is derived from the native *cry1Ac1* gene of Bt variety *kurstaki* strain HD73 (GenBank accession number AAA22331; Adang et al. 1985). Nucleotides 1-1844 of the coding sequence, encoding the first 614 amino acids, were taken from this gene (Narva et al. 2001b). The remainder of the gene, encoding the carboxy-terminal portion of the protein which is cleaved off in the insect gut, is derived from parts of other cry genes. Nucleotides 1845-1951, encoding amino acid residues 615-650, were derived from the *cry1Ca3* gene. The *cry1Ca3* gene was originally derived from Bt variety *aizawai* strain PS81I as described by Feitelson in 1993 (GenBank accession number AAA22343). Nucleotides 1952-3471, encoding amino acid residues 651-1156, were derived from *cry1Ab1*. This gene was originally isolated from Bt variety *berliner* strain 1715 and reported by Wabiko et al (1986; GenBank accession number AAA22330).

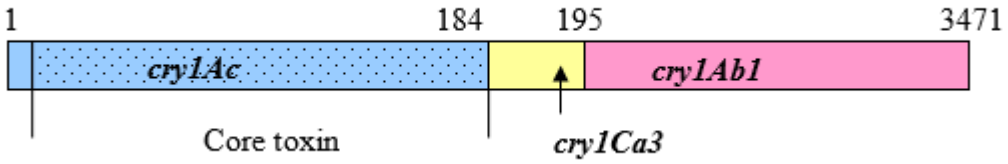

Figure 3 Structure of synthetic *cry1Ac(synpro)* gene

29. The *cry1Ac(synpro)* gene encodes a 131 kDa protein which is the full length protoxin (Gao et al. 2002a; Gao et al. 2002b). The core protein is approximately 65 kDa.

30. The coding sequence of the chimeric *cry1Ac(synpro)* gene has been further modified to achieve optimal expression in plants, without affecting the predicted protein sequence. This is needed as bacterial genes often contain some sequences with the potential to act as polyadenylation sites (often A+T rich), a higher G+C content than is frequently found in dicotyledonous plant genes, concentrated stretches of G and C, and codons that are not frequently used in dicotyledonous plant genes. To ensure the bacterial gene was expressed optimally in plants, a plant-preferred version of the *cry1Ac(synpro)* gene was synthesised.

31. The synthetic *cry1Ac(synpro)* gene encodes a protein toxin, Cry1Ac(synpro), which is very similar to the native Cry1Ac proteins.

32. Cry1 protoxins are activated by the action of specific proteases, and activated native Cry1Ac is generated by cleavages of 28 amino acids at the N-terminal and almost half the remaining protein from the carboxy-terminus, leaving a protease resistant core of approximately 600 amino acids (Lightwood et al. 2000; Bravo et al. 2007).

The introduced cry1F(synpro) gene and its encoded protein

33. The *cry1F(synpro)* gene in the GM cotton line is a synthetic gene, combining parts of three different *cry* genes isolated from Bt. The first portion of the synthetic *cry1F(synpro)* gene is derived from the native *cry1Fa2* gene of Bt var *aizawai* strain PS81I (nucleotides 1 – 1810, encoding the first 603 amino acid residues). The *cry1Fa2* gene was described by Feitelson in 1993 (GenBank accession number AAA22347). The remainder of the gene,

encoding the carboxy-terminal portion of the protein, is derived from parts of the *cry1Ca3* (nucleotides 1811-1917, encoding the amino acid sequence up to residue 639) and *cry1Ab1* (nucleotides 1918-3447, encoding the remaining amino acid residues) genes as described for Cry1Ac(synpro). The active core toxin is made up of the *cry1F* sequence, together with a small portion of the *cry1Ca3* gene sequence (see Figure 4) (Narva et al. 2001a). The coding sequence of the chimeric *cry1F(synpro)* gene has been further modified to achieve optimal expression in plants, without affecting the predicted protein sequence, as described above for *cry1Ac(synpro)*.

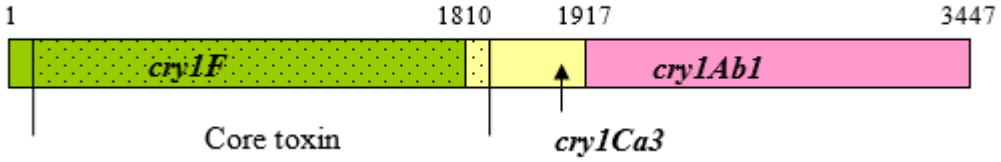

Figure 4 Structure of synthetic *cry1F(synpro)* gene

34. The *cry1F(synpro)* gene encodes the full length protoxin, a protein of approximately 130 kDa (Gao et al. 2006). The core protein is an approximately 65 kDa protein.

35. The synthetic *cry1F(synpro)* gene encodes a protein toxin, Cry1F(synpro), which is very similar to the native Cry1F protein.

36. As described for other Cry1 toxins (Bravo et al. 2007), specific proteases cleave off the carboxyl-terminal domain of Cry1F(synpro), as well as approximately 25-30 amino acids from the amino-terminal end, leaving an active protease-resistant core of approximately 600 amino acids. The applicant has stated that putative protease cleavage sites for Cry1F(synpro) are located at R28 or R31 (N-terminal) and R612 or K615. The second site thus may be within the sequence encoded by *cry1Ca3*, which is predicted to encode 8 or 11 amino acid residues of domain III of Cry1F(synpro). As noted above (Section 5.2.1), domain III is implicated in determining the range of susceptible organisms affected by Cry proteins (de Maagd et al. 1999).

5.2.3 Toxicity/allergenicity of the Cry proteins encoded by the introduced genes

37. General information on toxicity of the native Cry1Ac and Cry1F toxins, and the synthetic toxins, is presented here, with more specific information on the toxicity of the WideStrike™ cotton plant material in Section 5.5.2.

Equivalence of microbially produced proteins

38. In order to carry out the toxicity, biochemical and insecticidal studies, the applicant modified bacteria (*Pseudomonas fluorescens* strain MR872) to produce Cry1Ac(synpro) and Cry1F(synpro). These proteins were then compared to those produced *in planta*. It was concluded from a number of experiments that *in planta* and microbially produced proteins were biochemically equivalent. These experiments included Western blot and SDS-PAGE analysis, amino-terminal sequencing, glycosylation analysis, peptide mass fingerprinting and matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy.

39. Information on the degree of identity and similarity of bacterially-derived and plant-derived Cry1Ac(synpro) proteins has been provided and declared CCI by the Regulator.

40. The predicted amino acid sequences of the bacterially-derived and plant-derived Cry1F(synpro) proteins are identical for the first part of the Cry1F core toxin sequence (amino acids 1-603). For the remaining sequence, the plant derived sequence corresponds to

Cry1Ca3 and Cry1Ab1 as expected (Figure 2). However, there are four amino acid differences for the microbially derived sequence compared to the plant derived Cry1F(synpro) protein: an F604L substitution, resulting from a codon change to enable cloning of the chimeric carboxy-terminal part of the protoxin, and Y608S, I624S and I629L within the Cry1Ca3 portion of the carboxy-terminal domain (Gao et al. 2001). These changes render the microbial sequence identical to native Cry1F for this region. Two of the changes (F604L and Y608S) lie within the predicted carboxy-terminal domain of the core toxin, a region thought to be involved in receptor binding (de Maagd RA et al. 2000).

41. To assess the biological equivalence of plant-produced and microbially-produced Cry1F(synpro), separate toxicity studies were conducted for each, and the results compared (Herman 2001a). Each of the studies assessed toxicity to three lepidopteran pests with known varying sensitivity to Cry1F toxins, *H. virescens*, *S. exigua* and *H. zea* (species which are important pests of cotton in the USA but not in Australia). Mortality and insect-weight data were collected after feeding larvae on agar feeding trays spiked with a range of concentrations of Cry1F(synpro), after six days for the plant-produced protein and after seven days for the microbially-produced protein. The degree of purity of the microbially-produced Cry1F(synpro) was not indicated. Freeze dried leaf of cotton line 281-24-236, expressing Cry1F(synpro), was used as the source of plant-derived Cry1F(synpro). The concentration of active ingredient (ie Cry1F(synpro) in either plant or microbial preparations) giving 80% growth inhibition (GI_{80}) was calculated. For each species, similar GI_{80} values were found for each of the Cry1F(synpro) sources, while for the three species the GI_{80} values differed by an order of magnitude (*H. virescens* < *S. exigua* < *H. zea*). While insect weights in the negative controls differed across the studies and details of data analysis are not provided, the consistent response to the plant- and microbially-derived Cry1F(synpro) provides some support for their biological equivalence.

Toxicity/allergenicity to humans

42. *Bacillus thuringiensis* (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). Individual strains of Bt may produce up to six different Cry proteins.

43. Microbial preparations of Bt have also been used for decades as pesticides, being first commercialised as insecticidal products in France in the late 1930s. Since then there have been numerous commercial releases of Bt insecticidal products and of crops genetically modified to express delta-endotoxins for insect resistance (Sanchis & Bourguet 2008). Microbial Bt preparations are used on a variety of fibre crops as well as food crops, including grains, fruits and vegetables. In addition, they are used in controlling forest pests, mosquitoes and blackflies (OECD 2007). On this basis people and other organisms have a long history of exposure to Bt toxins.

44. The APVMA has approved a number of products containing various Bt subspecies for use as insecticides, including strains of the subspecies *aizawai* and *kurstaki*, from which parts of the introduced genes were originally derived.

45. Bt does not have a history of causing allergenicity in humans. There have been rare reports of occupational allergies associated with the use of Bt insecticidal products containing Bt.

46. 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.

47. The US EPA has since determined that the dermal allergic reactions reported by Bernstein et al. 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).

48. The Cry1Ac(synpro) and Cry1F(synpro) proteins are approximately 131 kDa and 130 kDa in size, respectively, and are therefore considerably larger than typical allergenic proteins.

49. Microbially produced Cry1Ac(synpro) was rendered inactive on *Heliothis virescens* after exposure to 90ºC for 30 min (Herman & Gao 2001a). Microbially produced Cry1Ac(synpro) was rapidly degraded in *in vitro* simulated gastric digestion experiments, ie in less than one minute (Korjagin 2001). In *in vitro* simulated intestinal fluid digestibility studies, microbially produced Cry1Ac(synpro) was degraded rapidly to the activated core toxin. The truncated protein was stable for the remainder of the test (4 hrs) (Korjagin 2003).

50. Cry1Ac is rapidly degraded (under 30 seconds) under simulated mammalian gastrointestinal conditions (Fuchs et al. 1993).

51. Microbially produced Cry1F(synpro) is rendered inactive on *Heliothis virescens* after exposure to 75ºC for 30 min (Herman & Gao 2001b). In *in vitro* simulated intestinal fluid digestibility studies, microbially produced Cry1F(synpro) was degraded rapidly to the activated core toxin. The truncated protein was stable for the remainder of the test (4 hrs) (Korjagin & Embry 2003).

52. The likelihood of a protein having toxic or allergenic properties can be predicted, on a purely theoretical basis, by bioinformatic analysis. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The applicant has compared the amino acid sequences of the proteins encoded by each of the introduced *cry* genes to databases of known toxins and allergens. The results of these analyses did not indicate that any of the encoded proteins shared any significant sequence homology with any known toxins or allergens (Stelman 2001a; Stelman 2001b).

53. FSANZ has approved food use in Australia of products, ie oil and linters, derived from plants expressing Cry proteins. These include products from WideStrike™ and other GM cotton lines, as well as different lines of corn expressing Cry1Ab, Cry1Ac, Cry1F or Cry3Bb1, and potato expressing Cry3Aa[10].

Toxicity to vertebrates

54. Vertebrates are not expected to be susceptible to Cry proteins. This is partly because the alkaline conditions required to activate the toxin do not exist in the guts of mammals and because the toxic effects of Cry proteins are mediated through binding to receptors in the mid gut of target insects, which are not present in mammals, birds and fish.

55. The toxicological database on Bt shows no mammalian health effects attributable to delta-endotoxins. In particular, the European Food Safety Authority (EFSA) evaluated the

[10] Source:, accessed 7 July 2009.

food safety of delta-endotoxins expressed in maize plants, such as Cry1Ab in Bt11 (EFSA 2005) and purified Cry proteins, including Cry1Ab. These studies and acute oral toxicity studies (Mendelsohn et al. 2003; OECD 2007) also confirm the low toxicity of the Cry proteins studied. In tests examining the morphology, secretion of albumin and release of lactate dehydrogenase of cultured bovine hepatocytes exposed for 24 h and 48 h to Cry1Ab, it was found that Cry1Ab has little acute toxicity to these mammalian cells (Shimada et al. 2003).

56. Three acute toxicity studies were conducted in mice using microbially produced Cry1F(synpro), microbially produced Cry1Ac(synpro) or a combination of both (Brooks & Andrus 1999; Brooks & Yano 2001a; Brooks & Yano 2001b). The three studies each used five male and five female mice. Parameters evaluated included detailed clinical observations and gross pathological changes. No mortality or adverse clinical signs, including pathological lesions were observed on any of the test animals given unpurified Cry1Ac(synpro) or Cry1F(synpro), or a purified (to approximately 15%) 50:50 mixture of the two proteins. However, one female mouse given Cry1F(synpro) had a moderate increase in reactivity to handling on one day.

57. An acute oral toxicity study was conducted with the northern bobwhite quail (*Colinus virginianus*) using microbially produced Cry1F(synpro) and Cry1Ac(synpro) (Gallagher & Beavers 2002b). Parameters evaluated included abnormal behaviour, physical injury, body weight/feed consumption and gross pathological changes. None of the animals died during the experiment. However, the majority of birds exhibited clinical signs, including wing drop, ruffled appearance and lethargy at some point in the study. Those clinical signs were present in both the test and control group. They were attributed to gossypol toxicity as the feed contained approximately 800 to 1,000 ppm gossypol. The safe limit of gossypol is considered approximately 100 ppm. The acute oral lethal dose of the Cry proteins that would kill 50% of animals was determined to be greater than 128 mg ai/kg body weight Cry1Ac(synpro) and Cry1F(synpro), the limit test dosage.

Toxicity to invertebrates

58. The toxicity of Cry proteins to insects relates to both efficacy on target species and effects on non-target species. The target organisms of the GMO proposed for release are listed in Chapter 1, Section 5.1.1 and the toxicity is addressed in the following sections. In addition, effects on non-target species are shown. The Cry1Ac and Cry1Fa toxins have a different but overlapping spectrum of toxicity regarding lepidopteran insect species. For example, Chambers et al. (1991) reported that Cry1F was toxic to *H. virescens*, *S. exigua* and *Ostrinia nubilalis*, whereas Cry1Ac was toxic to *H. virescens*, *H. zea* and *O. nubilalis*.

59. The Bt toxin database of the Canadian agency Natural Resources Canada (NRC) lists a number of studies which report on bioassays with Cry toxins. Overall these studies are in agreement with regard to the impacts of the individual Cry proteins on invertebrates.

60. However, in some instances, different results have been obtained with the same Cry protein using the same test species. For example, Avilla et al. (2005) noted that studies using Australian and Indian populations of *H. armigera* lead to results that differed from their Spanish population (see also sections below). Similarly, Hernandez-Martinez et al. (2008) found different results for Cry1Da and Cry1Ab towards different strains of *S. exigua*.

Cry1Ac

61. The literature on toxicity of Cry1Ac to invertebrates has been reviewed in previous RARMPs for insect resistant GM cottons, including WideStrike™ cotton (DIR 040/2003 and

DIR 044/2003), Bollgard II® cotton (DIR 012/2002, DIR 059/2005 and DIR 066/2006) and Bollgard II® pima cotton (*Gossypium barbadense*, DIR 074/2007). In addition, general information on Cry proteins is also provided in the more recent RARMP for DIR 087. The Cry1A proteins are a closely related group and their toxicity is highly specific to lepidopteran insects (Macintosh et al. 1990).

62. Herman (2001) investigated the toxicity of the microbially produced Cry1Ac(synpro) to eight insect pests of cotton, both lepidopteran and non-lepidopteran, three of which are pests of cotton in Australia. In these experiments, *H. virescens* and *Trichoplusia ni* were most susceptible to Cry1Ac(synpro), whereas *Pectinophora gossypiella*, *Aphis gossypii* and *Athonomus grandis grandis* were not susceptible (see Table 4). No LC_{50} data has been provided for the toxicity of Cry1Ac(synpro) to *H. armigera* or *H. punctigera*, the major pests of cotton in Australia.

Table 4 Summary table showing LC_{50}s of organisms tested with Cry1Ac(synpro)

Species – common name	Species – scientific name	Insect order	Developmental stage used	Number of concentrations tested (range)	Number of individuals	Number of tests	LC_{50} [ng ai/cm^2]
Cotton bollworm	*Helicoverpa zea*	Lepidoptera	Neonate larvae	11 (0.0213 – 1.260 ng ai/cm^2)	16	2	580
Tobacco budworm	*Heliothis virescens*	Lepidoptera	Neonate larvae	11 (0.000790 – 46.7 ng ai/cm^2)	16	2	1.2
Fall armyworm	*Spodoptera frugiperda*	Lepidoptera	Neonate larvae	11 (0.192 – 11,340 ng ai/cm^2)	16	2	5,600
Beet armyworm†	*Spodoptera exigua*	Lepidoptera	Neonate larvae	11 (0.0213 – 1.260 ng ai/cm^2) 4 (420 – 11,340 ng ai/cm^2)	16 16	2 2	880
Pink bollworm†	*Pectinophora gosypiella*	Lepidoptera	Neonate larvae	6 (46.7 – 11,340 ng ai/cm^2) 4 (420 – 11,340 ng ai/cm^2)	16 16	1 1	> 11,000
Cabbage looper	*Trichoplusia ni*	Lepidoptera	Neonate larvae	11 (0.192 – 11,340 ng ai/cm^2) 7 (0.192 – 140 ng ai/cm^2)	16 16	1 1	4.4
Cotton aphid†	*Aphis gossypii*	Hemiptera	Mixed stages	1 (14,000 ng ai/cm^2)	25 (test 1) and 20 (test 2)	2	>14,000*

Species – common name	Species – scientific name	Insect order	Developmental stage used	Number of concentrations tested (range)	Number of individuals	Number of tests	LC_{50} [ng ai/cm^2]
Boll weevil	*Athonomus grandis grandis*	Coleoptera	Early instar larvae	1 (11,340 ng ai/cm^2)	16	2	> 11,000

*Concentration in ng ai per mL; † cotton pest in Australia; LC50 s were scored after 6 days for all species with the exception of cotton aphid, which was scored after 3 days.

Cry1F

63. Herman and Young (Herman & Young 1999) investigated the toxicity of the microbially produced Cry1F(synpro) to six insect species, both lepidopteran and non-lepidopteran, one of which is a pest in Australian cotton fields. In these experiments, *Pseudoplusia includens* was most susceptible to Cry1F(synpro), whereas *P. gossypiella*, *A. grandis grandis* and *Lygus hesperus* were not susceptible (see Table 5). No LC_{50} data has been provided for the toxicity of Cry1F(synpro) to *H. armigera* or *H. punctigera*, the major pests of cotton in Australia.

Table 5 Summary table showing LC_{50}s of organisms tested with Cry1F(synpro)

Species – common name	Species – scientific name	Insect order	Developmental stage used	Number of concentrations tested (range)	Number of individuals	Number of tests	LC_{50} [ng ai/cm2]
Fall armyworm	*Spodoptera frugiperda*	Lepidoptera	Neonate larvae	8 (24.51 – 53,600 ng ai/cm^2)	16, except in test 2 only 8 for one concentration	2	190.0
Pink bollworm †	*Pectinophora gosypiella*	Lepidoptera	Neonate larvae	8 (24.51 – 53,600 ng ai/cm^2)	16, except in test 2 only 15 for one concentration	2	>53,600
Cabbage looper	*Trichoplusia ni*	Lepidoptera	Neonate larvae	8 (24.51 – 53,600 ng ai/cm^2)	16	2	145.0
Soybean looper	*Pseudoplusia includens*	Lepidoptera	Neonate larvae	8 (0.91 – 53,600 ng ai/cm^2)	16	2	1.2
Boll weevil	*Athonomus grandis grandis*	Coleoptera	Early instar larvae	1 (53,600 ng ai/cm^2)	16	2	>53,600
Western tarnishe	*Lygus hesperus*	Heteroptera	3 to 5 day old nymphs	1 (69,900 ng ai/cm^2)	25 in test 1,	2	>69,900*

Species – common name	Species – scientific name	Insect order	Developmental stage used	Number of concentrations tested (range)	Number of individuals	Number of tests	LC_{50} [ng ai/cm2]
d plant bug					20 in test 2		

† cotton pest in Australia; * The LC_{50} for Western tarnished plant bug is in ng ai/mL; LC_{50}s were scored after 7 days for all species with the exception of Western tarnished plant bug, which was scored after 3 days.

64. A number of other studies involving Cry1F proteins have also been conducted. Toxicity studies of native Cry1Fa1 have been performed on the target species *H. armigera* (Avilla et al. 2005). The authors concluded from the experiments that all of the concentrations tested (1 – 16 µg/mL) led to growth inhibition, but not mortality in *H. armigera*.

65. Encapsulated Cry1F protein was tested for mortality to *H. armigera* and *H. punctigera* using a surface contamination assay as well as a diet incorporation method (Liao et al. 2002). Cry1F was found to be more toxic to *H. punctigera* than to *H. armigera*.

66. Bioassays were conducted on neonate larvae of *H. zea*, *H. virescens*, *O. nubilalis* and *S. exigua* with purified Cry1F protein from Bt strain EG1945 (Chambers et al. 1991). The authors determined that the Cry1F protein was highly active against *O. nubilalis* and *H. virescens*, moderately active against *S. exigua*, and showed only little activity against *H. zea* (50% lethal concentration of greater than 5,700 ng Cry1F/cm^2 of diet surface)

67. Karim et al (2000) evaluated a number of Bt toxins for their toxicity to *H. zea* neonate larvae, by feeding with a modified artificial diet to which purified, activated core toxin had been added. After 5 days, the mortality rates were determined and the authors state that Cry1Fa did not result in toxic activity when fed at up to 400 ng/mg of diet.

Possible combination effects of Cry1Ac and Cry1F

68. A laboratory study has indicated that synergistic toxic effects may occur in *H. armigera* larvae upon ingestion of a mixture of Cry1Ac and Cry1F proteins (Chakrabarti et al. 1998). In the study, toxicity was defined as that causing 50% larval growth reduction. Feeding of a 1:1 mixture of the proteins led to 26 times higher toxicity than expected based on additive effects of the two toxins. These results suggest potential synergistic effects of the two Cry proteins. The mode of action responsible for this synergism and any implications for the spectrum of susceptible species is currently unknown.

69. Conversely, another study has shown only an additive effect of Cry1Ac and Cry1F against *H. armigera*. Ibargutxi et al (2008) investigated both individual as well as combination effects of Cry1Ac, Cry2Ab and Cry1Fa in *H. armigera* and *Earias insulana* (spiny bollworm). In their experiments, the authors used concentration-mortality assays and larval growth inhibition studies. All three toxins were more active against *E. insulana* than against *H. armigera* larvae. Cry1Ac was the most toxic against *H. armigera* while Cry1Fa was the least toxic and caused no mortality. In both test species, the effect of the Cry1Ac and Cry1Fa toxins combined was additive in both mortality and growth inhibition assays.

Toxicity to non-target invertebrates

70. A meta-analysis of the literature regarding non-target effects of GM crops containing Bt genes, including GM Bt cotton expressing Cry1Ac, has been performed (Marvier et al. 2007). The study especially considered the statistical validity and other experimental procedures in the analysed reports. It was concluded that non-target invertebrate groups were

present at lower levels in Cry1Ac cotton fields when compared to non-GM, insecticide-free fields. In contrast, non-target invertebrates were generally more abundant in Cry1Ac cotton when compared to insecticide treated non-GM cotton. There was no significant difference in the abundance of non-target invertebrates for studies where insecticide-treated Cry1Ac cotton was compared with insecticide-treated non-GM cotton.

71. The current literature considering how Bt cotton and other Bt crops affected the abundance of functional guilds (groups), such as predators, parasitoids, omnivores, detritivores and herbivores, and the relationships between predators and herbivores, and between predators and detritivores in field studies has been evaluated (Wolfenbarger et al. 2008). The study found slightly fewer predators in Bt cotton fields compared to unsprayed, non-GM cotton fields. This result was considered unrelated to the feeding style, but largely accounted for by the lower abundance of predators in the taxonomic groups of Nabidae and Coccinellidae in Bt fields. The abundance of common predatory genera, including *Chrysoperla*, *Orius* and *Geocoris* were similar in Bt and unsprayed non-Bt cotton. In non-Bt and Bt cotton fields treated with insecticides, similar abundances of non-target functional guilds occurred. In unsprayed Bt cotton fields the study found many more predators, herbivores and mixed-guild taxa than in insecticide sprayed controls.

72. In the laboratory, monarch butterfly (*Danaus plexippus* L.) larvae were used as a surrogate for indirect exposure of non-target arthropods to GM pollen (Hellmich et al. 2001). Larvae were fed on milkweed (a host plant) leaf discs treated with pollen from GM corn expressing Cry1Ac or Cry 1F. Pollen was applied at varying densities up to >1,000 grains/cm^2, which is at two to ten fold higher than commonly found in cornfields during anthesis, and milkweed leaves with no pollen or with pollen from near isoline hybrids were used as controls. After four days, the weights of larvae fed pollen from Cry1Ac or Cry1F corn lines did not differ significantly from control fed larvae.

73. The same study also examined toxicity to monarch butterfly larvae of purified Cry proteins incorporated into artificial diets over seven days. Cry1F was relatively non-toxic to first instars and did not cause mortality at any concentration tested. Cry1F produced a 50% growth inhibition only when present in high concentrations (5,220 ng/ml artificial diet). However, Cry1Ac was toxic to first instar larvae in terms of both mortality and growth inhibition. The concentrations required to cause 50% mortality (LC_{50}) or 50% growth inhibition (EC_{50}), respectively, were 13.8 and 0.9 ng/ml artificial diet.

74. Honey bee (*Apis mellifera* L.) survival was evaluated after a single dietary exposure of larvae aged 3 to 5 days to either a mixture of the microbially produced Cry proteins (10 µL of a 30% sucrose solution containing 11.9 µg Cry1Ac(synpro) plus 1.9 µg per mL Cry1F(synpro)), or pollen expressing Cry proteins (10µL of a 30% sucrose solution containing 2 mg of either Cry1Ac(synpro) or Cry1F(synpro) expressing pollen). One control group was fed 30% sucrose solution and another control group was fed 2 mg non-GM pollen in 30% sucrose solution. A positive control group was included that was fed 10 µL of a potassium arsenate solution containing 1,000 ppm arsenic (Maggi 2001). Mean time to emergence of adult bees was measured and found not to be significantly different between the treatment groups and the sucrose control group. Dead bees were found in the emergence cages but there was no significant difference in the number of dead bees between the treatments or the sucrose control group. The author indicated that level of dead bees found in the emergence cage is unusual for this type of experiment, however there was no statistical evidence for a treatment effect and no toxicity to the Cry proteins was identified.

75. A dietary toxicity study was conducted on adult ladybird beetles (*Hippodamia convergens*) using microbially produced Cry1Ac(synpro) or Cry1F(synpro) proteins (Porch

& Krueger 2001). Ladybirds were fed *ad libitum* over 15 days on a diet containing the Cry proteins either singly or in combination. Observations of mortality and other adverse effects, including lethargy, were conducted until the cumulative mortality in the control group exceeded 20% on day 15 of the test. The mortality in the negative control group was 21% on day 15. The cumulative mortality of the Cry1F(synpro) only group was 29%. The same cumulative mortality was obtained for the group receiving Cry1Ac(synpro) only. However, the group receiving a diet containing the combined Cry proteins showed mortality of 11%, less than the control group, and all live beetles appeared normal throughout the study. Both the LC_{50} and the 'no-observed-effect dose' were determined to be greater than the tested individual and combined concentrations of 22.5 µg Cry1Ac(synpro)/mL and 300 µg Cry1F(synpro)/mL.

76. A dietary toxicity study of microbially produced Cry1Ac(synpro) and Cry1F(synpro) was conducted on adult parasitic hymenoptera (*Nasonia vitripennis*) (Sindermann et al. 2002b). Four treatments were given: Cry1Ac(synpro); Cry1F(synpro); the two Cry proteins combined at the same concentrations as for individual testing; and a negative control diet. The concentrations used reflect approximately 32 times the concentration of Cry1Ac(synpro) and 58 times that of Cry1F(synpro) in pollen. The wasps were allowed to feed *ad libitum*. Observations of mortality and clinical signs were made until the cumulative mortality of the negative control exceeded 36% at day 10. On day 9, the cumulative mortality in the negative control was 20%, 29% in the Cry1Ac(synpro) group, 27% in the Cry1F(synpro) only group and 40% in the group receiving both Cry proteins. None of the differences were found to be statistically significant by the authors. Some wasps appeared lethargic in the Cry1Ac(synpro) group on day 7, and in the group receiving both Cry proteins in combination on days 7 and 8. Otherwise, the wasps were normal in appearance and behaviour.

77. A dietary toxicity study consisting of two tests of green lacewing larvae (*Chrysoperla carnea*) exposed to microbially produced Cry1F(synpro) and Cry1Ac(synpro) mixed with moth eggs, (*Sitotroga* sp.) was carried out (Sindermann et al. 2002a). In test 1, treatments consisted of Cry1F(synpro), Cry1Ac(synpro) and the two Cry proteins combined at the same concentrations as for individual testing. In test 2, treatments consisted of the two Cry proteins combined at the same concentrations as for individual testing in test 1, the two Cry proteins combined at $1/10^{th}$ of the concentrations as for individual testing and the two heat-treated Cry proteins at the same concentrations as for individual testing in test 1. The individual concentrations used reflect approximately 58 times the concentration of Cry1F(synpro) and 32 times that of Cry1Ac(synpro) in pollen. The lacewing larvae were allowed to feed *ad libitum*. Observations of mortality, clinical signs and pupation were made until the cumulative mortality of the negative control was 23% at day 16 for the first test and until pupation exceeded 50% on day 18 of the 2nd test. In test 1, observations of mortality and pupation for the control group were made at day 16 and for all other groups on day 15. In that test, mortality in the group receiving both Cry proteins in combination was significantly different (43%) from the control group (23%), the two other test groups were not significantly different (20% for both groups). In the control group, 15 larvae had pupated; in the Cry1F(synpro) group, 15 larvae; in the Cry1Ac(synpro) group, 19 larvae; and in the group receiving both Cry proteins 16 larvae. All surviving larvae were reported as normal in appearance and behaviour throughout the test period. In test 2, mortality was not significantly different for any group tested. The no-observed-effect concentration for the combined proteins was more than 5.2 µg Cry1F(synpro) /mL plus 46.8 µg Cry1Ac(synpro) /mL.

78. Acute toxicity of a mixture of microbially produced Cry1F(synpro) and Cry1Ac(synpro) to *Daphnia magna* Straus. was evaluated (Marino & Yaroch 2002a). Three replicates of 10 individuals each (less than 24-hour old *Daphnia* instars) were exposed to a

solution containing 2.5 µg Cry1Ac(synpro)/mL and 0.51 µg Cry1F(synpro)/mL for 48 hrs. *Daphnia* were observed 24 and 48 hours after initiation of the experiment. No immobility or other unspecified adverse effects were observed in *D. magna*.

79. A study comprising three experiments investigating chronic toxicity of diets containing microbially produced Cry1F(synpro) and/or Cry1Ac(synpro), lyophilised Cry1Ac cotton leaf tissue or PSC-355 control cotton leaf tissue to Collembola of the species *Folsomia candida* was conducted by Teixeira (2002).

80. In the first experiment, *F. candida* was exposed for a period of 28 days to either

- Brewer's dry granulated yeast only or
- Brewer's dry granulated yeast containing the microbially produced Cry proteins either individually or in combination.

81. Cry1Ac(synpro) was tested at a concentration of 22.6 µg a.i./g yeast, Cry1F(synpro) at 709 µg a.i./g yeast and the combination of the two Cry proteins consisted of 22.6 µg Cry1Ac(synpro)/g yeast plus 709 µg Cry1F(synpro)/g yeast. The number of individuals per group was 40. The Collembola were monitored for mortality and sublethal effects, including lethargic behaviour. In addition, the number of offspring produced was counted to assess potential effects on reproduction. Exposure to Cry1Ac(synpro), Cry1F(synpro) or the combined proteins resulted in no significant change in survival rate (95%, 93% and 98%, respectively; the control showed a survival rate of 98%). Exposure to diet containing Cry1F(synpro) microbial protein or the combined protein did not adversely affect reproduction of Collembola (431 and 410 offspring per replicate, respectively; the control produced 440 offspring). However, exposure to the Cry1Ac(synpro) diet did adversely affect reproduction of Collembola (243 offspring per replicate). This decrease in reproduction was thought to be due to impurities in the test substance.

82. In the second experiment, the Cry1Ac(synpro) only treatment was repeated as described for the first experiment with similar results (survival was 100% for both the control and treatment group and reproduction was 387 offspring for the control group versus 305 offspring for the Cry1Ac(synpro) group). This reduction in number of offspring was found to be statistically significant. To evaluate whether the decrease was due to Cry1Ac(synpro) or to impurities associated with the production of the toxin, a third experiment using lyophilised leaf material was carried out. Lyophilised leaf material obtained from the parental GM cotton line 3006-210-23 expressing Cry1Ac(synpro) was fed to Collembola at 5% or 50% of the diet. This corresponded to concentrations of 0.1 and 0.97 µg Cry1Ac(synpro)/g diet at the start of the experiment and 0.07 and 0.73 µg Cry1Ac(synpro)/g diet at the end of the experiment. Exposure to lyophilised Cry1Ac(synpro) cotton leaf or lyophilised PSC-355 control cotton leaf at 5% and 50% of the diet did not adversely affect survival (100%) or reproduction (396 to 406 offspring per test group) of Collembola.

83. An acute toxicity study on adult earthworms (*Eisenia foetida*) was conducted in an artificial soil substrate (Sindermann et al. 2001). Four treatments were given: 247 µg Cry1F(synpro)/g soil, 107 µg Cry1Ac(synpro)/g soil, the two Cry proteins combined at the same concentrations as for individual testing and a control consisting of artificial soil only. According to the researchers, these concentrations used reflect approximately 50 times the expected environmental concentration of Cry1F(synpro) and Cry1Ac(synpro) in a cotton field under US conditions (Sindermann et al. 2001, Appendix 4).

84. There was no mortality in the negative control group during the 14 day test, and behaviour and appearance was considered normal. One worm was not found and presumed

dead in the Cry1Ac(synpro) only group. With this exception, all worms were considered normal in appearance and behaviour throughout the test period. A slight loss in body weight from test initiation to test termination was noted in all groups, ie between 0.04 g for all test groups and 0.05 g for the control group, and was considered to arise from not adding feed to the artificial soil during the test. The 14-day LC_{50} and no-observed-effect estimations for earthworms exposed to the Cry proteins were determined to be greater than 247 µg Cry1F(synpro)/g soil, greater than 107 µg Cry1Ac(synpro)/g soil and greater than 247 µg Cry1F(synpro)/g soil plus 107 µg Cry1Ac(synpro)/g soil.

Toxicity of other Cry proteins represented in the introduced protoxins

85. As described previously, the introduced *cry* genes are synthetic genes. In each case the synthetic gene consists of the amino-terminal *cry* core toxin sequence from *cry1Ac* or *cry1F* and parts from *cry1Ab* and *cry1Ca3* genes. The latter form part of the gene sequence introduced into the GM plants. They encode the carboxy-terminal part of the protoxin, which is mostly (Cry1F(synpro)) or entirely (Cry1Ac(synpro)) cleaved off to yield the active core toxin.

Toxicity of Cry1Ca3

86. A search of the literature did not yield reports on toxicity testing of Cry1Ca3 in organisms other than insects[11]. Cry1Ca3 demonstrated mortality in bioassays on neonates of the lepidopteran species *Argyrotaenia citrana*, *Choristoneura occidentalis*, *Pandemis pyrusana* and *Platynota stultana* (Knight et al. 1998). In addition, Payne (US Patent 5246852 1993) reported the toxin as active, ie causing mortality, on larval stages of the Lepidoptera *Choristoneura occidentalis*, *Plutella xylostella*, *Spodoptera exigua* and *Trichoplusia ni*.

87. A protein described as Cry1Ca is listed in the *Bacillus thuringiensis* Specificity Database[12] with a number of bioassays regarding its toxicity: these include bioassays on the Diptera *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*, and on the Lepidoptera *Agrotis ipsilon*, *Cydia pomonella*, *Epinotia aporema* and *Helicoverpa zea*, all of which were relatively resistant to the toxin. For some lepidopteran species, including *Mamestra brassicae* and *Manduca sexta*, a number of larval stages have been tested, and younger stages were found more susceptible than later stages.

Toxicity of Cry1Ab

88. The literature on the toxicity of Cry1Ab has been reviewed recently in the RARMP for DIR 087, but will be summarised here.

89. There are no reports of toxicity to mammals, birds and fish. The toxicological database on *B. thuringiensis* shows no adverse mammalian health effects attributable to delta-endotoxins. In particular, the European Food Safety Authority (EFSA) evaluated the food safety of delta-endotoxins expressed in maize plants, such as Cry1Ab in Bt11 maize (EFSA 2005) and purified Cry proteins, including Cry1Ab, have been assessed in dietary toxicity studies. These studies and acute oral toxicity studies (Mendelsohn et al 2003; OECD 2007) also confirm the low toxicity for mammals of the Cry proteins studied. In tests examining the toxicity of Cry1Ab on mammalian cells, it was found that Cry1Ab has little acute toxicity to bovine hepatocytes (Shimada et al. 2003).

[11] Source; accessed on 17 July 2009
[12] Source; accessed on 16 July 2009

90. The toxicity of Cry1Ab to non-target arthropods has been considered in a number of large scale studies and meta-analyses. These concluded that the only susceptible non-target species were amongst the Lepidoptera (US EPA 2001; Marvier et al. 2007; Wolfenbarger et al. 2008; Mendelsohn et al 2003; Duan et al. 2008). Similarly, in studies on soil microorganisms there were found to be no consistent statistically significant differences in the numbers of different groups of microorganisms, the activities of the enzymes and the pH between soils planted with Bt and non-Bt corn over a four year study (Icoz et al. 2008).

5.2.4 The introduced herbicide tolerance gene (pat) and the encoded protein

The *pat* gene

91. The glufosinate ammonium tolerance trait was introduced into WideStrike™ cotton as a marker to identify and select for transformed plant cells and plants during tissue culture regeneration. This was achieved by the introduction of the *pat* gene from *Streptomyces viridochromogenes* (Strauch et al. 1988; Wohlleben 1988). The *pat* gene encodes the enzyme phosphinothricin acetyl transferase (PAT; Wohlleben 1988).

92. WideStrike™ cotton contains one complete copy of the *pat* gene from GM parental cotton line 3006-210-23 as well as one complete copy plus an additional fragment from GM parental cotton line 281-24-236.

93. The introduced gene constitutes a small part of the *Streptomyces* genome. The gene sequence was optimised for codon usage in plants to optimise expression.

The PAT protein

94. The introduced herbicide tolerance gene encodes the PAT protein, a protein of 183 amino acids. The amino acid sequence is identical to the native PAT sequence. PAT confers tolerance to the L-isomer of phosphinothricin (glufosinate ammonium), the active ingredient in various herbicides.

95. The *pat* gene is very similar to the *bar* gene which also encodes a PAT protein and has been used in GM crops. The two genes have an identity of 87% at the nucleotide sequence level and both encode PAT proteins of 183 amino acids with 85% amino acid sequence identity. Their molecular weights (~22 kDa) are comparable and they share a similar substrate affinity and biochemical activity (Wehrmann et al. 1996).

96. Glufosinate ammonium acts as an herbicide by inhibiting the plant enzyme glutamine synthetase, leading to ammonia accumulation. This inhibits amino acid synthesis and photosynthesis, leading to severe damage to plant tissues, ultimately killing the plant (Pline 1999). Glufosinate ammonium is the active ingredient of a number of proprietary herbicides. The terms glufosinate ammonium and phosphinothricin are often used synonymously.

97. The PAT enzyme detoxifies glufosinate ammonium by acetylation of the L-isomer into N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit the enzyme glutamine synthetase (Droge-Laser et al. 1994) and therefore confers resistance to the herbicide (OECD 1999; OECD 2002).

98. In *S. hygroscopicus* and *S. viridochromogenes*, PAT prevents autotoxicity from the antibiotic bialaphos (Kumada et al. 1988).

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

99. PAT proteins are widespread in the environment, through the presence of naturally occurring bacteria as well as in other GM crops approved for commercial release.

100. *Streptomyces* spp. are saprophytic, soil-borne microbes that produce useful compounds including antibiotic substances and herbicides, eg bialaphos. Streptomyces are generally not considered human or animal pathogens. In rare circumstances, they may cause localised, chronic suppurative infection of the skin and underlying soft tissue or visceral infections (reported and reviewed in Dunne et al. 1998). *S. viridochromogenes* has a history of causing allergenicity in humans.

101. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The applicant has compared the amino acid sequence of the protein encoded by the introduced *pat* gene to databases of known toxins and allergens. The results of these analyses did not indicate that any of the encoded proteins shared any significant sequence homology with any known toxins or allergens (Stelman 2001c).

102. The PAT protein expressed in the GM cotton plants proposed for release is similar to that present in InVigor® hybrid canola (DIR 021/2002) and Liberty Link® Cotton (DIR 062/2005), which have been assessed and approved by the Regulator for commercial release in Australia. FSANZ has approved the use of food derived from these and other GM plants containing either the *bar* or *pat* gene, including GM cotton, corn, canola, rice and soybean, concluding that the PAT protein is not toxic (eg ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; FSANZ 2003; FSANZ 2005a; FSANZ 2005b; FSANZ 2005d; FSANZ 2005c; FSANZ 2008). Products derived from WideStrike™ cotton have also been approved for food use by FSANZ.

5.3 The regulatory sequences

103. 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 is an mRNA termination region, including a polyadenylation signal. Information on the promoters and terminators for the transformation events used to generate WideStrike™ cotton are summarised in Table 3.

5.3.1 Regulatory sequences for the expression of the introduced cry genes

104. Expression of *cry1F(synpro)* is controlled by the (4OCS)Δmas 2' promoter, a synthetic promoter derived from the mannose synthase gene promoter (mas) and octopine synthase gene enhancer (OCS) of *Agrobacterium tumefaciens* (Barker et al. 1983; Ellis et al. 1987). The mRNA termination region is provided by the bidirectional polyadenylation signal of *A. tumefaciens* open reading frame 25 (Barker et al. 1983).

105. The OCS enhancer sequences have been demonstrated to function in both orientations depending on their distance to the target promoter sequences; the more proximal to the promoter the enhancer sequence is, the stronger the enhancing effects on gene expression that are observed.

106. Expression of *cry1Ac(synpro)* in this GM cotton is controlled by the Ubiquitin (Ubi) promoter derived from the *Zea mays* (maize) polyubiquitin gene (Christensen et al. 1992). The Ubi promoter sequences used consist of the promoter, exon 1 (untranslated enhancer) and intron 1 (Christensen & Quail 1996). The Ubi promoter is considered to be constitutive, resulting in expression of the genes it drives at relatively high levels in most tissues of the plant (reviewed in Christensen & Quail 1996), although expression levels may vary between tissues and depend on any stress applied to the plant (eg Takimoto et al. 1994).

107. The Ubi promoter is widely used in GM food crops. In Australia, FSANZ has approved the use of GM plants containing the Ubi promoter in food, eg insect resistant and herbicide tolerant maize (maize line DAS-59-122-7; FSANZ 2005a). Non-GM maize contains the Ubi promoter and has a long history of use as both human food and animal feed (eg Piperno & Flannery 2001; Matsuoka et al. 2002).

108. The mRNA termination region for *cry1Ac(synpro)* is provided by the bidirectional polyadenylation signal of *A. tumefaciens* open reading frame 25 (Barker et al. 1983).

5.3.2 Regulatory sequences for the expression of the introduced pat gene

109. The introduced *pat* gene is the synthetic plant optimised glufosinate resistance gene based on a phosphinothricin aceltyl transferase gene sequence from *Streptomyces viridochromogenes*. The two full length copies of the *pat* gene in WideStrike™ are under different promoters. Expression of the *pat* gene is under the control of the Ubi promoter in line 281-24-236 and (4OCS)Δmas 2' promoter in line 3006-210-23 (see Chapter 1, Section 5.4.2).

110. The mRNA termination region of all inserted genes is provided by the bidirectional polyadenylation signal of *A. tumefaciens* open reading frame 25 (Barker et al. 1983).

5.4 The generation of WideStrike™ cotton proposed for release

111. The GM cotton proposed for release was generated by conventional crossing of two GM cotton lines, the parental lines 3006-210-23 (containing *cry1Ac(synpro)* and *pat*) and 281-24-236 (containing *cry1F(synpro)* and *pat*). Each parental line was modified for increased insect resistance and herbicide tolerance by transformation with a final construct generated by inserting an expression cassette containing the insecticide resistance gene into a T-DNA vector backbone containing the herbicide tolerance gene, as detailed below.

5.4.1 Construct design

The cry1Ac(synpro) expression cassette

112. The *cry1Ac(synpro)* construct contains the core sequence derived from the Bt var *kurstaki* strain HD73 *cry1Ac* gene with the addition of partial sequences from the *cry1Ca3* and *cry1Ab1* genes. This protoxin gene was synthetically constructed with a plant-optimised coding sequence and inserted into a cloning vector, pMYC1680, containing the Ubi promoter and the polyadenylation signal from ORF25.

The cry1F(synpro) expression cassette

113. The *cry1F(synpro)* construct contains the core sequence derived from the Bt *cry1F* gene with the addition of partial sequences from the *cry1Ca3* and *cry1Ab1* genes. This protoxin gene was synthetically constructed with a plant-optimised coding sequence and inserted into a cloning vector termed pMYC2392 containing the (4OCS) mas 2' promoter and the ORF25 polyadenylation signal.

The pat expression cassettes

114. The *pat* gene was inserted into a T-DNA vector backbone between the T-DNA border sequences from *A. tumefaciens*. For use with the *cry1Ac(synpro)* expression cassette, the vector generated was pAGM294 and expression of the *pat* gene was driven by the (4OCS)mas 2' promoter and terminated with the ORF25 polyadenylation signal. For use with

the *cry1F(synpro)* expression cassette, the vector generated was pAGM277 and the *pat* gene was under the control of the *Z. mays* Ubi promoter and the ORF25 polyadenylation signal.

Final T-DNA transformation vector pMYC3006

115. The final transformation vector pMYC3006 (see Figure 5) was obtained by inserting the *cry1Ac(synpro)* expression cassette from pMYC1680 into pAGM294.

Final T-DNA transformation vector pAGM281

116. The final transformation vector pAGM281 (see Figure 5) was obtained by inserting the *cry1F(synpro)* expression cassette from pMYC2392 into pAGM277.

5.4.2 Method of genetic modification

117. The parental cotton lines were generated by *Agrobacterium tumefaciens*-mediated transformation (reviewed in Zambryski 1992). This method of transformation has been discussed in previous RARMPs (eg DIR 070/2006).

118. The two T-DNA transformation vectors pMYC3006 and pAGM281 were introduced into plant cells using standard *Agrobacterium* transformation protocols. The transformation vectors were introduced separately into *A. tumefaciens* strain LBA4404 and used to transform cells of the commercial American cotton variety, GC510 (see Figure 5).

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

120. Line 3006-210-23 or Cry1Ac(synpro) cotton refers to those plants that were regenerated from cells transformed with *A. tumefaciens* containing the vector pMYC3006. Line 281-24-236 or Cry1F cotton refers to plants regenerated from cells transformed with *A. tumefaciens* containing pAGM281. The latter event contains a complete copy plus an additional fragment of the *pat* gene.

121. The two GM cotton plants containing the single insecticidal traits, or their progeny from self pollination, were then crossed, and repeatedly backcrossed, to another elite American commercial cotton variety, PSC-355 (the 'recurrent parent' in the breeding program). The two GM lines 281-24-236 and 3006-210-23 were then combined by conventional breeding to generate WideStrike™ cotton (see Figure 5). Thus the GM WideStrike™ cotton contains two insecticidal genes, synthetic *cry1F(synpro)* and synthetic *cry1Ac(synpro)*, and two complete copies and a partial copy of the herbicide tolerance *pat* gene.

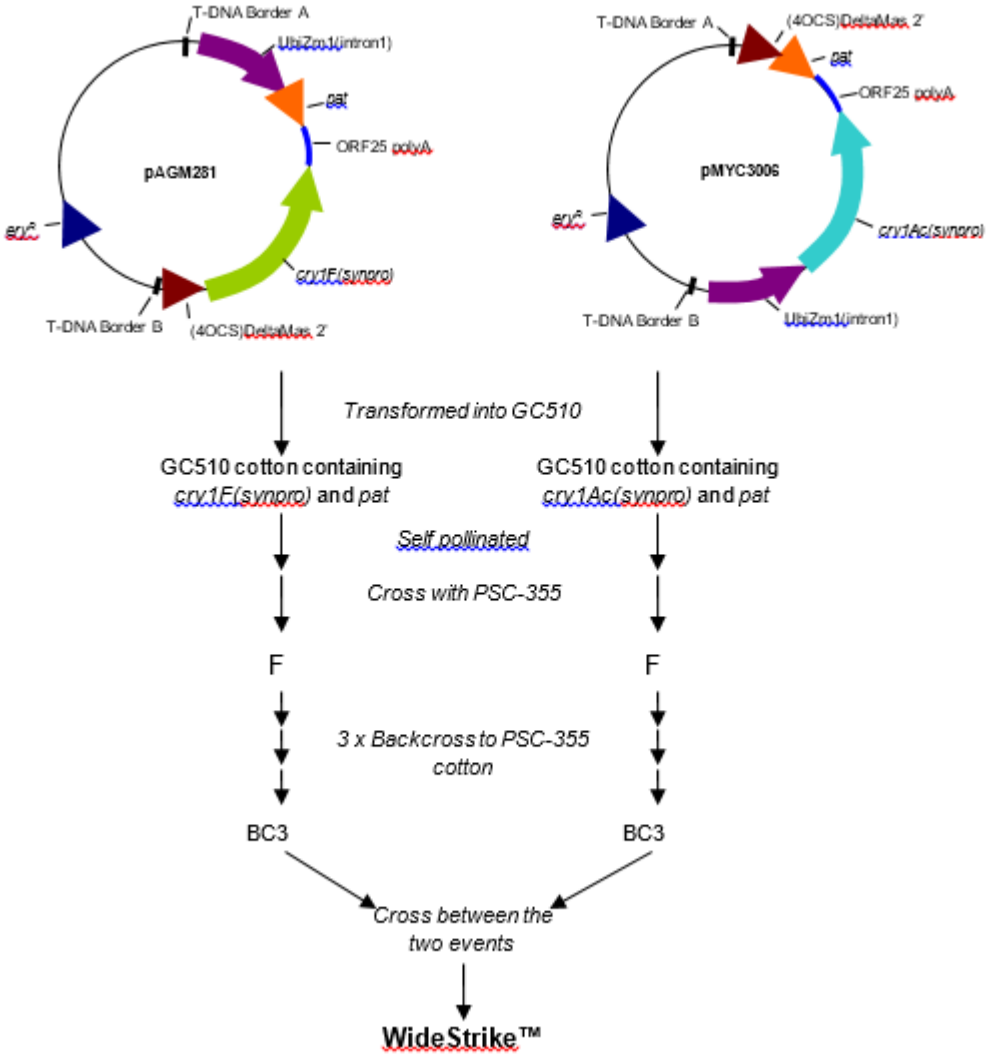

Figure 5Schematic diagram of the genetic modification and generation of WideStrike™ cotton.

5.5 Characterisation of the GMO

5.5.1 Stability and molecular characterisation

122. The parental cotton lines were developed through a series of backcrosses and self pollinations. Mendelian segregation analysis was performed on both the parental lines and the GMO proposed for release using presence or absence of glufosinate ammonium tolerance as well as *cry1Ac(synpro)* and *cry1F(synpro)* expression (as detected by qualitative ELISA) as scoring criteria. The applicant provided analyses that suggest inheritance of the introduced genes as single dominant genes.

123. Detailed molecular analysis of both parental GM lines was conducted by the applicant. This included sequencing of the inserted and flanking (cotton) DNA for both insertions. The presence of a single copy of the introduced *cry1Ac(synpro)* and *pat* genes in the GM parental cotton 3006-210-23 was suggested by Southern blot analysis and confirmed by DNA sequencing. Sequencing of the introduced DNA sequence, including the *cry1Ac(synpro)* gene and the flanking border sequences, of parental cotton 3006-210-23 indicated that the inserted genes are complete and match exactly to the expected DNA sequence (see Figure 6). The immediate flanking sequences to the site of insertion were from the cotton genome, which confirms that no vector sequence has been inserted. BLASTN searches in 2002 using the

flanking sequences did not generate any significant homologies to any known genes. Analysis of the cotton genome flanking sequence did not detect any novel open-reading frames. At the site of integration, 16 bp from the original locus are absent (Song 2002a).

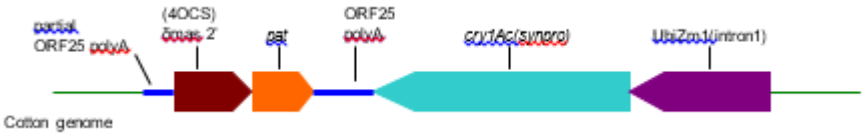

Figure 6Insert structure of 3006-210-23 cotton.

124. The presence of a single copy of the introduced *cry1F(synpro)* and *pat* genes and an additional *pat* fragment in the GM parent 281-24-236 was suggested by Southern blot analysis and confirmed by DNA sequencing. DNA sequencing showed that the sequence of the introduced genes in parental line 281-24-236 was identical to the expected sequence with the exception of two base changes in the ZmUbi-1 promoter sequences (Song 2002b). It was found that this parent contains an additional copy of the entire UbiZm1 promoter sequence and a 231 bp fragment of the *pat* gene (see Figure 7). This partial sequence is present downstream of the T-DNA border B at the 3' end of the complete introduced gene, in opposite orientation to the *pat* expression cassette of the complete insert. A deletion of 53 bp from the flanking cotton DNA is present in the GMO. The applicant has reported that a BLASTN search found that the majority of the 3' border sequences plus 37 bp in the 5' flanking border has more than 90% homology to a *G. hirsutum* cDNA encoding gibberellin 20-oxidase (GeneBank accession number AY603789). Gibberellin 20-oxidases are involved in the final steps of the gibberellin pathway. Gibberellins are tetracyclic diterpenes, some of which act as plant hormones implicated in plant growth and developmental processes, including flowering, seed germination and fruit development (as summarised in Fagoaga et al. 2007). Analysis of the insertion site and cDNA indicate that the introduced genetic elements have been inserted in the 3'UTR (untranslated region, ie non-protein coding) of the putative gene.

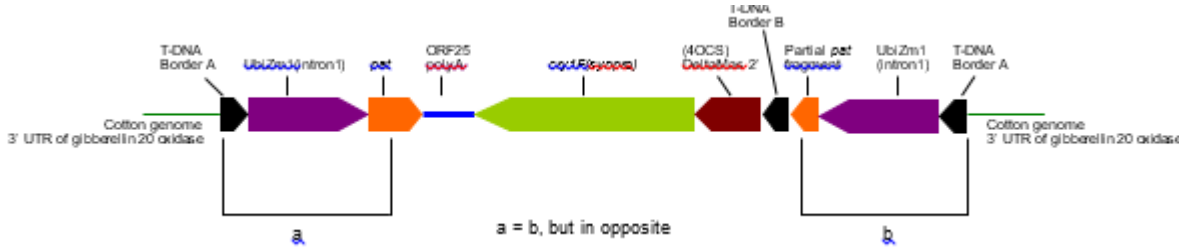

Figure 7Insert structure of 281-24-236 cotton

5.5.2 Phenotypic characterisation of the GMO

Physiological characterisation

125. The applicant stated that WideStrike™ cotton proposed for release has the same water, soil type, nutrient and climatic requirements as non-GM cotton. In this case, the GM cotton would be able to grow in regions suitable for cotton in the Australian environment, and would be expected to be limited by the same abiotic factors as non-GM cotton.

Agronomic characterisation

126. The applicant has provided limited, preliminary data on some agronomic characteristics of WideStrike™ and the non-GM parent variety, PSC-355 grown in Australia (Annetts 2006b). Four treatment groups were investigated: unsprayed PSC-355, PSC-355 sprayed with

Lepidoptera-specific insecticides, unsprayed WideStrike™ cotton and WideStrike™ cotton sprayed with Lepidoptera-specific insecticides. Plant characteristics, including plant hight, number of nodes, height to node ratio, number of bolls, yield and percentage of tipped out branches were determined. The author concluded that WideStrike™ cotton sprayed with Lepidoptera-specific insecticides out-yielded unsprayed WideStrike™ cotton and that WideStrike™ cotton sprayed with Lepidoptera-specific insecticides outperformed unsprayed WideStrike™ cotton (with some exceptions).

127. The applicant has provided data on the agronomic characteristics of WideStrike™ and the non-GM parent variety, PSC-355 grown in the USA. The results of the study are provided in Table 6. Agronomic performance of WideStrike™ did not show changes in survival characteristics, reproductive potential, seed production, plant vigour, ways of dissemination and ageing. While some statistically significant differences were found between WideStrike™ cotton and the non-GM parent variety, the applicant has stated that these differences are not considered to be biologically significant since the differences were within the range of variability commonly seen among commercial cotton varieties.

Table 6 Characteristics of WideStrike™ and the non-GM parent variety, PSC-355

Variable	Units	WideStrike™ cotton	PSC-355	Number of locations#
Plant height	Inches	40.2	41.5	17
Total nodes	Number per plant	17.4	17.6	16
Height to node ratio	Inches per node	2.32	2.35	17
Node of the 1st fruiting branch	Node	6.8	6.6	17
Fruiting branches	Number per plant	11.6 *	12.1	16
Total fruiting positions	Number per plant	24.7 *	26.6	17
Vegetative bolls	Number per plant	1.7	1.6	16
Field emergence	%	78.9	82.3	19
Cool vigour	%	36	38	20
4 day warm	%	64	65	20
7 day warm	%	80	82	20
Total germination	%	84	87	20
Dormant seed	%	0.5	0.3	20
Vegetative branches	Number per plant	2.8	2.6	16
Days to first flower	Days	61.4	60.6	18
Node of white flower – 15 days	Node	12.9	12.9	17
Node of white flower – 30 days	Node	16.9	16.8	15

Variable	Units	WideStrike™ cotton	PSC-355	Number of locations#
Percent retention – total	%	45.8	44.4	16
Percent retention – 1st position	%	62.4 *	54.3	16
Percent open bolls	% per plant	76.5	75.4	17
Seed cotton weight per boll	Grams per boll	5.3 *	5.1	19
Lint	%	37.1	37.3	19
Seed index (fuzzy)	Grams per 100 seeds	11.3 *	10.7	17
Lint per acre	Lbs per acre	1000	993	17
Length	Inches	1.177 *	1.147	19
Strength	Grams per tex	33.0	32.6	19
Micronaire	Micronaire units	4.51 *	4.96	19
Length uniformity	%	85.8	85.7	19
Reflectance	%	76.0 *	74.6	19
Yellowness	Hunter's +b scale	8.3	8.4	19

approximately 2,500 plants were used per location; * significantly different from the non-GM PSC-355 using means comparison according to Dunnett-Hsu at P = 0.05.

Biochemical characterisation

128. As part of the biochemical characterisation of WideStrike™ cotton, the applicant has provided data on the proximates, minerals, fatty acids, amino acids, vitamins, tocopherols and anti-nutrients. This is in comparison to either a null plants resulting from F1 segregation or PSC-355, and to literature values for cotton. This data was obtained from plants grown in six locations in the USA. No data has been presented from plants grown under Australian conditions.

129. A proximate analysis of cotton seed was carried out on control plants (null plants resulting from F1 segregation (Phillips et al. 2003)) or PSC-355 (McCormick & Phillips 2005) and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). For each study, plant material was harvested across six locations in the USA. The results of the study are provided in Table 7. All proximates were within or very similar to literature ranges except for moisture. Results for moisture for all treatments differed from the published literature and varied between the two datasets; but this is likely a consequence of sampling and preparation, since results were comparable between controls and WideStrike™. In one dataset, the crude fibre content for the GM cotton was significantly lower than the control, but was similar to the values reported in the literature for non-GM cotton, and differed from the control value by <10%.

Table 7 Proximate analysis of cotton seed on control and WideStrike™ cotton#

Proximate (% dry weight of sample)	Literature values*	Control	WideStrike™ cotton
Matrix	**Seed**	**Seed**	**Seed**
Ash	3.8 – 4.9	4.0 4.0	3.9 3.9
Total fat	15.4 – 23.8	22.6 21.9	22.9 22.2
Moisture	4.0 – 8.7	3.3 11.5	3.5 10.8
Protein	21.8 – 28.2	27.6 27.2	27.9 27.8
Carbohydrates	45.6 – 53.6	45.8 46.9	45.4 46.1
Calories (Kcalories/100 g)	Not available	497 493	499 496
Crude fibre	15.4 – 28.2	17.6 18.8	15.9 18.8
Acid detergent fibre (ADF)	35.5 – 37.7	25.2 28.4	25.2 27.3
Neutral detergent fibre (NDF)	42.1 – 54.8	35.9 34.7	34.1 34.3
Matrix	**Kernel**	**Kernel**	**Kernel**
Moisture	Not available	7.6	6.9
Matrix	**Hulls**	**Hulls**	**Hulls**
Ash	2.8	3.0	2.8
Total fat	2.5	3.0	2.0
Moisture	10.0 – 11.0	10.3	10.6
Protein	4.2 – 6.2	7.1	6.2
Carbohydrates	Not available	86.8	89.0
Energy (Kcalories/100 g)	Not available	403	399
Matrix	**Meal$**	**Meal**	**Meal**
Ash	4.6 – 9.8	6.0 6.42	6.7 6.24
Total fat	0.6 – 4.7	4.6 4.37	2.0 4.82
Moisture	9.0 – 13.3	2.2 4.67	9.2 4.78
Protein	43.0 – 52.4	47.2 48.5	51.3 50.0
Carbohydrates	Not available	42.1 40.7	40.0 38.9
Energy (Kcalories/100 g)	Not available	399 396	383 399
Crude fibre	8.4 – 15.3	12.4 11.3	9.3 10.6

Proximate (% dry weight of sample)	Literature values*	Control	WideStrike™ cotton
Acid detergent fibre (ADF)	12.2 – 23.9	18.5 16.6	14.1 14.7
Neutral detergent fibre (NDF)	15.8 – 32.4	24.2 23.7	20.2 21.5
Matrix	**Refined oil**	**Refined oil**	**Refined oil**
Ash	Not available	100.2	100.1
Total fat	Not available	< 0.1	< 0.1
Moisture	Not available	< 0.1	< 0.1

Some results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005). If only one value is displayed then the parameter was only examined by Phillips et al.(2003).

* Values from OECD (2004) are given.

$ Nutrient composition of solvent-extracted, 41% crude protein cottonseed meal from Forster and Calhoun (1995) are given. It is unclear if the meal was toasted or not. The term 'meal' refers to toasted meal in the case of Phillips et al.(2003).

130. A mineral analysis was conducted on seeds, hulls and toasted meal of control plants (null plants resulting from F1 segregation (Phillips et al. 2003)) or PSC-355 (McCormick & Phillips 2005)) and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). For each study, plant material was harvested across six locations in the USA. The results of the study are summarised in Table 8. No statistically significant differences were observed between the WideStrike™ cotton and the non-GM control, and all mineral results for cottonseed, hulls and meal were similar to reported literature values.

Table 8 Mineral analysis of seeds, hulls and toasted meal of control and WideStrike™ cotton[a]

	Literature values[b]	Control	WideStrike™ cotton
Matrix **Minerals (mg/100 g)**	**Seed**	**Seed**	**Seed**
Calcium	120 – 330	151 156	160 144
Copper	0.4 – 1.0	0.91 0.80	0.93 0.84
Iron	4.2 – 7.2	6.17 5.75	5.59 5.78
Magnesium	370 – 490	421 348	417 352
Manganese	1.1 – 1.8	1.42 1.26	1.51 1.28
Molybdenum	0.1 – 0.4	< 0.2 0.10	< 0.2 0.11
Phosphorus	610 – 860	699 636	687 621
Potassium	1080 – 1250	1237 947	1219 968
Sodium	5.4 – 300	15.6 65.1	26.5 78.0
Zinc	2.7 – 5.1	4.23	4.43

	Literature values[b]	Control	WideStrike™ cotton
		5.23	5.73
Sulphur	144 – 260	276 293	279 259
Matrix	**Hulls**	**Hulls**	**Hulls**
Calcium	150	146	150
Copper	0.36	0.33	0.36
Iron	3.0	2.97	2.14
Magnesium	150	181	183
Manganese	1.68	1.49	1.70
Molybdenum	0.037	< 0.2	< 0.2
Phosphorus	80	113	96
Potassium	1130	1215	1208
Sodium	0.9	16.1	12.9
Zinc	0.99	1.23	1.30
Sulphur	50	54	59
Matrix	**Meal[c]**	**Meal**	**Meal**
Calcium	160 –360	191 188	203 163
Copper	0.7 – 1.6	1.41 1.43	1.74 1.49
Iron	7.5 – 22.2	11.35 11.3	9.98 7.93
Magnesium	490 – 820	628 602	718 605
Manganese	1.4 – 2.5	1.89 1.73	2.05 1.65
Molybdenum	0.13 – 0.51	< 0.2 0.13	< 0.2 0.14
Phosphorus	860 – 1540	1155 1072	1388 1063
Potassium	1450 – 1980	1534 1458	1696 1487
Sodium	4 – 330	15.2 4.41	<10 4.51
Zinc	4.9 – 8.3	7.10 6.82	8.07 6.35
Sulphur	370 – 500	443 395	506 385

[a] Some results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005). If only one value is displayed then the parameter was only examined by Phillips et al.(2003).

[b] Values for cottonseed and meal as described in OECD (2004) are given, for hulls as described by the NCPA (2002).

[c] Nutrient composition of solvent-extracted, 41% crude protein cottonseed meal from Forster and Calhoun (1995) are given. It is unclear if the meal was toasted or not. The term 'meal' refers to toasted meal in the case of Phillips et al.(2003).

131. Fatty acid analysis was conducted on cottonseeds and oil of controls (null plants resulting from F1 segregation (Phillips et al. 2003)) or PSC-355 (McCormick & Phillips

2005) and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). For each study, plant material was harvested across six locations in the USA. The results of the studies are shown in Table 9. Overall, the fatty acid profile of cottonseed samples and refined oil from WideStrike™ cotton was not significantly different to that obtained for control samples harvested from the same field trial sites. The values vary between the two cotton seed datasets provided, with one dataset tending to be lower than the literature values and the other dataset being higher. For the oil samples, the values were consistent with the published ranges.

Table 9 Fatty acid analysis on cottonseeds and oil of control and WideStrike™ cotton[a]

	Literature values	Control	WideStrike™ cotton
Fatty acids (% fresh weight)			
Matrix	**Seed**	**Seed**	**Seed**
8:0 Caprylic	Not available	< 0.0200 < 0.0197	< 0.0200 < 0.0197
10:0 Capric	Not available	< 0.0200 < 0.0200	< 0.0200 < 0.0200
12:0 Lauric	Not available	< 0.0200 < 0.0202	< 0.0200 < 0.0202
14:0 Myristic	0.22 – 0.36[b]	0.185 0.836	0.198 0.839
14:1 Myristoleic	Not available	< 0.0200 < 0.0203	< 0.0200 < 0.0203
15:0 Pentadecanoic	0.11 – 0.2[b]	< 0.0200 < 0.0204	< 0.0200 < 0.0204
15:1 Pentadecenoic	Not available	< 0.0200 < 0.0204	< 0.0200 < 0.0204
16:0 Palmitic	8.31 – 9.31[b]	5.03 21.9	5.11 21.8
16:1 Palmitoleic	0.16 – 0.24[b]	0.113 0.487	0.117 0.462
17:0 Heptadecanoic	0.04 – 0.07[b]	< 0.0200 < 0.0205	< 0.0200 < 0.0205
17:1 Heptadecenoic	Not available	< 0.0200 < 0.0205	< 0.0200 < 0.0205
18:0 Stearic	0.78 – 1.09[b]	0.563 2.21	0.595 2.29
18:1 Oleic	4.96 – 5.36[b]	3.51 14.9	3.66 15.0
18:2 Linoleic	15.5 – 16.7[b]	11.7 53.0	11.6 52.0
18:3 Gamma linolenic	Not available	< 0.0200 0.072	< 0.0200 0.067
18:3 Linolenic	0.04 – 0.1[b]	0.0888 0.378	0.0900 0.393
20:0 Arachidic	0.09 – 0.10[b]	0.0638 0.247	0.0668 0.261
20:1 Eicosenoic	Not available	< 0.0200	< 0.0200

	Literature values	Control	WideStrike™ cotton
		< 0.0207	< 0.0207
20:2 Eicosadienoic	Not available	< 0.0200 < 0. 0207	< 0.0200 < 0. 0207
20:3 Eicosatrienoic	Not available	< 0.0200 < 0. 0207	< 0.0200 < 0. 0207
20:4 Arachidonic	Not available	< 0.0200 < 0. 0207	< 0.0200 < 0. 0207
22:0 Behenic	0.04 – 0.06[b]	0.0354 0.179	0.0361 0.131
Matrix	**Oil**	**Oil**	**Oil**
8:0 Caprylic	Not detectabled	< 0.208	< 0.208
10:0 Capric	Not detectabled	< 0.211	< 0.211
12:0 Lauric	Not detectable – 0.2[d]	< 0.214	< 0.214
14:0 Myristic	0.6 – 1.0[d]	0.868	0.882
14:1 Myristoleic	Not available	< 0.215	< 0.215
15:0 Pentadecanoic	Not available	< 0.216	< 0.216
15:1 Pentadecenoic	Not available	< 0.216	< 0.216
16:0 Palmitic	21.1 – 28.1 [c]	22.7	23.0
16:1 Palmitoleic	0.4 – 1.2 [c,d]	0.496	0.472
17:0 Heptadecanoic	Not detectable – 0.1[d]	< 0.218	< 0.218
17:1 Heptadecenoic	Not detectable – 0.1[d]	< 0.218	< 0.218
18:0 Stearic	2.1 – 3.3 [c,d]	2.26	2.43
18:1 Oleic	12.9 – 21.7 [c,d]	15.5	15.9
18:2 Linoleic	46.0 – 58.2 [c,d]	55.1	54.7
18:3 Gamma linolenic	Not available	< 0.0762	< 0.0762
18:3 Linolenic	Not detectable – 0.4 [c,d]	0.348	0.370
20:0 Arachidic	0.2 – 0.5[d]	0.241	0.258
20:1 Eicosenoic	Not detectable – 0.1[d]	< 0.219	< 0.219
20:2 Eicosadienoic	Not detectable – 0.1[d]	< 0.219	< 0.219
20:3 Eicosatrienoic	Not available	< 0.219	< 0.219
20:4 Arachidonic	Not available	< 0.219	< 0.219
22:0 Behenic	Not detectable – 0.6[d]	0.180	0.245

[a] Some results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005). If only one value is displayed then the parameter was only examined by McCormick and Phillips (2005).

[b] Values as described in Berberich (1996) for cottonseed are given. The values in Berberich (1996) were given as percent of fatty acids. These values were converted into percent dry weight of cottonseed (the lipid fraction of the tested cottonseed was given and represented 33.5%).

[c] Values as described in OECD (2004).

[d] Values as described in Codex Alimentarius (2001).

132. An analysis of the amino acid composition was conducted in two separate studies on cottonseeds and meal of null plants resulting from F1 segregation (Phillips et al. 2003) or PSC-355 (McCormick & Phillips 2005) as control plants and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). For each study, plant material was harvested across six locations in the USA. The results of the study are summarised in Table 10. Alanine and

tryptophan levels in WideStrike™ cotton seed were significantly higher than in control seed in one out of the two experiments, but values were still within the published range. Statistical analysis was only provided for one of the two datasets for meal, and showed that aspartic acid, alanine and lysine were all higher in the WideStrike™ cotton meal than in the control. Again, the levels of all amino acids were within or very close to the range published in the literature.

Table 10 Amino acid composition of cottonseed and meal of control and WideStrike™ cotton[a]

Amino acid [% dry weight]	Literature values[b]	Control	WideStrike™ cotton
Matrix	**Seed**	**Seed**	**Seed**
Aspartic acid	2.09 – 2.66	2.51 2.50	2.60 2.54
Threonine	0.74 – 0.96	0.766 0.639	0.787 0.673
Serine	0.94 – 1.32	1.21 1.26	1.26 1.28
Glutamic acid	4.33 – 5.28	5.41 5.44	5.49 5.62
Proline	0.82 – 1.14	1.03 1.22	1.04 1.27
Glycine	0.93 – 1.19	1.12 1.11	1.15 1.14
Alanine	0.85 – 1.13	1.05 0.969	1.08 1.01
Cysteine	0.38 – 0.48	0.404 0.488	0.423 0.481
Valine	1.01 – 1.28	1.19 1.03	1.23 1.05
Methionine	0.35 – 0.54	0.378 0.371	0.391 0.377
Isoleucine	0.71 – 0.88	0.867 0.804	0.888 0.827
Leucine	1.27 – 1.65	1.56 1.57	1.60 1.60
Tyrosine	0.48 – 0.79	0.691 0.600	0.718 0.613
Phenylalanine	1.13 – 1.45	1.40 1.42	1.44 1.44
Histidine	0.62 – 0.82	0.684 0.714	0.734 0.738
Lysine	1.01 – 1.33	1.08 1.25	1.16 1.26
Arginine	2.38 – 3.23	2.91 2.93	3.08 2.98
Tryptophan	0.23 – 0.36	0.258 0.305	0.275 0.317
Matrix	**Meal[c]**	**Meal**	**Meal**
Aspartic acid	3.99 – 4.25	4.15	4.70

Amino acid [% dry weight]	Literature values[b]	Control	WideStrike™ cotton
		4.57	4.88
Threonine	1.46 – 1.61	1.32 1.09	1.65 1.12
Serine	2.02 – 2.12	1.84 2.15	2.27 2.26
Glutamic acid	8.43 – 10.2	8.59 9.89	9.58 10.4
Proline	1.42 – 1.69	1.63 2.07	1.91 2.21
Glycine	1.80 – 1.91	1.88 1.86	2.15 1.91
Alanine	1.66 – 1.86	1.77 1.69	2.04 1.81
Cysteine	0.64 – 0.75	0.723 0.883	0.795 0.886
Valine	1.66 – 1.92	2.11 1.74	2.28 1.82
Methionine	0.58 – 0.79	0.683 0.823	0.760 0.821
Isoleucine	0.67 – 0.79	1.50 1.36	1.65 1.42
Leucine	2.45 – 2.63	2.65 2.65	3.02 2.78
Tyrosine	0.94 – 1.06	1.12 1.09	1.39 1.11
Phenylalanine	2.19 – 2.41	2.41 2.47	2.79 2.56
Histidine	1.39 – 1.51	1.31 1.29	1.51 1.32
Lysine	1.56 – 1.97	2.01 2.16	2.26 2.31
Arginine	4.35 – 5.03	5.00 5.09	5.86 5.32
Tryptophan	0.49 – 0.60	0.468 0.573	0.548 0.595

[a] The results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005).

[b] Values as described in OECD (2004) are given.

[c] Amino acid composition (% of dry matter) of cottonseed meals from Forster and Calhoun (1995) are given. It is unclear if the meal was toasted or not. The term 'meal' refers to toasted meal in the case of Philips et al (2003).

133. An analysis of the vitamin content was conducted in a single study on cottonseeds of controls (null plants resulting from F1 segregation) and WideStrike™ cotton (Phillips et al. 2003). Plant material was harvested across six locations in the USA. No significant differences were found, and no literature values were available for comparison. The results of the study are summarised in Table 11.

Table 11 Vitamin content of cottonseed of control and WideStrike™ cotton

	Control	WideStrike™
Matrix	**Cottonseed**	**Cottonseed**
Vitamins [mg/kg dry weight]		
Vitamin A	< 0.6	< 0.6
Vitamin B1 (thiamin)	3.61	3.34
Vitamin B2 (riboflavin)	2.59	2.45
Vitamin B6 (pPyridoxine)	4.90	4.89
Vitamin C (ascorbic acid)	4.15	4.90
Folate (folic acid)	2.41	2.41
Niacin (nicotinic acid)	25.8	25.3
Alpha Tocopherol	110	112
Beta Tocopherol	< 7.0	< 7.0
Gamma Tocopherol	130	119
Delta Tocopherol	< 7.0	< 7.0
Total Tocopherols	240	231

134. An analysis of the tocopherol composition was conducted in two separate studies on oil of controls (null plants resulting from F1 segregation (Phillips et al. 2003) or PSC-355 (McCormick & Phillips 2005)) and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). For each study, plant material was harvested across six locations in the USA. The results of the studies are summarised in Table 12. Tocopherol results for the control and the GM cotton are very similar and fall within Codex standards for occurrence of alpha-, beta-, gamma-, and delta-tocopherols in crude cottonseed oil (CODEX 2001). However, it should be noted that the oil obtained from the processing in this study was refined and deodorized, which typically removes tocopherols from the oil.

Table 12 Tocopherol composition of control and WideStrike™ cotton[a]

	Literature values[b]	Control	WideStrike™
Matrix	**Refined oil**	**Refined oil**	**Refined oil**
Tocopherol [mg/kg]			
Alpha tocopherol	136 – 674	549 452	515 449
Beta tocopherol	Not detected – 29	< 60.0 < 20.0	< 60.0 < 20.0
Gamma tocopherol	138 – 746	344 458	372 432
Delta tocopherol	Not detected – 21	< 60.0 < 20.0	< 60.0 < 20.0

[a] The results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005).

[b] Values as described in the Codex Alimentarius (2001) are given.

135. Anti-nutrient analysis was conducted in two separate studies on seed, meal and refined oil of controls (null plants resulting from F1 segregation (Phillips et al. 2003) or PSC-355 (McCormick & Phillips 2005) and WideStrike™ cotton (Phillips et al. 2003; McCormick & Phillips 2005). In addition, Phillips et al. (2003) also analysed kernels. For each study, plant material was harvested across six locations in the USA. The results of the study are summarised in Table 13. WideStrike™ cotton seed did not differ significantly in anti-nutrients compared to the control. Aflatoxins were only detectable in one control sample of

cotton seed, and all other anti-nutrients measured in seed were within literature ranges. Results for analysis of kernel, meal and refined oil for gossypol (free and total) did not differ significantly between WideStrike™ and control cotton. For meal, all values are within the literature range. No literature values for gossypol are available for kernels, but the obtained results are similar to the values reported for cottonseed. Only low total gossypol levels were found in the oil samples analysed. The cyclopropenoid fatty acids (sterculic, malvalic, and dihydrosterculic) were also determined for oil and very similar levels were obtained for the control and WideStrike™ samples. When comparing results of the cyclopropenoid fatty acid analysis to literature values, the malvalic results for both WideStrike™ and the control were lower than the literature value. Since these lower levels were seen in both the GM and non-GM cotton, it is not considered to be an effect of the genetic modification. Results for sterculic and dihydrosterculic fatty acids were comparable to the literature values.

Table 13 Anti-nutrient composition of fractions of control and WideStrike™ cotton[a]

	Literature values[b]	Control	WideStrike™
Matrix	**Seed**	**Seed**	**Seed**
Cyclopropenoid fatty acids (%)			
Sterculic acid	0.13 – 0.70	0.321 0.155	0.292 0.156
Malvalic acid	0.17 – 0.61	0.397 0.271	0.344 0.283
Dihydrosterculic acid	0.11 – 0.50	0.220 0.152	0.209 0.162
Aflatoxins (ppb dry weight)			
AHB1	Not available	< 1.0 12.3	< 1.0 < 1.0
AHB2	Not available	< 1.0 < 1.0	< 1.0 < 1.0
AHG1	Not available	< 1.0 < 1.0	< 1.0 < 1.0
AHG2	Not available	< 1.0 < 1.0	< 1.0 < 1.0
Free gossypol (%)	0.47 – 0.70	Not available 0.695	Not available 0.670
Total gossypol (%)	0.51 – 1.43	0.870 0.973	0.791 0.914
Matrix	**Kernel**	**Kernel**	**Kernel**
Free gossypol (%)	Not available	0.908	1.028
Total gossypol (%)	Not available	1.056	1.085
Matrix	**Meal[c]**	**Meal**	**Meal**
Free gossypol (%)	0.02 – 1.77	0.045 0.954	0.057 0.989
Total gossypol (%)	0.93 – 1.43	0.927 1.36	1.078 1.46
Matrix	**Refined oil**	**Refined oil**	**Refined oil**

	Literature values[b]	Control	WideStrike™
Free gossypol (%)	Not available	< 0.002 < 0.03	< 0.002 < 0.03
Total gossypol (%)	0.00 – 0.09	< 0.002 < 0.01	< 0.002 < 0.01
Cyclopropenoid fatty acids (%)			
Sterculic acid	0.08 – 0.58	0.217 0.207	0.237 0.192
Malvalic acid	0.422 – 1.44	0.272 0.364	0.263 0.353
Dihydrosterulic acid	0.00 – 0.22	0.212 0.202	0.204 0.204

[a] The results are from two independent experiments. The top lines for each parameter displays the results obtained by Phillips et al.(2003) and the results in the second line displays the results obtained by McCormick and Phillips (2005).

[b] Values as described in OECD (2004).

[c] The term 'meal' refers to toasted meal in the case of Phillips et al.(2003).

136. In conclusion, only five compounds (crude fibre and four amino acids) were found to differ between WideStrike™ cotton and controls, but these were still very similar to published values. There were a limited number of instances when results from compositional analyses were found to be either higher or lower than reported literature ranges. These differences were not considered significant since results for control and GM samples did not differ in these instances. Therefore, the results from the compositional analyses demonstrate that WideStrike™ cotton is similar to non-GM cotton.

5.5.3 Expression of the encoded proteins in the GM cotton

137. Western blotting of lyophilised leaf extract of parental cotton 3006-210-23 with polyclonal anti-truncated Cry1Ac antibodies showed only truncated Cry1Ac(synpro). It has been suggested that protein cleavage may have occurred *in planta* or during processing for western blotting (Gao et al. 2002b).

138. Western blotting of lyophilised leaf extract of parental cotton 281-24-236 with polyclonal anti-Cry1F antibodies showed only truncated Cry1F(synpro). However, an immunoreactive band co-migrating with microbially-produced truncated protein was observed. In addition, an immunoreactive band between 30 and 35 kDa was obtained (Gao et al. 2001). It has been suggested that protein cleavage may have occurred *in planta* or during processing for western blotting.

139. Field studies documenting expression of Cry1F(synpro), Cry1Ac(synpro) and PAT in WideStrike™ cotton, in different organs and at different stages of development, have been conducted in both the USA and Australia.

140. All samples, with the exception of pollen, nectar, seed, kernel, hull, meal and oil, were lyophilised prior to protein expression analysis. Soluble and extractable Cry1F(synpro), Cry1Ac(synpro) and PAT proteins were measured using quantitative enzyme-linked immunosorbent assay (ELISA) methods with detection limits ranging from 0.001 – 0.4 ng protein/mg sample weight. Results were reported on a fresh weight basis for cottonseed, pollen, nectar and processed products and dry weight for all other tissues.

141. In the USA, WideStrike™ cotton was planted at six locations across the country together with non-GM controls (null plants obtained from the F1 segregating generation

(Phillips et al. 2003)). At each site, three GM plots and one control plot were established, with each GM plot consisting of three replicate plots. Plant material was collected from two or six locations over time and protein expression levels recorded for tissues, organs and whole plants, as shown in Table 14. In a later USA study, McCormick and Phillips (2005) also analysed cottonseed and processed products for expression of the three introduced genes.

Table 14 Expression levels of Cry1F(synpro), Cry1Ac(synpro) and PAT in WideStrike™ cotton

Tissue	Cry1Ac(synpro) [ng/mg tissue dry weight][a,*]	Cry1F(synpro) [ng/mg tissue dry weight][a,*]	PAT [ng/mg tissue dry weight][a,*]
Young leaf (3 – 6 weeks)	1.82	6.81	0.43
Terminal leaf (68-87 Days after planting (DAP))	1.31	8.19	0.23
Flower (59-87 DAP)	1.83	5.44	0.35
Square (68-87 DAP)	1.82	4.88	0.52
Boll – early (74-84 DAP)	0.64	3.52	0.27
Whole plant – seedling	1.37	14.1	0.35
Whole plant – at pollination	1.05	25.3	0.30
Whole plant – at defoliation	0.6	21.1	0.34
Root – seedling	0.17	0.88	(0.06)
Root – at pollination	(0.07)	0.54	Not detected
Root – at defoliation	Not detected	0.51	(0.05)
Pollen (74-95 DAP)	1.45	(0.06)	(0.05)
Nectar	Not detected	Not detected	Not detected
Seed	0.55	4.13	0.54
Cottonseed	0.46 0.46†	3.1 2.34†	0.53 0.53†
Kernel	0.51 0.69†	3.9 3.9†	0.78 0.70†
Hulls	nd 0.18†	0.16 1.6†	nd 0.05†
Toasted meal	nd 0.38†	nd 1.0†	nd nd†
Refined oil	nd nd†	nd nd†	nd nd†

[a] The means across all samples of the individual tissues are given; Values in brackets indicate that the calculated concentration is less than the limit of quantitation (LOQ) of the method; nd: not detected; *Phillips et al.(2003); †McCormick and Phillips (2005)

142. Whole plants, collected at pollination stage, were found to express the highest levels of Cry1F(synpro), followed by leaves, flowers and seeds. There was little expression in the roots, and levels of expressed protein in pollen and nectar were extremely low to undetectable. In processed cotton products, Cry1F(synpro) was present at low levels in cottonseed, kernel and hulls and not detected in toasted meal and refined oil (Table 14).

143. The USA studies indicated that the expression level of Cry1Ac(synpro) is markedly lower than that of Cry1F(synpro) in WideStrike™ cotton. This may be because the *cry1F(synpro)* and *cry1Ac(synpro)* genes are controlled by different promoters (Phillips et al. 2003). There was little difference in expression of Cry1Ac(synpro) between tissues, with the highest concentration being found in young leaves, flowers and squares (1.82 – 1.83 ng/mg) and the lowest in roots (0.17 ng/mg).

144. Analysis of processed cotton products showed that Cry1Ac(synpro) was present in cottonseed and kernels and not detected in refined oil (Table 14).

145. Expression studies have also been carried out in Australia, using lyophilised tissue of WideStrike™ cotton plants grown in three locations, summarised in Table 15 and Table 16 (Litzow 2004; Dixon 2006; Annetts 2006a; Annetts 2006b; Annetts 2006c).

Table 15 Expression levels of Cry1F(synpro) in WideStrike™ cotton grown in Australia

Location	Tissue	Average expression level [ng Cry1F(synpro)/mg tissue dry weight]			
		69 – 86 DAP	**99 – 104 DAP**	**134 – 148 DAP**	**153 DAP**
Boggabri, NSW	4th node leaves	30.9	61.9	121.3	na
Breeza, NSW		31.8	nd	82.1	na
Breeza, NSW		27.9	48.9	76.1	197.5
Darling Downs		17.3	39.7	124.6	na
Darling Downs		21.6	79.7	172.6	na
Breeza, NSW	Terminal leaves	21.9	30.8	82.0	120.1
Darling Downs		22.4	32.1	148.9	na
Darling Downs		32.7	79.4	159.7	na
Breeza, NSW	Pin squares	16.7	27.2	39.5	26.9
Darling Downs		16.4	15.1	67.8	na
Darling Downs		17.3	63.8	nd	na
Breeza, NSW	Mature squares	19.6	81.3	113.7	173.3
Darling Downs		20.6	91.6	152.3	na
Darling Downs		31.7	98.1	128.6	na
Breeza, NSW	Roots	2.45	2.51	2.53	10.73
Darling Downs		1.9	3.0	2.7	na

DAP: days after planting; nd: not detected; na : not assessed

Table 16 Expression levels of Cry1Ac(synpro) in WideStrike™ grown in Australia

Location	Tissue	Average expression level [ng Cry1Ac(synpro)/mg tissue dry weight]		
		69 – 86 DAP	**99 – 104 DAP**	**134 – 148 DAP**
Boggabri, NSW	4th node leaves	0.7	1.1	1.5
Breeza, NSW		0.8	nd	1.4
Breeza, NSW		1.5	2.0	2.5
Darling Downs		1.3	2.2	1.9
Darling Downs		1.9	2.6	3.3
Breeza, NSW	Terminal leaves	0.9	2.34	3.1
Darling Downs		1.3	3.0	1.9
Darling Downs		2.2	2.9	3.5
Breeza, NSW	Pin squares	0.5	0.8	0.4
Darling Downs		0.3	0.9	0.5
Darling Downs		1.1	0.9	nd
Breeza, NSW	Mature squares	1.1	1.7	2.3
Darling Downs		1.1	1.6	2.0
Darling Downs		1.9	1.8	2.1
Breeza, NSW	Roots	0.08	0.1	0.07
Darling Downs		0.04	0.07	0.04

DAP: days after planting; nd: not detected

146. The general pattern of expression of Cry1F(synpro) and Cry1Ac(synpro) is similar to that noted in the USA studies, with maximum expression occurring in leaves and the least in roots for both proteins. Again, the level of expression of Cry1F(synpro) was found to be substantially higher than that of Cry1Ac(synpro) (Table 15 and Table 16), and expression of both proteins generally increased over the course of the growing season.

147. Comparing values across all studies, the average expression level of Cry1F(synpro) at 69-86 DAP (first data column, Table 15) was notably higher in the Australian study than for USA plant tissue at an equivalent stage (Table 14). In terminal leaves, for example, Cry1F(synpro) expression levels ranged from 21.9 – 32.7 ng/mg in the Australian study,

compared with 8.19 ng/mg in the USA study. Values for Cry1Ac(synpro) expression in the Australian and USA studies are not markedly different (Table 14 and Table 16).

148. USA studies showed the PAT protein to be present in seeds of WideStrike™ cotton. Expression data for the PAT protein in WideStrike™ cotton is summarised in Table 14. Data on PAT expression in plants grown under Australian field conditions was not supplied by the applicant.

149. Levels of the PAT protein ranged from 0.27 ng/mg dry weight in early bolls to 0.54 ng/mg dry weight in seeds and expression in roots, pollen and nectar was at the lower limits of detection. For processed products, the highest levels were found in the kernels (0.7 – 0.78 ng/mg fresh weight).

5.5.4 Toxicity of the GM cotton plant material

Toxicity/allergenicity of GM cotton plant material to humans

150. The OECD consensus document on compositional considerations for new cotton varieties (2004) suggests the following parameters to be analysed in both cottonseed and oil that is to be used in human food: tocopherol (vitamin E), fatty acids, gossypol (free and total), malvalic acid, sterculic acid and dihydrosterculic acid. In addition, proximates should be analysed in cottonseed. The applicant has provided analyses for all those parameters (see Table 7, Table 9, Table 11, Table 12 and Table 13), demonstrating that they were within or similar to ranges published in the literature. The exception to this is for some fatty acids which showed high variability between datasets, and malvalic acid which was slightly lower in both WideStrike™ and non-GM cotton seed oil than published values (see Table 13). In addition, all measured parameters did not differ significantly between WideStrike™ cotton and controls, with the exception of crude fibre, which was lower in WideStrike™ cotton seed in one dataset but still within the literature range (see Table 7).

151. Cotton seed, meal and hulls are not generally used for human consumption because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropenoid fatty acids, but may be used for animal feed, particularly cattle, which are less affected by gossypol (OGTR 2008). Processed fractions of WideStrike™ cotton, such as oil and linters, have been approved by FSANZ for use in human food since 2004 (FSANZ 2004; FSANZ 2005c).

152. The applicant has stated that none of the dealings conducted under licences DIR 040/2003 and DIR 044/2003 have resulted in adverse effects from occupational exposure in staff dealing with WideStrike™ cotton.

Toxicity of GM cotton plant material to other vertebrates

153. The OECD consensus document on compositional considerations for new cotton varieties (2004) suggests the following parameters to be analysed in both cottonseed and meal that is to be used in animal feed: proximates, calcium, phosphorus, gossypol (free and total), sterculic acid, malvalic acid and dihydrosterculic acid. In addition, amino acids and fatty acids should be analysed in cottonseed. The applicant has provided analyses demonstrating that those parameters do not differ between WideStrike™ cotton and non-GM cotton (see Section 5.5.2, Table 7, Table 9, Table 12 and Table 13). In addition, results were within or similar to published values, with the exception of fatty acids (see Table 9).

154. The potential for toxic effects as a result of feeding broiler chickens meal derived from WideStrike™ cottonseed was investigated (McNaughton 2003). Ten percent of the diet

consisted of cotton seed meal in all treatments. The animals were observed for mortality and for the onset, severity and duration of any behavioural changes or evidence of toxicity. The rate of mortality was very low throughout the trial and was not significantly different for WideStrike™ cottonseed or controls. Similarly, body weight gain was not affected by feeding WideStrike™ cottonseed compared to non-GM controls, with no significant differences in weight measured at the end of the feeding trial (42 days).

155. A dietary study was conducted with the northern bobwhite (*Colinus virginianus*) using cotton meal prepared from WideStrike™ cotton seeds (Gallagher & Beavers 2002a). The test group received meal from GM cotton seed over five days, making up 10% of the diet, and for another three days untreated basal diet. Animals were observed for clinical signs of toxicity and abnormal behaviour, and weight was recorded. Both the test group and the non-GM cotton seed control group showed signs of toxicity that was attributed to the gossypol content in the feed (including wing droop, ruffled appearance, loss of coordination and lethargy), however there was no mortality observed during the study. Cotton seed meal contains gossypol, a substance with both cardiotoxic and hepatotoxic properties. While feed with a gossypol content of up to 100 ppm is considered safe, the diet used in this study contained approximately 800 to 1,000 ppm free gossypol.

156. An eight-day dietary toxicity study was conducted on Rainbow trout (*Oncorhynchus mykiss* Walbaum.) using 10% cotton meal prepared from WideStrike™ cotton seed (Marino & Yaroch 2002b). No mortality or signs of toxicity were observed during the study.

Toxicity to target invertebrates

157. The applicant conducted a study on the efficacy of WideStrike™ cotton against *S. litura* (Dow AgroSciences 2006b). *S. litura* neonates were placed onto leaves collected from field grown WideStrike™ cotton at the boll-maturation stage, or onto leaves from non-GM cotton as a control. Five replicate tests were performed in which mortality was measured at varying intervals over 21 days. By day six, all *S. litura* larvae on WideStrike™ cotton leaves had died compared to only 6% of the control larvae. It could not be concluded from this study whether it was the Cry1Ac(synpro) or the CryF(synpro) protein causing the observed effect, although INGARD® cotton, which contains only the Cry1Ac insecticidal protein, has been shown to only poorly control *S. litura* (Strickland et al. 2003). The information provided by the applicant lacks some details, eg it is not clear how the data were adjusted or how many insects were exposed to each treatment.

Field trials in the USA

158. A pilot field trial involving four independent tests was conducted in the USA to evaluate the efficacy of WideStrike™ cotton to tobacco budworm (*H. virescens*) and pink bollworm (*P. gosypiella*) where control PSC-355, both parental lines and WideStrike™ cotton plants were established in experimental plots (Pellow 2001). The GM lines gave better control of *H. virescens* than the unsprayed non-GM plants and similar control to the sprayed non-GM plants. In preliminary data from the trial evaluating efficacy against *P. gossypiella*, the Cry1Ac parental line and the WideStrike™ cotton provided excellent control of these insects, while the Cry1F(synpro) parental line does not appear to provide any control.

Field trials in Australia

159. The applicant conducted a field trial in Breeza Plain, NSW, under DIR 040/2003. Both PSC-355 (control) and WideStrike™ cotton were planted and artificially infested with *H. armigera*. Part of the control and WideStrike™ cotton were sprayed during the growing

season for the control of lepidopteran pests when the threshold of two larvae per metre row was exceeded, part of both remained unsprayed. Sprayed and unsprayed WideStrike™ cotton plants retained more of the top five squares than the non-GM controls. An analysis of freeze dried leaf material (collected below the 4th node) indicated that average Cry1Ac(synpro) levels increased from 1.0 to 1.4 ng/mg tissue from 69 to 140 days after planting (DAP) and that average Cry1F(synpro) levels increased from 31.8 to 82.1 ng/mg tissue in the same time span (Dixon 2006).

160. In another field trial conducted in Boggabri, NSW (Litzow 2004), both PSC-355 (control) and WideStrike™ cotton were planted. Part of the control and WideStrike™ cotton were sprayed to control lepidopteran pests. At the site, artificial infestation of plants was carried out in the early season, at the 10 – 13/squaring growth stage. Ten unsprayed control and WideStrike™ cotton plants were artificially infested. Later in the season, natural infestation with *H. armigera* and *H. punctigera* was considered sufficiently high to conduct an efficacy trial. Sprayed WideStrike™ cotton retained the highest percentage of top 5 squares. Unsprayed WideStrike™ cotton performed as well as the sprayed non-GM control, while unsprayed non-GM plants retained the fewest top 5 squares. A similar trend was observed for lint yield, with the order of highest to lowest yield being: sprayed WideStrike™ cotton; unsprayed WideStrike™ cotton; sprayed non-GM cotton; unsprayed non-GM cotton. An analysis of freeze-dried leaf material (collected below the 4th node) indicated that average Cry1Ac(synpro) levels increased from 0.72 to 1.54 ng/mg tissue from 86 to 134 DAP and that average Cry1F(synpro) levels increased from 30.9 to 121.3 ng/mg tissue in the same time span. Larger sized larvae were only observed in unsprayed WideStrike™ cotton when the crop was 'cutting out'. Possible reasons for the observation of larger larvae may include either a drop in the expression of insecticidal proteins or avoidance of tissues with high *cry* gene expression by the insects.

161. The applicant submitted a report on a preliminary study investigating the efficacy of WideStrike™ cotton in controlling *H. armigera* and *H. punctigera* under Australian field conditions. The small scale trial was conducted under *Helicoverpa* pressure that exceeded the nominal *Heliothis* spray threshold of two larvae/m row ten times during the growing season. The treatments included WideStrike™ cotton (not managed for lepidopteran pests), the parental non-GM variety PCS-355 (not managed for lepidopteran pests), WideStrike™ cotton (managed for lepidopteran pests) and PCS-355 (managed for lepidopteran pests). Five replicates of two rows with a length of 10 m each were used per treatment. Fewer *Helicoverpa* larvae were present and less damage consistent with lepidopteran pressure was observed on WideStrike™ cotton plants (sprayed or unsprayed) compared to unsprayed non-GM plants.

Toxicity to non-target invertebrates

Field trials in the USA

162. Field trials were conducted at Winnsboro, Louisiana, and Maricopa, Arizona, USA to evaluate the effects of WideStrike™ cotton on non-target arthropods (Mahill & Storer 2002). In these trial areas the target pests are tobacco budworm (*H. virescens*) and cotton bollworm (*H. zea*) at the former, and pink bollworm (*P. gossypiella*) at the latter site. The studies consisted of three and two replications per treatment, respectively, in a randomised complete block design. All cotton was managed for non-lepidopteran pests. Care was taken to use active ingredients that were not lepidopteran active and that would minimise damage to beneficial arthropods. WideStrike™ cotton and PSC-355 were grown, and these were either sprayed for lepidopteran and non-lepidopteran pests or only sprayed for non-lepidopteran

pests. A number of sampling methods were used to determine the abundance of beneficial insects in the test and control plots. Preliminary analysis of the data showed that no consistent adverse effects were observed for WideStrike™ cotton on non-target arthropods in either location when compared to unsprayed non-GM cotton. Significant negative effects were detected in plots of non-GM plants sprayed for Lepidoptera.

163. In another series of surveys in Winnsboro and Maricopa in 2002 and 2003, effects of WideStrike™ cotton on non-target beneficial arthropods were investigated (Storer 2003). The parental variety PSC-355 was used as a control. All treatments included control of non-lepidopteran pests. Two to three replicates per treatment per location were included in both years. A number of sampling methods were used.

164. The results indicate that the abundance and diversity of non-target arthropods tended to be lower in non-Bt cotton that received treatment with insecticides to control Lepidoptera than in non-Bt cotton that did not receive insecticides to control Lepidoptera. By contrast, the non-target arthropod abundance and diversity in WideStrike™ cotton was similar to non-Bt cotton when both were managed for other pests but received no sprays to control Lepidoptera. Some minor but statistically significant differences were detected but these were not consistent across years, across locations or across sampling methods. The only major or consistent differences in arthropod abundance between WideStrike™ cotton and unsprayed non-Bt cotton were in lepidopteran larvae, which are the target of the GMO proposed for release.

Field trials in Australia

165. A comparative arthropod census on unsprayed WideStrike™ cotton and on the unsprayed parental cultivar PSC-355 was performed near Dalby, Australia (Murray 2005). The two treatments were unreplicated in 40 x 40 m blocks.

166. Invertebrate samples were obtained by suction sampling at 4-leaf, squaring, flowering and cut-out stage. Identification of some samples was to the species level, although for many genus and species names could not be assigned. The results indicate that similar numbers of arthropods were present in both treatments, with approximately 19% more arthropods present, 5.4% fewer species represented and 2.5% fewer families represented on WideStrike™ cotton compared to the non-GM plants. In samples that were pooled over the growing season, the number of Lepidoptera was lower in WideStrike™ and the number of dipteran species was slightly reduced. Twenty families of Diptera were represented in the non-GM cotton *vs* 12 families of Diptera in WideStrike™ cotton. No analysis of the significance of those reductions was carried out.

Degradation of the introduced Cry proteins in soil

167. The applicant supplied a study in which the microbially produced Cry1Ac(synpro) was tested for degradation in soil representative for the USA (Herman et al. 2001). Based on bioassays assessing toxicity to *H. virescens*, the Cry1Ac(synpro) protein did not decay significantly over the 28 days of the experiment. However, when the applicant subsequently analysed the soil, it was found to have low microbial activity and biomass, indicating that the soil was of poor viability and it was thus considered not representative of soils in cotton growing areas and unsuitable for degradation experiments.

168. In a second study, lyophilised leaf material of WideStrike™ cotton was incubated in representative soils to determine degradation (Herman & Collins 2001). Non-GM cotton plant material was included as a control. The soil used was collected from a cotton growing region in the USA, and it was characterised according to the USDA classification as fine-

silty, mixed, non-acid, thermic aeric fluvaquents. Samples were incubated at approximately 25ºC and tested at various intervals for biological activity against *H. virescens*. Bioassays were conducted on neonate larvae and mortality and insect weights were measured. Based on the increase observed in the concentration of treated soil sample required to reduce growth of the larvae by 50% (GI_{50}) over time, the half life of the Cry proteins was 1.3 days, indicating a rapid decay rate.

169. Shan et al. (2007) conducted a study in the USA on soil samples collected from plots where WideStrike™ cotton was grown over three consecutive years. In the final year, three types of samples were taken and analysed for the presence of Cry1Ac(synpro) and Cry1F(synpro): root tissue samples, rhizosphere samples and bulk soil samples (taken at least 4 inches away from the plant). The proteins were not detected in the soil samples, but were present in the WideStrike™ cotton root tissue samples at mid-bloom and post harvest. Bioassays using *H. virescens* larvae showed no significant weight differences among insects fed on diet containing diluted soil samples. However, diet containing diluted WideStrike™ cotton root tissue, included as a positive control, reduced the growth of *H. virescens* larvae compared to diet without root tissue. Non-GM cotton root tissue also significantly reduced growth of the larvae, although to a lesser extent than WideStrike™ cotton root tissue.

170. Shan et al. (2008) conducted a study on soil samples collected from plots where GM maize expressing truncated Cry1F (event DAS-0150701), was grown over three consecutive years. The results were similar to those found for WideStrike™ cotton with the exception that non-GM maize root tissue did not reduce the growth of larvae.

Section 6 The receiving environment

171. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, location and duration of the dealings, 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 2007).

172. For this particular licence application, it is considered that the receiving environment would be primarily the areas south of latitude 22º South. The applicant has proposed growing WideStrike™ cotton commercially in Australia, south of latitude 22º South, and for harvested plant material to enter general commerce. The applicant also proposes that no restrictions be placed on the use of the GM cotton seed, cottonseed oil and meal in animal feed or human food and GM plant material may be transported and used Australia-wide.

6.1 Relevant abiotic factors

173. The abiotic factors relevant to the growth and distribution of cotton currently suitable for commercial production in Australia are discussed in *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* document (OGTR 2008). In brief, cotton cultivation is limited to areas in Australia where abiotic factors are appropriate. These factors include temperature, water availability (ie the right amounts at optimal times of the growth cycle via irrigation or rainfall), the length of the growing season and the suitability of the soil (good water retention qualities are required). These same factors may limit growth of cotton outside of the agricultural setting.

174. The typical climate for summer cotton growing regions in Australian areas south of latitude 22º South is warm summers and mostly higher summer than winter rainfall. Climatic data for some of the current cotton growing areas in southern Australia are given in Table 17.

Table 17 Climatic data for some of the current cotton growing regions in Australia.

	Emerald Post Office (central QLD)	Narrabri West Post Office (northern NSW)	Bourke Post Office (northern NSW)	Hillston Airport (southern NSW)
Average daily max/min temperature (summer)	34.1ºC/21.0ºC	33.3ºC/18.7ºC	35.6ºC/20.3ºC	32.5ºC/17.7ºC
Average daily max/min temperature (winter)	23.3ºC/7.8ºC	18.8ºC/4.5ºC	19.0ºC/5.6ºC	15.9ºC/4.6ºC
Average monthly rainfall (summer)	98.0 mm	73.9 mm	38.8 mm	28.8 mm
Average monthly rainfall (winter)	27.8 mm	46.0 mm	23.6 mm	32.2 mm

Source: http://www.bom.gov.au; (summer: December – February; winter: June – August; monthly means collected over at least 40 years were averaged over each season).

6.2 Relevant biotic factors

6.2.1 Presence of related plants in the receiving environment

Commercial non-GM and GM cotton in Australia

175. 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).

176. GM cotton plants 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). This includes herbicide tolerant and/or insect resistant GM cotton plants that have previously been approved for commercial release. In contrast, non-GM *G. hirsutum* comprised approximately 5% of commercially grown cotton.

177. Approvals for commercial releases of other GM cotton lines in Australia are as follows:

- 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)
- 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)
- 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).

178. To date, the Regulator has not received any reports of adverse effects caused by these authorised releases.

Cotton in the natural Australian environment

179. In southern Australia ephemeral populations of cotton may be present. 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).

180. Many of these naturalised populations have morphological characteristics including poor architecture, small bolls and tufted seed with sparse, grey lint, suggesting that they are not derived from modern cotton cultivars (Curt Brubaker and Lyn Craven, CSIRO, pers. comm., 2002). Some of the naturalised populations appear to have derived from cultivars which would have been introduced in the 1800s, others are more recent but none seem to have originated from the current commercial types of *G. hirsutum* that have been cultivated since the 1970's (Eastick 2002).

181. There are 17 native species of *Gossypium* in Australia, most of which can be found in the NT and the north of Western Australia (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). 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).

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

6.2.2 Presence of other biotic factors

183. 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)* document (OGTR 2008).

184. Vertebrates, invertebrates and other organisms in areas suitable for the GM cotton proposed for release may be exposed to its introduced genetic material. While lepidopteran insects do not seem to be identified as beneficial organisms, they play a major role as pests.

Lepidopteran pests in Australian cotton fields include *Helicoverpa armigera* (Hubner) and *H. punctigera* (Wallengren) (eg Macintosh et al. 1990), *Agrotis infusa* (Boisduval), *Bucculatrix gossypii* (Turner), *Pectinophora gossypiella* (Saunders), *Spodoptera exigua* (Hubner) and *S. litura* (Fabricius). Some of these are targets of the GMO proposed for release. Beneficial organisms include various spiders as well as insects, eg some belonging to the insect orders Hymenoptera, Coleoptera, Hemiptera and Diptera (Cotton Catchment communities CRC 2007).

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

Cry genes and proteins

185. *B. thuringiensis* (Bt) is an aerobic gram-positive endospore-forming bacterium that is ubiquitous in the environment (reviewed by Roh et al. 2007). Bt is insecticidal and was first described isolated from an infected flour moth, *Anagasta kuehniella*. During sporulation, proteinaceous inclusions are produced by Bt. The inclusions contain the insecticidal crystal proteins, also called Cry proteins or δ-endotoxins. The Cry proteins are encoded by *cry* genes and over 380 of these genes have been cloned and sequenced (Crickmore et al. 2009). The genes and their encoded proteins are widespread in the soil environment and have also been found associated with plant products and insects (reviewed by Schnepf et al. 1998).

186. Cry proteins are present in Bt microbial sprays which are used to protect crops from insect herbivory. Bt microbial sprays have been in use since 1938 in France (reviewed by Roh et al. 2007). A number of Bt subspecies, including ssp *kurstaki* and *aizawai*, as well as *Bacillus* species are listed as authorised active ingredients by the APVMA (accessed on 28 January 2009). Residues of Bt proteins, including Cry1Ac, are present on a wide variety of foods, including organically or conventionally produced fresh foods, with no reported toxic or allergic responses in humans (ANZFA 1999).

187. Commercial GM cotton plants such as Bollgard II® containing *cry1Ac* and *cry2Ab* contribute to the presence of *cry* genes and their products in Australia.

188. This information forms the baseline data for assessing the risks from exposure to these proteins as a result of the commercial release of the GM WideStrike™ cotton.

The introduced *pat* gene and PAT protein

189. The *pat* gene was derived from *S. viridochromogenes* (Thompson et al. 1987; De Block et al. 1987). *Streptomycetes* are common soil bacteria (Lawrence 2000), which can naturally develop the ability to detoxify glufosinate ammonium (Bartsch & Tebbe 1989). Different versions of PAT protein have also been expressed in other GM crop plants trialled (DIRs 010/2001, 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003) or commercially approved (canola DIR 021/2002 and cotton DIR 062/2005) in Australia.

6.3 Relevant agricultural practices

190. Agronomic management of the GM WideStrike™ cotton would differ from the management of non-GM cotton in that fewer applications of pesticide sprays are expected to be used since it is resistant to the major lepidopteran pests of cotton. These management practices are assumed to be similar to those used for the commercially grown GM Bollgard II®, Roundup Ready®/Bollgard II® and Roundup Ready Flex®/Bollgard II® cotton lines. These commercially released GM cotton lines currently constitute the majority of cotton produced in Australia.

191. A resistance management plan (RMP) for Bollgard II® cotton varieties has been developed by the Transgenic and Insect Management Strategy (TIMS) committee of the Australian Cotton Growers' Research Association in consultation with the APVMA (Monsanto Australia Limited 2004; Farrell & Johnson 2005). The APVMA requires implementation of this plan as a condition of the Bollgard II® registration. The RMP is designed to minimise the development of resistant insects and requires growers to employ a number of measures designed to achieve this objective. As part of the resistance management strategy, refuge crops must be grown, to allow Bollgard II®-sensitive insects to survive.

192. A similar RMP is expected to be proposed for WideStrike™ cotton. The applicant has applied to the APVMA for use of WideStrike™. Cultivation of WideStrike™ cotton varieties would need to comply with this RMP and any other relevant conditions that may be imposed by the APVMA.

193. Other relevant crop management practices, including application of herbicides and fertilizer, are expected to be similar to those for non-GM cotton. These are outlined in the reference document *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). At this stage, the applicant does not intend to market the herbicide tolerance trait of WideStrike™ cotton proposed for release and hence has not applied to the APVMA for use of herbicides containing glufosinate ammonium on the GM cotton.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM WideStrike™ cotton

7.1.1 Previous releases approved by the Gene Technology Regulator or authorised by the Genetic Manipulation Advisory Committee

194. The Regulator has previously issued Licences DIR 040/2003 and DIR 044/2003 to Dow AgroSciences Australia Pty Ltd for the limited and controlled release of cotton genetically modified for insect resistance and herbicide tolerance. The GM cotton line authorised for release under DIR 040/2003 was WideStrike™ cotton. The licence permitted release during one growing season on an area of 0.04 ha. Licence DIR 044/2003 authorised the release of three cotton lines, including two lines containing a construct with either the *cry1Ac(synpro)* or the *cry1F(synpro)* gene introduced with the *pat* gene and the third line was WideStrike™. The licence permitted release during two growing seasons on a maximum area of 12.8 ha. There were no reports of adverse effects on human health and safety or the environment from these limited and controlled releases.

7.1.2 Approvals by other Australian government agencies

195. 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) and FSANZ. This is discussed further in Chapter 5.

196. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances, and therefore these plants are also subject to regulation by the APVMA.

197. Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend this herbicide to be applied to the GM cotton in the field. Herbicides must only be used in accordance with APVMA approvals.

198. FSANZ is responsible for human food safety assessment, including GM food. The oil and cotton linters derived from this GM cotton event have been approved by FSANZ for use in human food (FSANZ 2004). FSANZ reviewed their decision and reaffirmed their previous conclusion that oil and linters derived from the GM cotton is fit for human consumption (FSANZ 2005d).

199. FSANZ has also approved use of a GM maize (corn) line, 1507, from Dow for use in food in 2003 (FSANZ 2003). The maize line contains both the *pat* gene and a truncated version of a *cry1F* gene with plant-optimised codon usage.

200. An AQIS permit has been granted to allow import of seed.

7.2 International approvals

201. WideStrike™ Insect Protection Cotton has been approved for field trial planting in a number of countries including Argentina, Brazil, China, Japan, Mexico and Spain (information provided by the applicant).

202. Commercial planting was approved in the USA in 2004. WideStrike™ cotton is not known as a noxious weed in the USA. In the USA, approximately 142,000 ha were planted to GM cotton modified with Cry1Ac and Cry1F in the 2008 growing season (Dow AgroSciences, as cited in Siebert et al. 2008). To date, no adverse effects from those releases are known.

203. Food and feed approval has also been granted in several countries including Mexico, the USA, Canada, Japan (eg Japanese Biosafety Clearing House 2005; US EPA 2005). In Korea, use in food was approved in 2005. In addition, WideStrike™ cotton varieties stacked with Roundup Ready® and Roundup Ready Flex® have been approved for use in food and/or feed in other countries, including Japan, Mexico and Korea. To date, no adverse effects from the food / feed use are known.

204. Similarly, both parental lines are approved for environmental release and/or use in human food or animal feed in various countries. Crops modified with either Cry1Ac or Cry1F have been approved for commercial release overseas. These include cotton, maize and tomatoes in the case of Cry1Ac and cotton and maize in the case of Cry1F.

Chapter 2 Risk assessment

Section 1 Introduction

205. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 8) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP.

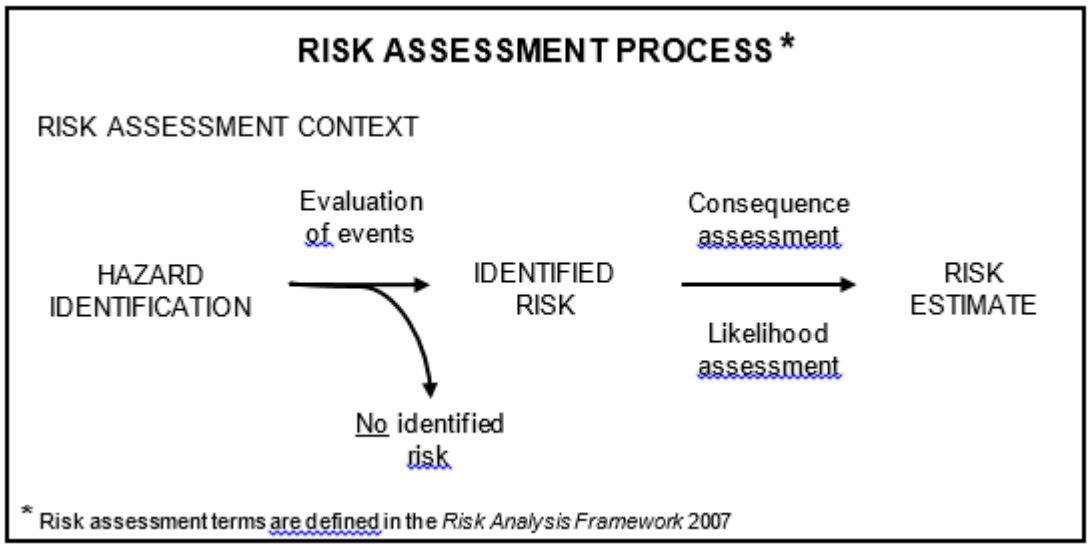

Figure 8The risk assessment process

206. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of this GMO into the environment.

207. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of potential harm. There is no implication that the hazard will necessarily lead to harm. A hazard may be an event, a substance or an organism (OGTR 2007).

208. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

209. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

210. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation and the identification of risk

211. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

212. The criteria used by the Regulator to determine harm are described in Chapter 3 of the Risk Analysis Framework (OGTR 2007). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

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

- the proposed dealings and duration of the proposed dealings
- characteristics of the non-GM parent
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s) in the short term and long term
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs in the short term and long term
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic factors at the site of release
- agricultural management practices for the GMOs.

214. Under section 10 of the Regulations, the Regulator must consider potential risks both in the short term and the long term. Attempts to assign durations for short and long term are not practical and, instead, the Regulator considers the likelihood and consequence of an adverse outcome over the foreseeable future. Long term consideration also involves the identification of specific indicators of risk (see Section 5, Chapter 5) upon which research and testing of credible hypotheses can be undertaken post-licence if a licence were to be issued.

215. The fourteen events that were characterised are discussed in detail later in this Section. They are summarised in Table 18 where events that share a number of common features are grouped together in broader hazard categories. Three events were considered to lead to an identified risk that required further assessment (Refer to Chapters 3 and 4).

Regulatory elements

216. All of the introduced gene regulatory sequences (gene promoters and gene terminators) operate in the same manner as regulatory elements endogenous to cotton plants, and are sourced from regulatory elements which occur naturally and to which humans and other organisms are normally exposed. Any adverse impacts from the introduced regulatory elements are considered equivalent to and no greater than those from any endogenous regulatory elements.

Table 18 Summary of events that may give rise to an adverse outcome

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic or allergenic to people, or toxic to other organisms	1. Exposure of people, other vertebrates and microorganisms to GM plant material containing proteins encoded by the introduced insect resistance and herbicide tolerance genes.	Allergic reactions in people, or toxicity in people, other vertebrates and microorganisms	No	• The encoded proteins are widespread in the environment and therefore vertebrates and microorganisms are already exposed to them. • The introduced proteins have very low acute oral toxicity for mammals, birds and fish. • Products derived from WideStrike™ are approved for use in human food by FSANZ. • Compositional analysis indicates that cotton seed and raw cotton seed meal from WideStrike™ cotton are similar to that derived from non-GM cotton (see Event 12).
	2. Exposure of invertebrates to GM plant material containing proteins encoded by the introduced genes.	Toxicity in non-target invertebrates	Yes	• PAT is widespread in the environment and is not considered to cause toxicity in invertebrates. • However, toxicity of Cry proteins to non-target invertebrates may pose a risk. **See Chapter 3, Identified Risk 1.**
	3. Altered metabolism of glufosinate ammonium in the GM cotton plants expressing the PAT protein resulting in the production of toxic compounds.	Toxicity in people and other organisms	No	• The applicant does not intend glufosinate ammonium to be used on the GM cotton plants. • The toxicity of metabolites from the metabolism of glufosinate ammonium in the GM plants is comparable to or less than that of the parent compound, which is of low acute oral toxicity.
Section 2.2 Spread and persistence (weediness) of the GM cotton line in the environment	4. Expression of the introduced genes for insect resistance or herbicide tolerance improving the survival of GM cotton plants in the agricultural environment.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	• Agronomic characteristics are similar for the GMO and non-GM parent. • Agriculture is highly managed so volunteers will be destroyed. • Glufosinate ammonium tolerant cotton volunteers are effectively controlled by mechanical means or, if still at the seedling stage, by the use of alternative herbicides. • The GMO only has toxicity to certain insect groups.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	5. Expression of the introduced genes for insect resistance and/or herbicide tolerance improving the survival of GM cotton plants outside of cotton fields south of latitude 22ºS.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	• Cultivated cotton is not considered to be weedy. • Resistance to Lepidoptera and tolerance to herbicide is unlikely to increase weediness as other abiotic factors limit the spread and persistence of cotton in areas south of latitude 22ºS. • The toxicity of the GMO is limited to certain insect groups. • There is no evidence of interaction between the proteins encoded by the introduced genes for insect resistance and herbicide tolerance.
	6. Expression of the introduced genes for herbicide tolerance improving the survival of GM cotton plants north of latitude 22ºS.	Weediness	No	• Tolerance to glufosinate ammonium is unlikely to increase weediness as the herbicide is only used in limited situations and other abiotic factors limit the spread and persistence of cotton. • Applicant does not intend the GM cotton to be grown in areas north of latitude 22º South which reduces the likelihood of the GM cotton reaching these areas.
	7. Expression of the introduced *cry* genes improving the survival of GM cotton plants in the natural environment north of latitude 22º South.	Weediness Toxicity in non-target invertebrates	Yes	• **See Chapter 4, Identified Risk 2.**
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	8. Expression of the introduced genes for insect resistance and herbicide tolerance in descendants of non-GM cotton plants.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	• Only low levels of gene transfer to plants in close proximity are likely to occur. • The resulting GMO will be similar to WideStrike™, so no new adverse outcomes would occur. • Naturalised cotton does not occur in close proximity to areas of proposed commercial plantings of the GM cotton. • The GMO only has toxicity to certain insect groups.
	9. Expression of the introduced genes for insect	Weediness Allergic reactions in	No	• Tolerance to glufosinate ammonium and glyphosate is unlikely to increase weediness as other abiotic factors limit the spread and persistence of cotton.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	resistance and herbicide tolerance in other herbicide tolerant GM cotton plants.	people, or toxicity in people or other organisms		• Cotton plants with tolerance to both glufosinate ammonium and glyphosate can still be controlled by mechanical means.
	10. Expression of the introduced genes for insect resistance and herbicide tolerance in other insect resistant GM cotton plants.	Weediness Allergic reactions in people, or toxicity in people or other organisms	Yes	• Tolerance to glufosinate ammonium is unlikely to increase weediness as the herbicide is only used in limited situations and other abiotic factors limit the spread and persistence of cotton. • However, expression of the *cry* genes in other insect resistant cotton plants may pose a risk. **See Chapter 4, Identified Risk 3.**
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	11. Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	• The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via natural mechanisms.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	12. Unintended changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM cotton line resulting from altered expression or random insertion of the introduced genes.	Weediness Allergic reactions in people, or toxicity in people or other organisms	No	• Phenotypic and compositional analyses demonstrate that WideStrike™ cotton is similar to non-GM cotton indicating that biochemical pathways and plant physiology are not altered in the GM plants. • WideStrike™ plants have been grown for several years without any unintended effects being reported.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.6 Development of insect and/or herbicide resistance	13. Development of insects resistant to Cry1Ac(synpro) and Cry1F(synpro) proteins or development of herbicide resistant weeds	Loss of insecticidal efficacy Emergence of weeds that are more difficult to control / development of herbicide resistant weeds	No[13]	• The issue of insect resistance development is being considered by the APVMA. Cultivation of WideStrike™ cotton may require the implementation of a resistance management plan and/or other conditions that may be imposed by the APVMA. • Development of herbicide resistant weeds would be considered by the APVMA should the applicant apply to register use of glufosinate ammonium on WideStrike™. Currently, this is not the case.
Section 2.7 Unauthorised activities	14. Use of the GMOs outside the proposed licence conditions (non-compliance).	Potential adverse outcomes mentioned in Sections 2.1 to 2.6	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.

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

217. Toxicity is the adverse effect(s) of exposure to a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000). Allergenicity is the potential of a substance to elicit an immunological reaction following exposure, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

218. There continues to be considerable discussion in the scientific literature on the characteristics of protein allergens and whether or not one can predict allergenic potential based on these characteristics (Hileman et al. 2002; Stadler & Stadler 2003; Thomas et al. 2005; Silvanovich et al. 2006). For the assessment of GM food crops, the source of the introduced gene, ie is it derived from a source known to be allergenic, is an important characteristic for consideration, as is its reactivity to sera from patients known to be allergic to the source material (Kimber et al. 1999). Other characteristics commonly used to predict a protein's potential for allergenicity include amino acid sequence homology with known human allergens, the stability of the protein, resistance to digestion in the gastrointestinal tract and post translational glycosylation (Metcalfe et al. 1996; Huby et al. 2000).

219. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for insect resistance and herbicide tolerance. Workers cultivating the GM cotton would be exposed to all plant parts. Products derived from the GM cotton plants have been approved for use in food by FSANZ (FSANZ 2004; FSANZ 2005d) and therefore this is a potential source for exposure to people. Other organisms may be exposed directly to the proteins through biotic interactions with GM cotton plants (vertebrates, insects, symbiotic or pathogenic microorganisms and/or fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include

[13] This risk was not identified for further assessment by the Regulator as it is actively managed under the AgVet Code Act.

feeding by vertebrates, insects, fungi and/or bacteria on organisms that feed on GM cotton plant material.

220. As well as the primary consideration of the likely allergenic/toxic potential of the proteins encoded by the introduced genes, consideration of the possible long term persistence of the proteins in the environment is also undertaken. There have been a number of studies on the presence and degradation in soil of proteins (produced from introduced genes) from GM plant residues. These studies have largely focussed on *Bacillus thuringiensis* proteins. Two points have emerged from these studies. Firstly, while it is evident that some proteins from plant residues can be degraded quickly in soil (eg Prihoda & Coats 2008) those that readily bind to soil constituents such as clay and humic acid may accumulate and persist (Crecchio & Stotzky 2001). Secondly, the method of detection needs to be optimised for the protein being considered (Coats et al. 2006).

Event 1: Exposure of people, other vertebrates and microorganisms to GM plant materials containing proteins encoded by the introduced insect resistance and herbicide tolerance genes

221. Presence and expression of the introduced genes and regulatory elements could potentially result in the production of novel toxic or allergenic compounds in the GM cotton plants, or alter the level of endogenous cotton compounds, including anti-nutritional and toxic factors. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of GM plant materials, this may give rise to adverse biochemical or physiological effects on the health of these organisms. Consideration of exposure of invertebrates to the Cry proteins is given in Event 2.

222. Cotton tissues, particularly seeds, contain anti-nutritional and toxic factors, the most studied being gossypol and cyclopropenoid fatty acids (OGTR 2008 and references therein). As a result, cotton plants can be toxic to animals if ingested in excessive quantities. Levels of these natural defensive chemicals have been measured in WideStrike™ cotton and were within the range found in commercial cotton varieties. Additionally, compositional analysis of WideStrike™ cotton showed that most compounds were present at similar levels as in the non-GM parent and were within the range found in commercial cotton varieties (Chapter 1, Section 5.5.2). Therefore, exposure to the GM plant materials is not expected to adversely affect the health of humans or other vertebrates and microorganisms.

223. The GM cotton proposed for release differs from non-GM cotton in that it expresses three additional proteins: Cry1Ac(synpro), Cry1F(synpro) and PAT.

PAT – potential for toxicity or allergenicity to humans and for toxicity to other organisms

224. PAT enzymes are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). *Streptomyces* spp. are saprophytic, soil-borne bacteria and generally not considered to cause adverse effects in humans and other organisms (refer to Chapter 1, Section 5.2.4).

225. The WideStrike™ cotton plant contains two copies of the *pat* gene and another partial fragment of the gene.

226. PAT is not considered harmful to humans and database searches found no significant homologies of the proteins encoded by the introduced genes with known toxins and allergens. In addition, the applicant has supplied data that demonstrate that the PAT protein is not

glycosylated, is heat-labile and easily digested in in-vitro digestibility assays. These characteristics are considered to limit any allergenic potential for humans.

227. A search of the GenBank database reveals that other genes encoding PAT or similar enzymes are present in a wide variety of bacteria. The class of enzymes to which PAT belongs, acetyltransferases, are common enzymes in microorganisms, plants and animals. Similar PAT enzymes are expressed in GM crop plants either commercially approved or trialled in Australia (eg cotton, under licences DIR 015/2002, DIR 038/2003 and DIR 062/2005; and canola, under DIR 010/2001 and DIR 021/2002) and no adverse reactions to those have been reported to the Regulator.

228. In addition, products from WideStrike™ cotton as well as a range of other crops expressing PAT enzymes have been approved for use in food by FSANZ as well as food regulators in other countries (refer to Chapter 1, Section 7).

229. Therefore, it is not expected that exposure to cotton material containing the *pat* gene or its product would cause adverse effects, such as toxicity or allergenicity.

Cry proteins – potential for toxicity or allergenicity to humans and for toxicity to other organisms (excluding invertebrates)

230. Each of the synthetic *cry* genes consists of the core toxin gene region from either *cry1Ac* or *cry1F*, and parts from *cry1Ab* and *cry1Ca3* genes. The latter encode the carboxy terminal part of the protoxins produced, however most or all of this part of the protoxin is cleaved off during activation of the toxin and degraded.

231. The native Cry1Fa and Cry1Ac proteins are naturally produced by the bacterium *B thuringiensis* (Bt), varieties *aizawai* (Bta) and *kurstaki* (Btk), respectively. The presence of Cry1 proteins in food has increased over the past 30 years due to the commercial use of Bt microbial sprays to protect food crops, including so called 'organic' crops, from insect attack (ANZFA 1999). Insecticidal products containing Bta or Btk as the active ingredient are registered in Australia, including for use on cotton, vegetables, vines and fruit trees (APVMA). The native Cry proteins are known to be present in the environment through both natural occurrences of the bacteria as well as the commercial and private application of Bt sprays on food plants. Therefore, the residues of Bt proteins, including Cry1Fa and Cry1Ac, may be present on a wide variety of fresh foods such as cabbage, lettuce and tomato. No reported toxic or allergic responses have resulted from this use.

232. Purified Btk toxins had no effect on *in vitro* growth of pure or mixed cultures of a range of organisms including gram positive and negative bacteria, yeast, filamentous fungi and diatoms (Stotzky 2000). A study using purified Cry1Ac added to soil did not cause any detectable changes to numbers of culturable microorganisms (bacteria or fungi) (Donegan et al. 1995; Donegan & Seidler 1998).

233. Modified versions of Cry1Ac and other Cry proteins have also been produced. These have been used in GM plants, including Bollgard II® cotton which is being grown commercially in Australia under licence DIR 066/2006. No adverse effects from these releases have been reported to the Regulator.

234. Although the introduced *cry1* genes in the GM cotton proposed for release are synthetic genes, the encoded Cry protein sequences are derived from native Cry proteins. Bt is not considered harmful to humans and database searches found no significant homologies of the proteins encoded by the introduced genes with known toxins and allergens (refer to Chapter 1, Section 5.2.2). In addition, the applicant has supplied data that demonstrate that the proteins encoded by the introduced genes are not glycosylated, are heat-labile and easily

digested in *in-vitro* digestibility assays. The absence of these common allergenic characteristics suggests low allergenic potential for humans.

235. In vertebrates, a number of toxicity studies were conducted using either the microbially produced Cry proteins, both individually and in combination, and/or relevant GM plant materials (see Chapter 1, Sections 5.2.3 and 5.5.3). These studies found no detectable adverse effects on mice, chicken, Northern Bobwhite quails and rainbow trout.

236. The stacking of two *cry* genes in WideStrike™ cotton raises the possibility of combination effects of Cry1Ac(synpro) and Cry1F(synpro). Cry proteins are not considered toxic to humans and other organisms (except some invertebrates) and there is no evidence or reasonable expectation that the presence of these two Cry proteins together will result in a toxic effect in these organisms.

Combination of PAT and the Cry proteins – potential for toxicity or allergenicity to humans and for toxicity to other organisms (excluding invertebrates)

237. There is no evidence or reasonable expectation that synergistic effects arising from the combination of the two traits are likely to occur, or that they would result in new or increased risks relating to toxicity or allergenicity. Herbicide tolerance and insect resistance in WideStrike™ cotton operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the introduced *cry* and *pat* genes or their metabolic pathways and no reason to expect that this is likely to occur.

Exposure considerations

238. Basic characteristics of all cotton, such as the presence of toxins and anti-nutrients, limit its deliberate use as a food source by humans and higher animals. In Australia, humans usually only consume processed products of cotton plants (oil and linters) which do not contain detectable levels of protein or genetic material (Sims & Berberich 1996; Sims et al. 1996; USDA 2004). FSANZ has approved the use of oil and cotton linters derived from this GM cotton event for use in human food (2004; 2005d).

239. The meal, hulls and whole cotton seed can be used for cattle feed because gossypol is detoxified by digestion in the rumen. Cotton seed is a valuable foodstuff for cattle as it combines high energy, high fibre and high protein (Ensminger et al. 1990b). Cattle and sheep may also be fed cottonseed hulls, which are an important source of roughage. Gin trash is also fed to ruminants, and is thought to have approximately 90% of the food value of cottonseed hulls (Ensminger et al. 1990a). The use of cotton as stockfeed is limited, nonetheless, to a relatively small proportion of the diet (10 – 20%) and it must be introduced gradually, to avoid potential toxic effects. Sheep may also be fed cottonseed, generally at a maximum of 100 – 300 g/day/sheep (Knights & Dunlop 2007) especially during drought (Leaping Sheep 2006). Cotton stubble is not used for grazing either cattle or sheep due to pesticide residue concerns (Ansell & McGinn 2009). Inactivation or removal of gossypol and cyclopropenoid fatty acids during processing enables the use of some cotton seed meal for catfish, poultry and swine.

240. Mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll (Llewellyn & Fitt 1996). There are no reports of mammals, including rodents, feeding on mature cotton bolls. Similarly there is no evidence of avian species ingesting cotton seeds so birds are not likely to be exposed to the proteins expressed in the seeds of the GM plants.

241. The basic biology of cotton also limits accidental exposure of humans and possibly other organisms. For example, cotton pollen is comparatively heavy, sticky and not easily

dispersed by wind (McGregor 1976; Moffett 1983) which is expected to limit the potential for cotton pollen to act as an airborne allergen for humans and prevent exposure to some animals via skin contact and inhalation.

242. Depending on the location of the plants, cotton may be pollinated by insects, such as honey bees, and this could lead to presence of WideStrike™ cotton pollen in honey. However, honey bees visit cotton flowers primarily to collect nectar and rarely collect cotton pollen, but pollen grains do accidentally adhere to the hairs on their bodies (Moffett et al. 1975). Generally, only a small amount of pollen (< 0.1%) is present in honey (Agrifood Awareness Australia 2001). For a review of this subject see RARMP for DIR 012/2002, and therefore, exposure would be low. Occupational exposure of workers in cotton fields, gins or during transport of cotton may occur; however, this is considered limited as the processes involved in cotton growing and processing are mainly carried out mechanically.

243. Irrigation practices used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways (Good Management Practice of the cotton industry). These practices indicate that GM cotton materials would not regularly enter aquatic habitats in large quantities, and therefore aquatic species are not expected to be exposed on a regular basis.

244. Exposure of soil organisms, including fungi and bacteria could occur during the growing season as well as post-harvest. Studies on the persistence of the proteins encoded by the introduced genes in soil would not suggest their long-term persistence in soil (Chapter 1, Section 5.5.4). The literature on Cry proteins suggests that persistence in soil may depend on soil characteristics, for example clay soils may facilitate absorption of the proteins, which would increase their half-life. As the introduced proteins are not known to be harmful to soil organisms (Chapter 1, Section 5.2.2), except potentially some invertebrates (Event 2), an adverse outcome over the baseline of current cotton farming is not expected.

Conclusion

245. The potential for allergic reactions in people or toxicity to people or other organisms (excluding invertebrates) as a result of exposure to GM plant materials containing the proteins encoded by the introduced genes is **not an identified risk** and will not be assessed further.

Event 2: Exposure of invertebrates to GM plant material containing proteins encoded by the introduced genes.

246. Presence and expression of the introduced genes for insect resistance and herbicide tolerance could potentially result in the production of toxic compounds in the GM cotton that give rise to adverse biochemical or physiological effects on the health of invertebrates as a result of direct or indirect ingestion of the Cry proteins present in WideStrike™ cotton.

247. As indicated in Chapter 1, Section 5.2.4, PAT is widespread in the environment through the presence of naturally occurring bacteria and as well as other GM crops approved for commercial release. There is no data to suggest that PAT is toxic to invertebrates.

248. 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 all commercial cotton was GM cotton, of which 83% was Bollgard II® cotton (pers. comm. Cotton Australia, 2009). The applicant proposes to grow the GM WideStrike™ cotton

in the cotton growing regions of Australia south of latitude 22º South and accordingly, invertebrates would be exposed to the Cry1Ac(synpro) and Cry1F(synpro) proteins.

249. The primary purpose of the Cry1Ac(synpro) and Cry1F(synpro) proteins are to provide resistance to insect herbivory. Thus, the toxicity of the Cry proteins for insect pests of cultivated cotton is not considered to be an adverse outcome but rather the intent of the genetic modification. However, other non-target invertebrate species may also be sensitive to these toxins. Evidence suggests that expression of different Cry proteins in combination can have combination effects on the toxicity for invertebrates (Schnepf et al. 1998; del Rincon-Castro et al. 1999). For Cry1Ac(synpro) and Cry1F(synpro) both synergistic and additive effects have been observed in *H. armigera* (Chakrabarti et al. 1998; Ibargutxi et al. 2008). Therefore, **a risk is identified** for toxicity in non-target invertebrates resulting from the direct or indirect ingestion of the GM cotton. The level of risk of toxicity to non-target invertebrates from this event is estimated in Chapter 3 as **Identified Risk 1**.

Event 3: Altered metabolism of glufosinate ammonium in the GM cotton plants expressing the PAT protein resulting in the production of toxic compounds.

250. If WideStrike™ cotton were sprayed with a herbicide containing glufosinate ammonium, the metabolites produced by the GM cotton as a result of expression of the introduced *pat* genes could be toxic to people and other organisms.

251. Dow does not intend that the GM cotton is sprayed with glufosinate ammonium and, consequently, has not applied to the APVMA to register the use of glufosinate ammonium on WideStrike™ cotton. Therefore, at this stage, application of herbicides containing glufosinate ammonium on WideStrike™ could occur if the herbicide was used during, for example, weed control, and accidentally applied to volunteer WideStrike™ cotton plants. Whether glufosinate ammonium may be intentionally applied to WideStrike™ cotton in the future is unknown.

252. A risk assessment regarding the production of toxic compounds in the process of metabolising glufosinate ammonium by the PAT protein has been conducted previously (DIR 062/2005; the RARMP for DIR 062/2005).

253. The herbicide glufosinate ammonium is comprised of a racemic mixture of the L- and D- enantiomers. The L- enantiomer is the active constituent and acts by inhibiting the enzyme glutamine synthetase. D-glufosinate ammonium does not exhibit herbicidal activity and is not metabolised by plants (Ruhland et al. 2002).

254. Phosphinothricin acetyl transferase (PAT), inactivates the L-isomer of glufosinate ammonium by acetylating it to N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit glutamine synthetase (Droge-Laser et al. 1994; OECD 2002).

255. The metabolism of glufosinate ammonium in tolerant GM plants and in non-GM (non tolerant) plants has been reviewed (Food and Agriculture Organization 1998; OECD 2002). While in non-GM plants the metabolism of glufosinate ammonium is low to non existent because of plant death due to the herbicidal activity, some metabolism does occur (Muller et al. 2001) and is different to that in GM plants expressing the PAT protein (Droge et al. 1992).

256. Two pathways for the metabolism of glufosinate ammonium in non-GM plants have been identified. The first step, common to both pathways, is the rapid deamination of L-phosphinothricin to the unstable intermediate 4-methylphosphonico-2-oxo-butanoic acid (PPO). PPO is then metabolized to either:

- 3-methyl-phosphinico-propionic acid (MPP, sometimes referred to as 3-hydroxy-methyl-phosphinoyl-propionic acid) which may be further converted to 2-methyl-phosphinico-acetic acid (MPA); or
- 4-methyl-phosphonico-2-hydroxy-butanoic acid (MHB), which may be further converted to 4-methyl-phosphonico-butanoic acid (MPB), a final and stable product (Droge-Laser et al. 1994; Ruhland et al. 2002; Ruhland et al. 2004).

257. The main metabolite in non-GM plants is MPP (Muller et al. 2001; OECD 2002).

258. The metabolism of glufosinate ammonium has been investigated in herbicide tolerant, GM canola, maize, tomato, soybean and sugar beet (Thalacker, as cited in Food and Agriculture Organization 1998; OECD 2002). The findings were that the major residue present in the GM crops after glufosinate ammonium herbicide application was N-acetyl-L-glufosinate ammonium, with lower concentrations of glufosinate ammonium and MPP.

259. Studies using cell cultures of GM canola gave similar results, with N-acetyl-L-glufosinate ammonium being the major metabolite (Ruhland et al. 2002).

260. N-acetyl-L-glufosinate and MPP are non-toxic to both plants and mammals, including humans (OECD 1999; OECD 2002). The toxicity of these metabolites was comparable to or less than that of the parent compound, and all were considered to be of low acute toxicity.

Conclusion

261. The potential for increased toxicity as a result of altered metabolism of glufosinate ammonium in the GM cotton plants is **not an identified risk** and will not be assessed further.

2.3 Spread and persistence (weediness) of the GM WideStrike™ cotton in the environment

262. Baseline information on the weed status as well as on abiotic and biotic interactions of non-GM cotton in Australia is provided in the review document *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008). In summary, cotton lacks most characteristics that are common to many weeds, such as the ability to produce a persistent seed bank, rapid growth to flowering, continuous seed production, very high seed output, high seed dispersal and long-distance seed dispersal. However, there are no models or short-term experiments that can accurately predict whether a plant may become invasive (Auer 2008).

263. 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 both non-GM and GM cotton, including herbicide tolerant and/or insect resistant GM cotton, has been considered in previous RARMPs produced during the assessment process of proposed dealings for commercial releases with GM cotton lines (DIR 012/2002, DIR 059/2005, DIR 062/2005 and DIR 066/2006) or limited and controlled releases (DIR 073/2007, DIR 074/2007 and DIR 087 are available on the OGTR Website).

264. In 2004, WideStrike™ cotton was approved for food and feed use as well as for environmental release in the USA. In the USA, the area planted to GM cotton containing Cry1Ac and Cry1F in the 2008 growing season has been estimated to be 142,000 ha. From these releases there have been no reports of increased weediness compared to the non-GM parent.

Baseline cottons relevant to the current application

265. In the current cotton growing areas of Australia, most cotton grown is GM. In the 2008/09 growing season, 95% consisted of 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 stacks with Bollgard II® added up to 83%. Considering the abundant presence of these GM cottons, they will form part of the baseline in addition to non-GM cotton when assessing the weed potential of WideStrike™ cotton.

266. The weediness of the GMO may be increased compared to the baseline cottons, if the expression of the introduced genes leads to an increase of the likelihood of its establishment, reproduction or dispersal when compared to the baseline cottons; or to an increase in severity of any adverse impacts as a result of the proposed dealings; or to an increase in its potential distribution when compared to the baseline cottons.

267. This potential for increased weediness may be across a number of environments or particular to a certain environment, depending on the vulnerability, ie invasibility, of the environment. An increased weed potential could only be realised under circumstances where dealings with the GMO would facilitate the GMO reaching the vulnerable environment.

Event 4 Expression of the introduced genes for insect resistance and/or herbicide tolerance improving the survival of GM cotton plants in the agricultural environment

268. The applicant is seeking approval for a number of dealings, including potential commercial scale planting without specific containment measures of WideStrike™ cotton in cotton growing areas south of latitude 22º South in Australia.

269. In the agricultural environment, cotton volunteer plants may occur in the field following cultivation of cotton when environmental conditions are favourable. 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 non-GM cotton plants, and they were able to establish and persist, it could increase the exposure of humans and other organisms to the GM plant material. The potential for allergenicity and toxicity to humans and for toxicity to other organisms (excluding invertebrates) of the GMO, was assessed in Event 1 and there was no identified risk. Toxicity to invertebrates was assessed in Event 2 and a risk was identified for non-target invertebrates. The identified risk will be further discussed in Chapter 3. While toxicity may be an adverse outcome associated with weediness, toxicity is assessed in more detail elsewhere in this document, so is not included in the current section.

270. If the GM cotton plants were able to establish and persist this could adversely affect agricultural environments by giving rise to a lower abundance of desirable species.

271. The GM cotton expresses two genes expected to confer insect resistance to the plants as well as a gene conferring herbicide tolerance. The phenotype of the GMO proposed for release was described in Chapter 1, Section 5.5.2. Evaluation of the available information indicates that the agronomic characteristics of WideStrike™ cotton are highly similar to the non-GM parent when grown in an agricultural environment in the USA. The few significant differences identified in the data, ie number of fruiting branches, number of total fruiting positions, percent boll retention at the first position, seed cotton weight per boll, seed index (fuzzy) and some fibre quality characteristics (fibre length, Micronaire and reflectance) are generally within the range of other commercial cottons and are not expected to contribute to the weediness potential in an agricultural environment. Therefore, the only characteristics of

WideStrike™ cotton that may increase its potential for weediness in an agricultural environment compared to the baseline cottons are herbicide tolerance and insect resistance.

272. Although the applicant states that the presence of the *pat* gene does not provide sufficient levels of tolerance to commercial herbicide sprays containing glufosinate ammonium, data has not been provided for the concentrations of glufosinate ammonium that the WideStrike™ cotton plants are tolerant to over various developmental stages. Therefore, for this risk assessment, it is assumed that WideStrike™ cotton is potentially tolerant to commercial concentrations of glufosinate ammonium throughout the development of the cotton plant.

273. In the agricultural setting, cotton volunteer plants are actively managed. This high degree of management in agriculture in general indicates that the invasibility of agricultural environments is low. Despite the presence of some characteristics in agricultural environments that would indicate vulnerability to invasion by weeds, such as monoculture and a high level of ecosystem disturbance through cultivation, there is a high degree of weed management in agricultural environments.

274. Volunteer management occurs by mechanical methods involving mulching, root cutting and cultivation, burning or application of herbicides (at the seedling stage) (Australian Cotton Cooperative Research Centre 2002; Charles 2002; Roberts et al. 2002). Volunteer WideStrike™ cotton plants may not be controlled by the application of glufosinate ammonium but could easily be controlled by other herbicides (at the seedling stage) or other methods. Herbicides containing carfentrazone-ethyl or paraquat and diquat as active constituents are currently registered by the APVMA for control of volunteer cotton (APVMA).

275. Integrated weed management strategies are currently recommended in Australia. They stress the need to avoid relying on one control method (Roberts & Charles 2002). For example, to avoid development of glufosinate ammonium-resistant weeds, it is recommended that the application of glufosinate ammonium alone should not be used as the sole management strategy. Currently, the applicant does not intend to market herbicide tolerance in WideStrike™ cotton. However, should the applicant intend to seek approval to apply glufosinate ammonium, an appropriate integrated weed management strategy is likely to be implemented.

276. WideStrike™ cotton expresses two *cry* 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. However, as discussed previously, commercial cotton crops are highly managed and in non-GM cotton insecticides are used to limit insect populations.

277. There is no evidence or reasonable expectation that synergistic effects arising from the combination of the two traits are likely to occur, or that they would result in new or increased risks relating to increased spread and persistence of WideStrike™ cotton. Herbicide tolerance and insect resistance in WideStrike™ cotton operate through independent, unrelated biochemical mechanisms. There is no evidence of any interaction between the introduced genes for insect resistance and herbicide tolerance or their metabolic pathways, and no reason to expect that this is likely to occur.

Conclusion

278. The potential for improved survival of the GM cotton through the expression of the introduced genes leading to increased spread and persistence in agricultural environments is **not an identified risk** and will not be assessed further.

Event 5 Expression of the introduced genes for insect resistance and herbicide tolerance improving the survival of GM cotton plants outside of cotton fields south of latitude 22º South

279. The applicant is seeking approval for a number of dealings, including commercial scale planting without specific containment measures of WideStrike™ cotton in cotton growing areas south of latitude 22º South in Australia; transportation of GM plant material; and use of seed as stockfeed. Thus, the GMOs could be dispersed away from the cotton fields in areas south of latitude 22º South where it could potentially spread and persist.

280. If the GM cotton was to be dispersed into the natural environment and established and persisted in the environment it could increase the exposure of humans and other organisms to the GM plant material. The effects of exposure to GM cotton materials have been assessed in Events 1 and 3 and were not an identified risk. The introduced genes improving survival of the GM cotton in the agricultural environment was assessed in Event 4 and was also found not to be an identified risk. The impact of the GM cotton on invertebrates is discussed in Event 2 and is Identified Risk 1.

281. In a natural situation cotton does not reproduce vegetatively (Sheelavantar et al. 1975; OGTR 2008), 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, digestibility by animals and decomposition by microorganisms are not expected to be altered in WideStrike™ cotton compared to non-GM cotton.

282. Seed dispersal may occur through a number of mechanisms, including the following:

- Animals may eat the GM seed and disperse it outside the agricultural environment.
- Irrigation or adverse weather conditions such as flooding may wash seed into drains, creeks and rivers.
- Harvested bolls may be dispersed during transport on the way to gins.
- Delinted or black seed may be dispersed during transport from gins to further processing facilities or to the agricultural environment where it may be stored, or used for planting or as cattle feed.

283. 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).

284. Extremes of weather may cause dispersal of plant parts into waterways. Much of this dispersed seed is not expected to survive as modern cotton varieties have been bred to be soft-seeded (Mauncy 1986; Hopper & McDaniel 1999). The viability of *G. hirsutum* seed is affected by moisture (Halloin 1975) and extended soaking in water generally reduces cotton seedling emergence and results in smaller seedlings (Buxton et al. 1977). Areas that get flooded regularly may not be favourable for commercial production, as cotton plants are

poorly adapted to waterlogging (Hodgson & Chan 1982). Irrigation practices used by cotton growers in southern Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways (Good Management Practice of cotton industry). This practice would reduce the dispersal of seed into the natural environment. In the event of cotton seed reaching the sea, experiments using seawater showed that the viability of modern cultivated cottons with thin seed coats decreased markedly after one week, probably due to the thin seed coat enabling rapid water uptake (Stephens 1958).

285. Dispersal during transport would occur along the transport routes and would be mainly into roadsides or ditches. Transport of ginned cotton seed is conducted in covered vehicles to minimise loss of seed. Details of the results of roadside surveys over three years in two traditional cotton growing regions, in the Lower Namoi Valley, NSW, and the Darling Downs, QLD, were reviewed in the RARMPs prepared for DIR 023/2002 and DIR 059/2005 and are summarised here:

- The results indicate that cotton was not a problem roadside weed in the regions surveyed. The number of volunteer cotton seedlings was highly variable between seasons, indicating that it is probably dependent on environmental conditions for germination with the majority of cotton volunteer plants resulted from new germination rather than the survival of plants from the previous season.
- The survival of cotton plants seemed to be limited by plant competition and roadside slashing. Slashing appeared to be the common method of roadside weed control, and herbicide use tended to be limited to around fixtures and drainage points where slashing is difficult. As a number of different weed management methods were used, it is considered unlikely that the genetic modifications would provide a significant selective advantage in the roadside environments.

286. While some GM cotton plants may establish on roadsides, in ditches or in the wider natural environment after dispersal, there is no indication that the genetic modification would lead to altered susceptibility to the major abiotic factors that limit the establishment and persistence of cotton in areas south of latitude 22º South. These limiting factors include reliable availability of water and nutrients and, importantly, frosts in winter. It is therefore, expected that any WideStrike™ cotton entering the natural environment south of latitude 22º South would only be able to establish ephemeral populations, similar to the baseline cottons.

Conclusion

287. The potential for improved survival of the GM cotton as a result of the expression of the introduced genes leading to increased spread and persistence outside of cotton fields south of latitude 22º South is **not an identified risk** and will not be assessed further.

Event 6 Expression of the introduced gene for herbicide tolerance improving the survival of GM cotton plants north of latitude 22º South

288. The applicant is seeking approval for the commercial scale planting of WideStrike™ cotton in all current cotton growing areas south of latitude 22º South in Australia, and for harvested material to enter general commerce. This would include conventional breeding, sale of seed for commercial planting, use in human food and stockfeed, sale of lint, export of seed and transport. Therefore, GM cotton plants may establish and persist in the wider environment, ie north of latitude 22º South, as a result of GM cotton seed dispersal via transport, stock feeding, animals or flooding.

289. If the GM cotton were to spread and persist in the environment north of latitude 22º South it could increase the exposure of humans and other organisms to the GM plant material. The potential for allergenicity and toxicity to humans and for toxicity to other organisms (excluding invertebrates) of the GMO, was assessed in Event 1 and there was no identified risk. Toxicity to invertebrates was assessed in Event 2 and a risk was identified for non-target invertebrates. The identified risk will be further discussed in Chapter 3. As these toxicity considerations are assessed in more detail elsewhere in this document, they are not included in the current section.

290. The applicant has not proposed to grow WideStrike™ cotton in areas north of latitude 22º South, so the amount of seed that reaches northern Australia via transport and adverse weather conditions is expected to be limited.

291. GM cotton seed may be dispersed around sites where the cotton seed is stored, during transportation, stock feeding or extreme weather conditions, or by animals. As cotton does not compete well with other plants and has high water and nutrient requirements, volunteer establishment is mainly expected in disturbed, favourable habitats such as ditches, feedlots and roadside drains. If the expression of the introduced *pat* gene was to provide the GM cotton plants with a significant selective advantage over the current baseline cottons, they may spread and persist more in favourable natural environments north of latitude 22º South than other cottons.

292. As discussed earlier (Event 4), although Dow has stated that WideStrike™ is not tolerant to commercial levels of glufosinate ammonium, no data has been supplied. Therefore, for this event it is assumed that WideStrike™ cotton plants are tolerant to glufosinate ammonium.

293. Glufosinate ammonium is not currently registered for use on non-GM cotton. Products containing glufosinate ammonium are registered for use on crops of InVigor® hybrid canola, Liberty Link® Cotton, around various fruit trees and vines, in home gardens and in some non agricultural settings such as roadsides. However, glufosinate ammonium may not be entirely effective in the control of cotton seedlings, and is not considered fully effective on established cotton plants, irrespective of whether they are GM or non-GM (Roberts et al. 2002).

294. Assessment of the risk from expression of the *pat* gene increasing the weediness of cotton plants in Australia is provided in the previous risk assessments for the limited and controlled releases of WideStrike™ (DIR 040/2003 and DIR 044/2003). The *bar* gene which also encodes a PAT protein has been assessed for other GM cotton (eg limited and controlled DIR 015/2002, DIR 038/2003, DIR 056/2004, and DIR 087; commercial DIR 062/2005; available on OGTR Website). These risk assessments concluded that expression of the *pat* or *bar* genes does not enhance the weed potential of these GM cotton plants (in comparison to non-GM cotton plants) in the cotton growing regions of Australia or, in the case of Liberty Link® Cotton (DIR 062/2005), Australia-wide. Experience with field trials and the commercial release of Liberty Link® Cotton (DIR 015/2002, DIR 038/2003, DIR 056/2004 and DIR 062/2005) have not shown any difficulties in controlling volunteer plants with tolerance to glufosinate ammonium.

295. A number of studies have investigated whether the introduction of glufosinate ammonium tolerance results in increased weediness. Glufosinate ammonium tolerant crops, such as canola, potato, maize and sugar beet were not found to be more invasive or more persistent than their conventional counterparts (Poulsen et al. 1999; Crawley et al. 2001).

296. In addition, evaluation of the available information indicates that the agronomic characteristics of WideStrike™ cotton are highly similar to the non-GM parent. The few significant differences identified in the data are generally within the range of other commercial cotton and are not expected to contribute to the weediness potential. There is no reason or reasonable expectation that WideStrike™ cotton should behave differently to other glufosinate ammonium tolerant GM plants in Australia. Similarly, WideStrike™ cotton has not been reported as a weed in the USA. Therefore, it is not expected that the herbicide tolerance of WideStrike™ cotton would confer a significant advantage over the baseline cottons anywhere in Australia.

297. The expression of the *pat* gene is not expected to alter susceptibility to the abiotic and biotic factors that limit the spread and persistence of cotton in areas north of latitude 22º South, such as plant competition, soil type, fire, herbivory and variable availability of water and nutrients (Farrell & Roberts 2002; Eastick & Hearnden 2006; OGTR 2008).

Conclusion

298. The potential for improved survival as a result of expression of the introduced genes for herbicide tolerance leading to increased spread and persistence of the GM cotton, north of latitude 22º South, is **not an identified risk** and will not be assessed further.

Event 7 Expression of the introduced genes for insect resistance improving the survival of GM cotton plants north of latitude 22º South

299. If the expression of the introduced genes for insect resistance was to improve the survival of WideStrike™ cotton in favourable natural environments north of latitude 22º South, this could lead to increased exposure of humans and other organisms to the GM plant material. This may give rise to adverse impacts such as toxicity to non-target invertebrates or lower abundance of desirable species compared to the current baseline non-GM and commercially approved GM cotton.

300. No data is available from field trials for the potential weediness of WideStrike™ cotton compared to the baseline cottons in northern Australia, also little information is available on the effect of the introduced genes on invertebrates present in northern Australia.

Conclusion

301. **A risk is identified** for improved survival of WideStrike™ cotton north of latitude 22ºSouth due to the expression of the genes conferring insect resistance. The level of risk of weediness from this event is estimated in Chapter 4, **Identified Risk 2**.

2.4 Gene flow of the genetic elements introduced into WideStrike™ cotton to sexually compatible plants (vertical gene transfer)

302. Vertical gene flow is the transfer of genes 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 (reviewed in Waines & Hedge 2003). For GM plants, vertical gene flow could therefore occur via successful cross pollination between the plant and nearby cotton plants, related weeds or native plants (Glover 2002).

303. 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. Before an increased potential for adverse effects could be realised as a result of gene flow of the

introduced genetic elements from WideStrike™ to sexually compatible plants, both of the following steps must occur:

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

304. Baseline information on the characteristics relating to vertical gene transfer in non-GM cotton 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. From the data provided there is no indication that the basic reproductive characteristics of WideStrike™ cotton relating to vertical gene transfer would be different to non-GM cotton.

305. 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 (OGTR 2008). Therefore, for this risk assessment it is considered that the only sexually compatible species present in Australia that could receive genes from WideStrike™ cotton are *G. hirsutum* and *G. barbadense*. For both of these species this includes both cultivated GM and non-GM, as well as naturalised cotton.

306. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the cotton plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. However, the impacts from the introduced regulatory elements are considered equivalent to and no greater than the endogenous regulatory elements and so will not be considered further.

307. Vertical gene flow from WideStrike™ cotton to other *G. hirsutum* and *G. barbadense* plants may occur through a number of avenues in the course of the proposed dealings.

308. Outcrossing from WideStrike™ cotton may occur while being cultivated in the cotton growing areas south of latitude 22º South. In addition, outcrossing may occur from any WideStrike™ cotton volunteer plants. As the proposed dealings include cotton seed entering general commerce, WideStrike™ cotton volunteer plants could potentially establish anywhere in Australia in the vicinity of transport routes and where environmental conditions are suitable for cotton.

309. Outcrossing may occur to GM or non-GM cotton plants that are either commercially grown or are present as volunteers. WideStrike™ cotton volunteer plants or their offspring may form ephemeral populations or, in some niches in the natural environment, permanent self-sustaining populations. Outcrossing of WideStrike™ cotton to, eg Bollgard II®/Roundup Ready Flex®, could result in plants expressing Cry1Ac, Cry1Ac(synpro), Cry1F(synpro) and Cry2Ab, conferring insect resistance, and PAT and CP4 EPSPS, conferring tolerance to glufosinate ammonium and glyphosate, respectively.

310. Perpetuation of the WideStrike™ cotton trait/s in populations of sexually compatible plants is only expected in circumstances where there is an advantage to the plants containing the trait/s.

311. It should be noted that WideStrike™ was generated from two genetic modification events and, as expected, the *cry1Ac(synpro)* and *pat* genes from one transformation event and the *cry1F(synpro)* and *pat* genes from the other transformation event have been inserted into different regions of the plant genome and therefore segregate independently of one another.

This means, after any initial outcrossing of WideStrike™ to non-GM cotton, any subsequent generations of cotton volunteers may contain either both *cry* and *pat* genes, one *cry* and *pat* gene or no *cry* or *pat* genes. However, this does not impact on the assessment for weediness as a result of gene transfer of the introduced *cry* and *pat* genes to non-GM cottons because any GM cotton produced from outcrossing containing either one *cry* and *pat* gene or no *cry* or *pat* genes will have equivalent or less insecticidal efficacy or herbicide tolerance than a GM cotton volunteer with both *cry* or *pat* genes. Therefore, segregation of the genes will not be considered further.

Event 8 Expression of the introduced genes for insect resistance and herbicide tolerance in descendants of non-GM cotton plants

312. The prevalence of non-GM cotton in commercial cropping has decreased dramatically since the commercial release of GM cotton. In the 2008/09 season approximately 5% of cotton grown was non-GM. Both non-GM *G. hirsutum* and *G. barbadense* are used, with *G. barbadense* only representing a very small proportion of the commercial crop, especially in drought years (pers. comm. Cotton Australia, 2009). *G. barbadense* is commercially grown in a few cotton growing regions in areas south of latitude 22º South, around Bourke, Tandou and Hillston in NSW.

313. In southern areas of Australia only transient volunteer populations of cotton occur, mainly due to the impact of frost. However, naturalised cotton can occur in tropical areas and small, naturalised cotton (*G. hirsutum* and *G. barbadense*) populations 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 NT, while naturalised *G. barbadense* occurs mainly along the eastern regions of QLD (data from Australian Virtual Herbarium). Both *Gossypium* species are commonly found in littoral and watercourse habitats (Eastick 2002).

314. Outcrossing of WideStrike™ cotton into those non-GM cottons could occur either by deliberate planting of WideStrike™ cotton in the nearby agricultural environment, or by WideStrike™ cotton volunteer plants occurring in the agricultural or natural environment.

315. Expression of the introduced genes for insect resistance and herbicide tolerance in non-GM cotton plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants. This in turn could confer a fitness advantage in environments where cotton plants are limited by the respective stressors.

316. As discussed in Events 1 and 3, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM cotton plants by the introduced genetic material. This would be the same if the introduced genes were expressed in non-GM cotton plants. Toxicity to invertebrates was considered in Event 2 (and Identified Risk 1). The potential for increased weediness was assessed in Events 4 – 7 and a risk was identified for the insect resistance genes improving survival of cotton plants in northern Australia (as Identified Risk 2).

Gene transfer to commercial non-GM *G. hirsutum*

317. Should gene transfer occur to commercially planted non-GM *G. hirsutum*, the resulting plant would be highly similar to the GMO proposed for release. Therefore, any adverse outcomes expected for those offspring would be comparable to WideStrike™ cotton (see previous Events for details).

Gene transfer to commercial non-GM G. barbadense

318. *G. barbadense* is the closest relative of *G. hirsutum* occurring in Australia (OGTR 2008). Hybridisation can occur naturally between these two species (Brubaker et al. 1999). Hybrid progeny exhibit characteristics intermediate to the parents but typically with a lower capacity to produce cotton bolls. *G. barbadense* and hybrids are not weedier or more difficult to control than *G. hirsutum* (Warwick Stiller & Greg Constable, CSIRO, pers. comm., 2002).

319. Should gene transfer occur to commercially planted non-GM *G. barbadense*, the outcome is less certain than for transfer to *G. hirsutum*. The relative impact of environmental factors such as insect predation and herbicide use on the potential for spread and persistence of non-GM *G. barbadense* have not been determined, adding uncertainty to the risk assessment. However, it is expected that the species is limited by the same factors as *G. hirsutum*.

320. Therefore, any adverse effects in *G. barbadense* would be expected to be similar to those in *G. hirsutum*. In addition, the likelihood of gene transfer to *G. barbadense* is considered lower than gene transfer to non-GM *G. hirsutum* as it is currently considerably less prevalent in the environment.

Gene transfer to naturalised non-GM cotton

321. In areas south of latitude 22º South cotton volunteer plants may occur, but do not generally persist and naturalise. Naturalised cotton populations occur mainly in areas north of latitude 22º South. As the applicant does not intend to plant WideStrike™ cotton north of latitude 22º South, the presence of WideStrike™ volunteer plants and subsequent gene transfer to naturalised cottons is highly unlikely. However, if GM cotton volunteers establish in areas near to existing naturalised populations, eg along certain transport routes, the chance of transfer of the introduced genes to these naturalised populations would increase, providing they were flowering simultaneously. The different ways of GM seed dispersing and the chance of GM cotton volunteers establishing and surviving are discussed for Identified Risks 2 and 3.

322. The likelihood of gene transfer to other cotton plants is dependent on successful pollination. Cotton is primarily self-pollinating with pollen that is large, sticky and heavy and not easily dispersed by wind (Jenkins 1993; OGTR 2008). Overseas studies have shown that insect pollinators can transfer pollen to other nearby cotton plants at rates up to 80% (eg Oosterhuis & Jernstedt 1999). However, cotton pollen dispersal studies conducted in Australia consistently show that outcrossing is localised around the pollen source and decreases significantly with distance (OGTR 2008). For example, levels of outcrossing between cotton plants in adjacent rows are in the order of 1 – 2% (Thomson 1966; Mungomery & Glassop 1969; Llewellyn & Fitt 1996). Therefore, gene transfer from the GM cotton to other cotton plants is only expected to occur in close proximity and at low frequencies.

323. Following transfer of the *pat* and/or the *cry1Ac(synpro)* and *cry1F(synpro)* genes to naturalised cotton plants, the likelihood of it causing weediness in these plants is expected to be similar to the GM cotton plants. As naturalised *G. hirsutum* and *G. barbadense* species are commonly found in littoral and watercourse habitats (Eastick 2002), glufosinate ammonium is not expected to be widely used in these areas, offering no selective advantage (Events 4 – 6). GM cotton volunteers can be effectively controlled by mechanical means, or if still at the seedling stage, by the use of alternative herbicides, similar to the GMO.

324. The effect of the expression of the introduced *cry* genes in WideStrike™ cotton is discussed in Identified Risks 2 and 3. Some naturalised cotton populations may be better adapted to environmental stresses than cultivated modern cultivars. However, the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes is not expected to alter susceptibility to the abiotic factors that limit the spread and persistence of naturalised cotton populations in northern Australia, where reliable water availability in particular is known to be a major limitation. Biotic factors which may influence spread and persistence include plant competition, herbivory by lepidopteran or non lepidopteran insects and animal grazing (see Chapter 1 and Identified Risk 2), so reduced lepidopteran herbivory may provide a selective advantage to survival in some environments in some years. However, there is a large geographical isolation that exists between where the GM cotton is proposed to be planted and the occurrence of naturalised cotton. For gene transfer to naturalised cotton to occur, WideStrike™ volunteers would have to establish north of latitude 22º South, within pollination distance of naturalised cotton and be flowering at the same time. The chance of naturalised cotton plants that express the *cry1Ac(synpro)* and *cry1F(synpro)* genes establishing as weeds by finding suitable ecological niches is expected to be no greater than for naturalised non-GM cotton, which would again limit their occurrence.

Conclusion

325. The potential for adverse outcomes due to expression of the introduced genes in descendants of non-GM cotton is **not an identified risk** and will not be assessed further.

Event 9 Expression of the introduced genes for insect resistance and herbicide tolerance in other herbicide tolerant GM cotton plants

326. Herbicide tolerant GM cottons include those that have been approved for either:

- limited and controlled release (DIR 064/2006, DIR 073/2007, DIR 074/2007, DIR 081/2007 and DIR 087)
- commercial release, ie Roundup Ready®, Roundup Ready Flex® and Liberty Link® cottons (DIR 012/2002, DIR 059/2005, DIR 062/2005 and DIR 066/2006).

327. Outcrossing of WideStrike™ cotton into those herbicide tolerant cottons could occur either by deliberate planting of WideStrike™ cotton in the agricultural environment or by WideStrike™ cotton volunteer plants occurring in the agricultural or natural environment.

Gene transfer to herbicide tolerant GM cottons released under limited and controlled conditions

328. Licence conditions for limited and controlled GM cotton releases include measures to limit gene flow to other cotton plants. This is generally achieved by surrounding the release site by a pollen trap of a commercially released cotton cultivar, which could be WideStrike™ if it were approved for commercial release. Thus, pollen from WideStrike™ cotton plants could transfer to the GM cotton trial at low rates. However, controls placed on GM cottons released under limited and controlled conditions include not using the GMOs or the cotton pollen trap plants in food or feed and destroying any GMOs and cotton volunteer plants in the areas of the release in accordance with the licence. Therefore, the potential for any adverse outcome from gene transfer to these limited and controlled releases of herbicide tolerant GM cottons as a result of the proposed dealings is considered negligible.

Gene transfer to herbicide tolerant GM cottons released under commercial licences

329. Commercially released herbicide tolerant GM cottons are grown widely, ie 90% in the current cotton growing regions of Australia in the 2008/09 season (pers. comm. Cotton

Australia, 2009). Therefore, WideStrike™ cotton is expected to be grown in close proximity to other herbicide tolerant GM cottons in many circumstances and outcrossing from WideStrike™ cotton is likely to occur at levels characteristic for all cottons (OGTR 2008).

330. The introduced genes in the commercially approved herbicide tolerant GM cotton lines include the *cp4 epsps* gene (one copy in Roundup Ready® cotton, which confers tolerance to glyphosate only up to the four-leaf stage of growth, and two copies in Roundup Ready Flex® cotton, which confers tolerance to glyphosate throughout the growing season) and the *bar* gene in Liberty Link® Cotton which confers tolerance to glufosinate ammonium throughout the growing season.

331. Transfer of the introduced *cry1Ac(synpro)*, *cry1F(synpro)* and *pat* genes to Liberty Link® Cotton plants, which are tolerant to the herbicide glufosinate ammonium, could result in the expression of the *pat*, *bar*, *cry1Ac(synpro)* and *cry1F(synpro)* genes in the same cotton plant. This combination of genes could confer some selective advantage which could result in spread and persistence of these cotton plants in environments where the use of glufosinate ammonium and lepidopteran herbivory are the major constraints on cotton survival.

332. However, these plants would not have any new traits compared to the parent GMOs, ie they would still be insect resistant and herbicide tolerant. Stacking may result in plants with two copies of the *pat* gene and one copy of the *bar* gene. This may result either in increased or decreased (due to gene silencing) expression of the PAT protein. Decreased expression would reduce the risk of weediness as compared to the GM cotton. Plants with increased expression may be tolerant to higher concentrations of glufosinate ammonium. However, glufosinate ammonium has limited effectiveness in controlling cotton, as discussed below. Furthermore, Liberty Link® Cotton is, to date, not widely planted in Australia (425 ha in 2008/09; pers. comm. Cotton Australia, 2009) so the potential for gene transfer is currently limited.

333. Transfer of the introduced *cry1Ac(synpro)*, *cry1F(synpro)* and *pat* genes to Roundup Ready® or Roundup Ready Flex® cotton plants, which are tolerant to the herbicide glyphosate, could result in the expression of the *pat*, *cp4 epsps*, *cry1Ac(synpro)* and *cry1F(synpro)* in the same cotton plant. These genes in combination could confer some selective advantage which could result in spread and persistence of these cotton plants in environments where the use of glyphosate and/or glufosinate ammonium and lepidopteran herbivory are the major constraints on cotton survival.

334. The control of cotton volunteers is important both in cotton fields and outside the fields such as along roadsides and drains. There are three stages of cotton volunteers that need to be controlled: seedling cotton, established cotton, and regrowth or 'ratoon' cotton.

335. Herbicides can be used to control seedling cotton volunteers. Glyphosate has been the most common herbicide used to control these volunteers but, with the uptake of Roundup Ready® GM cotton since 2000, alternative herbicides are being used, including glufosinate ammonium. However, the use of glufosinate ammonium is limited on cotton volunteers as on cotton seedlings at the 4 and 8 leaf stage it offers only incomplete control. Other herbicides such as bromoxynil, carfentrazone and a combination of paraquat and diquat have been shown to be effective (Roberts et al. 2002). Cultivation is also a very effective method to control seedling cotton volunteers (Australian Cotton Cooperative Research Centre 2002).

336. Established or ratoon cotton plants, whether GM or non-GM, are difficult to control by herbicides alone. Instead, established or ratoon cotton plants are most effectively controlled by mechanical methods involving mulching, root cutting and cultivation (Roberts et al.

2002). Thus, the combination of glyphosate and glufosinate ammonium tolerance is not likely to impact on the control of cotton volunteer plants.

337. Herbicides containing glyphosate are classified into Group M and herbicides containing glufosinate ammonium are in Group N and these herbicides affect different biochemical pathways in plants. Therefore, it is unlikely that the presence of both the different herbicide tolerance genes in cotton will result in unintended biochemical interactions and that the plants will develop resistance to a different type of herbicide. A study on combining glyphosate and glufosinate ammonium herbicide tolerance traits in GM canola showed no tolerance to other, unrelated herbicides and no gene silencing (Senior et al. 2002).

338. Therefore, the potential for risks from the gene transfer of the WideStrike™ genes to other commercially available herbicide tolerant cottons are considered similar to that from WideStrike™ cotton (refer to all other relevant Sections of the current Chapter as well as Chapters 3 and 4).

Conclusion

339. The potential for an adverse outcome due to the expression of the introduced genes in other herbicide resistant GM cotton is **not an identified risk** and will not be assessed further.

Event 10 Expression of the introduced genes for insect resistance and herbicide tolerance in other insect resistant GM cotton plants

340. Insect resistant GM cottons in Australia include those that have been approved for either:

- limited and controlled release (DIR 048/2003, DIR 065/2006, DIR 073/2007 and DIR087)
- commercial release, ie Bollgard II® cotton varieties (DIR 012/2002, DIR 059/2005 and DIR 066/2006).

341. Outcrossing of WideStrike™ cotton into other GM cottons with insect resistance could occur either by deliberate planting of WideStrike™ cotton in the agricultural environment or by WideStrike™ cotton volunteer plants occurring in the agricultural or natural environment.

342. Transfer and expression of the introduced genes for insect resistance and herbicide tolerance in other insect resistant GM cotton plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

Gene transfer to insect resistant GM cotton released under limited and controlled conditions

343. As discussed in Event 9, the potential for any adverse outcome from gene transfer to these insect resistant GM cottons as a result of the proposed dealings is considered negligible.

Gene transfer to insect resistant GM cotton released under commercial licences

344. Bollgard II® cotton varieties are grown widely, ie 83% in the current cotton growing regions of Australia in the 2008/09 season (pers. comm. Cotton Australia, 2009). Therefore, Bollgard II® cotton is expected to be grown in immediate proximity to WideStrike™ cotton in many circumstances. Outcrossing from WideStrike™ cotton is likely to occur at levels characteristic for all cottons (OGTR 2008).

345. GM cotton plants expressing the *cry1Ac(synpro)*, *cry1F(synpro)* and *pat* genes in combination with the *cry1Ac* and *cry2Ab* genes present in Bollgard II® GM cotton as a result of vertical gene transfer, could have altered resistance to lepidopteran insects and herbicide

tolerance. This could confer a fitness advantage on the plants in environments where cotton plants are limited by lepidopteran insects and/or herbicides.

346. The presence of the *pat* gene in Bollgard II® GM cotton would have the same herbicide tolerance as the WideStrike™ cotton plants, so the potential for risks is considered similar to that discussed in Events 4, 5 and 6 where no risks were identified.

347. However, the presence of the *cry1Ac(synpro)*, *cry1F(synpro)* and *pat* genes in combination with the *cry1Ac* and *cry2Ab* genes present in Bollgard II® GM cotton, could confer a fitness advantage on the plants in environments where cotton plants are limited by lepidopteran insects susceptible to the expressed Cry proteins.

348. Therefore, **a risk is identified** for weediness as a result of vertical gene transfer of the *cry1Ac(synpro)* and *cry1F(synpro)* genes into Bollgard II® GM cotton. The level of risk of weediness from this event is estimated in Chapter 4 as **Identified Risk 3**.

2.5 Gene flow of the introduced genetic elements to sexually incompatible organisms (horizontal gene transfer)

349. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). 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 an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

350. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008, available on OGTR Website). 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.

351. Baseline information on the presence of the introduced or similar genetic elements in the environment is provided in Chapter 1, Sections 5.3 and 6.2.3. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

352. Possible adverse outcomes from the proposed dealings with the GM cotton and/or its products that might arise as a result of horizontal gene transfer include adverse reactions, such as allergenicity/toxicity or increased spread and persistence of the organism that has acquired the introduced genetic elements.

Event 11: Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer

353. Examination of the possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material.

HGT from WideStrike™ cotton plants to bacteria

354. Bacteria are afforded many opportunities to encounter DNA from GM plants. These include, exposure to GM plant material in the soil or in aquatic environments where GM

plant material is present, through a bacterial species' natural interactions with the GM plants as commensals, symbionts or parasites, or through the interactions of GM plant material and gut bacteria in herbivores (Keese 2008). Plant DNA from decaying plant material may persist in the soil under field conditions for several months and maybe up to 2 years (Gebhard & Smalla 1999; see also discussion in De Vries & Wackernagel 2004).

355. The mechanisms by which genetic material could be transferred to bacteria from plants are natural transformation (active uptake and integration of free DNA) and transduction (transfer of DNA following its accidental packaging into bacteriophage particles) (De Vries & Wackernagel 2004). However, only limited transfer and persistence of DNA from plants to bacteria has been shown in laboratory studies (Nielsen et al. 1998) and few examples of HGT to bacteria from eukaryotes resulting in an evolutionary advantage exist (Andersson 2005).

356. Bacteria that occur naturally in an environment are the best source for genes that may cause an adverse effect as a result of HGT (Keese 2008). It is suggested that introduced bacterial genes are the only genes in GM plants likely to transfer successfully to bacteria (Pontiroli et al. 2007). Bacterial antibiotic resistance genes (such as *nptII*) are commonly used in the process to generate GM plants. However, these genes are often abundant in the environment in a number of bacterial species and are more readily transferable by conjugation and transduction from other bacteria than from GM plants (Keese 2008).

HGT from WideStrike™ cotton plants to animals

357. DNA entry across the gastrointestinal tract is the most likely route of HGT from GM plants to animals (Keese 2008). This could occur for invertebrates and vertebrates that feed on GM plants, animals that feed on herbivores, or plant pollinators. The potential for transient gene transfer into somatic cells has been shown, but gene transfer to the germ line cells of animals has not been detected. The analysis of genomic sequences has shown only rare examples of HGT from plants to animals (Lambert et al. 1999; Bird & Koltai 2000). For HGT from GM plants to animals to be evolutionarily significant it must affect the germline cells and be passed on to the subsequent generation.

HGT from WideStrike™ cotton plants to viruses

358. While plant viruses have the capacity to acquire new genetic material as a result of recombination with the genetic material from the plants they infect or other pathogens infecting the plant, the vast majority of recombination that occurs involves other viral sequences (Keese 2008). The genome size of plant viruses is small and only rare examples have been found of host plant sequences in the genomes of viruses (Mayo & Jolly 1991; Khatchikian et al. 1989; Agranovsky et al. 1991; Meyers et al. 1991; Masuta et al. 1992). This suggests that the HGT from a GM plant to viruses is likely to be restricted to GM plants transformed with viral sequences and the viruses that naturally infect that plant species. Examples of HGT resulting from recombination between a virus and a homologous viral gene introduced into a GM plant have been documented. However, in most cases a selective advantage to the virus was favoured by the use of a defective virus as the infecting agent, for which recombination with the introduced genetic material in the GM plant would restore full infectivity (Keese 2008).

359. There are potentially far greater background levels of HGT to plant viruses from non-GM donor sources due to co-infections in plants by two or more viruses and from a broad range of viral sequences that occur naturally in plant genomes (Bejarano et al. 1996; Ashby et al. 1997; Harper et al. 1999; Peterson-Burch & Voytas 2002; Harper et al. 2002). As the GMO proposed for release does not contain viral sequences it is considered highly

unlikely that recombination with GM plant DNA would be more frequent than recombination with non-GM cotton genetic material.

HGT from WideStrike™ cotton plants to other eukaryotes

360. Algae, fungi and a range of protists are other potential eukaryotic HGT recipients of the introduced genetic material. However, HGT from plants to these organisms is exceedingly rare. Opportunities for these organisms to obtain genes with related sequences or functions to the introduced genes are more likely to occur by mutation or HGT from non-GM donor organisms (Keese 2008).

Nature of introduced genetic material

361. The introduced genes are present amongst common bacteria and plants. In light of the discussion above, it is far more likely that HGT will occur from naturally occurring *B. thuringiensis*, *S. viridochromogenes* or *A. tumefaciens* to soil microorganisms than from the GM cotton plants. Furthermore, the introduced *cry* and *pat* genes in the GM cotton plants have been modified for plant codon usage so in the unlikely event that gene transfer were to occur, only relatively low levels of gene expression in bacteria would be expected. Furthermore, the gene sequences expressed from the introduced genetic material are not expected to assist the process of HGT by facilitating gene movement across cell membranes or recombination with a host genome. 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.

362. A key consideration in the risk assessment process should be consideration of the safety of the protein product(s) resulting from the expression of the introduced gene(s) rather than the frequency (likelihood) of horizontal gene transfer *per se* (Thomson 2000). If the introduced gene sequences or their end products are not associated with any risk then even in the unlikely event of horizontal transfer occurring, it should not pose any risk to humans, animals or the environment. Events 2, 7 and 10 were associated with identified risks. Upon further analysis the risk from Event 2 and 7 were considered low (Chapter 3 and 4), and the risk from Event 10 was considered negligible. Management conditions have been proposed to address these low risks (Chapter 5).

Conclusion

363. The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.6 Unintended changes in biochemistry, physiology or ecology

364. All methods of plant breeding can induce unanticipated changes in plants, including through pleiotropy (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 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 the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription

- increased metabolic burden associated with high level expression of the introduced genes
- novel traits arising from interactions of the protein encoded by the introduced gene products with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced genes.

365. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity, allergenicity, weediness, and altered pest or disease burden 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). This means that there is low likelihood of such changes leading to harm as a result of a commercial/general release in the long term.

Event 12: Unintended changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM cotton line resulting from altered expression or random insertion of the introduced genes

366. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, are already discussed for Events 1 – 7. In particular, compositional analysis did not reveal great changes compared to the non-GM parent (Chapter 1 Section 5.5.2). An analysis of anti-nutrient compounds, including gossypol and cyclopropenoid fatty acids also showed values similar to the non-GM parent cotton and were within the range of literature values (see Table 13). FSANZ has assessed the oil and cotton linters derived from this GM cotton and has approved them for use in human food (FSANZ 2004; FSANZ 2005c).

367. Previous releases of this GM cotton in Australia (performed under DIR 040/2003 and DIR 044/2003) did not show any unintended secondary effects from sites in NSW, QLD and WA.

368. Phenotypic evaluation, including growth habit, germination and emergence, vegetative vigour, reproductive potential and fibre quality of WideStrike™ cotton was conducted in the USA (Chapter 1, Section 5.5.2.). There was no statistically significant difference in many of the characteristics measured between WideStrike™ and the non-GM parent cotton. However, there were statistically significant differences observed with regards to number of fruiting branches, number of total fruiting positions, percent boll retention at the first position, seed cotton weight per boll, seed index (fuzzy) and some fibre quality characteristics (fibre length, Micronaire and reflectance). These were generally within the range of other commercial cottons.

Conclusion

369. The potential for an adverse outcome as a result of unintended changes in biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

2.7 Development of insect and/or herbicide resistance

Event 13: Development of insects resistant to Cry1Ac and Cry1F proteins or development of herbicide resistant weeds

370. Widespread and long-term use of WideStrike™ cotton varieties, could result in the emergence of resistance to the Cry1Ac(synpro) and Cry1F(synpro) proteins in the target species and other susceptible lepidopteran species feeding on cotton. This would result in a reduction in efficacy of these, and possibly other, GM cotton varieties for the control of insect pests, and could also have impacts on the efficacy of Bt microbial sprays to control insects in other agricultural systems. Potential adverse effects include attenuation of the potential benefits of growing insect resistant cotton for the environment and human health.

371. The APVMA has a complementary regulatory role in respect to this application due to its responsibility for agricultural chemicals in Australia, including insecticides and herbicides, under the *Agricultural and Veterinary Chemicals Code Act 1994* (Ag Vet Code Act 1994). WideStrike™ cotton falls under the Ag Vet Code Act 1994 definition of an agricultural chemical product, due to its production of two insecticidal substances and a substance conferring herbicide tolerance, and is thus subject to regulation by the APVMA.

372. The APVMA is currently assessing an application from Dow to register the use of the insecticidal proteins as produced by the *cry1Ac(synpro)* and *cry1F(synpro)* genes in WideStrike™ cotton. Cultivation of WideStrike™ cotton, if registered, may require the implementation of a resistance management plan and/or other conditions that may be imposed by the APVMA.

373. The use of glufosinate ammonium on the WideStrike™ cotton could result in the development of herbicide resistant weeds through selection. Changes in agricultural practices such as adoption of minimal tillage or changes in herbicide use may cause changes to weed populations. For example, weed species that are inherently more resistant to a herbicide than other weed species may become more abundant. The development of herbicide resistant weeds may occur where glufosinate ammonium herbicide is used to replace other weed management practices, and this could result in the emergence of weeds that are more difficult to control.

374. Currently, the applicant does not intend to register glufosinate ammonium for use on WideStrike™ cotton. However, development of herbicide resistant weeds would be considered by the APVMA should the applicant apply to register use of glufosinate ammonium on WideStrike™.

Conclusion

375. The potential for an adverse outcome as a result of development of resistance to Cry proteins or herbicide resistant weeds is **not an identified risk** in the context of this assessment as it is assessed and actively managed through the application of the Ag Vet Code Act 1994, and therefore will not be assessed further by the Regulator.

2.8 Unauthorised activities

Event 14: Use of GMOs outside the proposed licence conditions (non-compliance)

376. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the WideStrike™ GM cotton outside of the proposed growing areas. The adverse outcomes that this event could cause are the same as

those discussed in the sections above. The Act provides for substantial penalties for non-compliance or unauthorised dealings with GMOs. The Act also requires that the Regulator have regard to 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.

Conclusion

377. The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process

378. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

379. Fourteen events 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 this occurred were also assessed.

380. A risk is only identified when a hazard is considered to have some chance of causing harm. Events 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.

381. Three events from the hazard identification process (Identified Risks 1, 2 and 3 in Table 18) are considered to lead to an identified risk with the adverse outcomes of toxicity to non-target invertebrates and/or weediness.

382. Chapters 3 and 4 give detailed consideration to the consequences and likelihood of these identified risks in order to obtain estimates of the level of risk. The risk is assessed against baselines established by reference to the characteristics of the parent organism and aspects of the receiving environment (including agronomic practices).

383. Information contained in the application (including information required by the Act and the Regulations on the GMOs, the parent organism, the proposed dealings and potential impacts on the health and safety of people and the environment), current scientific knowledge, and submissions received during consultation on the application and RARMP with experts, agencies and authorities (summarised in Appendices B, C and D) were also considered.

384. The consequence assessment considers the seriousness of the harm that could potentially result from an event, while the likelihood assessment considers the chance of the event resulting in harm. Consequence and likelihood assessments are then combined to give an overall risk estimate using the Risk Estimate Matrix (Figure 9). During the consequence and likelihood assessments, consideration is also given to areas of uncertainty that arise from a lack of data.

		RISK ESTIMATE			
⊐ ↘	Highly likely	**Low**	**Moderate**	**High**	**High**

	Likely	**Negligible**	**Low**	**High**	**High**
	Unlikely	**Negligible**	**Low**	**Moderate**	**High**
	Highly unlikely	**Negligible**	**Negligible**	**Low**	**Moderate**
		Marginal	Minor	Intermediate	Major
		CONSEQUENCES			

Figure 9 The OGTR Risk Estimate Matrix (OGTR 2007)

Risk Estimate Matrix: A *negligible* risk is considered to be insubstantial with no present need to invoke actions for mitigation. A *low* risk is considered to be minimal but may invoke actions for mitigation beyond normal practices. A *moderate* risk is considered to be of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. A *high* risk is considered to be unacceptable unless actions for mitigation are highly feasible and effective.

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

385. After an estimate is obtained for an identified risk, risks higher than negligible are evaluated to determine if risk treatment measures are required to mitigate potential harm (see Chapter 5 – Risk Management).

Section 4 Uncertainty

386. 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, ie consequence and likelihood, are always uncertain to some degree.

387. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability. For commercial/general releases, where there may not be limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, it is important that uncertainty is minimised. In the RARMP for DIR 044/2003, several information gaps were identified as requiring possible consideration if Dow were to submit an application for a larger scale release of the three GM cotton lines.

388. These were as follows:

- levels of expression of the insecticidal and herbicide tolerance genes in the GM cotton tissues under Australian field conditions
- effect of the GM cottons on non-target organisms under Australian field conditions
- potential for the introduced proteins to accumulate in the soil under Australian field conditions.

389. Additional data required were:

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

390. In preparing the application for DIR 091 Dow provided information in relation to these points. These have been discussed in relevant areas in this RARMP and are summarised here.

391. Gene expression data has been provided for the insecticidal genes under Australian conditions (see Table 15 and Table 16). No data has been provided for the herbicide tolerance

genes. While the applicant does not intend that glufosinate ammonium will be sprayed onto the WideStrike™ cotton plants, this remains an area of uncertainty.

392. The applicant has provided a single comparative study of arthropod census data from unsprayed non-GM and WideStrike™ cotton in Australia (Murray 2005). This study is discussed further in Chapter 3.

393. Data supplied on the effects of the introduced genes on non-target organisms, which includes soil organisms, is discussed in Chapter 2, and under Events 1 and 2 in Chapter 3.

394. The potential for accumulation of the introduced proteins in the soil under Australian field conditions was examined in an Australian study on the effect that continuous cropping of WideStrike™ might have on subsequent crops (Dow AgroSciences 2006a). The available overseas data on accumulation of Cry1Ac and Cry1F (Shan et al. 2007) does not suggest that these proteins accumulate to any great extent. However, published values on the persistence of Bt proteins in soil show a wide variation, eg less than one day up to more than 7 months (as reviewed in Clarke et al. 2005). The variation is thought to be due to the properties of different soils, the different environmental behaviour of microbially produced *versus* plant produced Bt toxin, the shortcomings of the methodologies/biological systems used for conducting bioassays and the use of bioassays versus biochemical methods.

395. Data has been provided on agronomic characteristics of the WideStrike ™ cotton plants grown in the USA, and most of these were within the range of the non-GM parent cotton (see Chapter 1, Section 5.5.2). Data has also been provided from studies conducted in the USA on biochemical characterisation of the WideStrike™ cotton plants (see Chapter 1, Section 5.5.2), to identify any unintended effects of the genetic modification. This is discussed further in Chapter 2, Event 12.

396. For all commercial or long-term releases uncertainty exists in relation to changes in the context surrounding the release. The risk assessment has been prepared in the context of current agricultural practices, climate and weather patterns, and the conclusions are appropriate in this context, however, over time if these were to change significantly then these conclusions are less certain.

397. In the long term, climate change may lead to a climate that is more conducive to the establishment of self-sustaining permanent populations of cotton in areas south of latitude 22º South. It has been suggested that there may be an increase in temperature from 0.4 – 2ºC by 2030, with an increase in the average number of extreme hot days, and a decrease in the average numbers of frosts (McRae et al. 2007). Rainfall predictions are less certain with a 10% increase or decrease forecast, but with more storm events.

398. Any identified uncertainty in aspects of the risk assessment or risk treatment measures must be addressed in determining the appropriate risk management and in considering recommendations for post release review (see Section 5, Chapter 3). Uncertainty in risk estimates may be due to insufficient or conflicting data regarding the likelihood or severity of potential adverse outcomes. Uncertainty can also arise from a lack of experience with the GMO itself. To a degree, the level of uncertainty about WideStrike™ cotton is low given the now several years of growing it overseas, eg in the USA. However, there are differences in the agricultural practices, pest species, soil composition and climatic conditions between Australia and the USA which are not addressed by releases overseas. WideStrike™ cotton was trialled on a limited scale in Australia. Further uncertainties are discussed in Chapters 3 and 4 in relation to risk estimates for the identified risks.

Chapter 3 Risk estimate for toxicity in non-target invertebrates

399. This Chapter estimates the risk associated with one event (Identified Risk 1 from Chapter 2) that could lead to the adverse outcome of toxicity in non-target invertebrates arising from this proposed release. The risk estimate is based on consequence and likelihood assessments for this event.

Section 1 Background

400. WideStrike™ cotton proposed for release expresses two insecticidal proteins and a herbicide tolerance protein as a result of genetic modification. Events that may give rise to toxicity for non-target organisms as a result of the proposed release were considered in Chapter 2. Expression of the PAT protein is not expected to provide a novel source of harm to organisms, as this and similar proteins are naturally present in the environment and are expressed by common bacterial species without any indication of toxicity for any organism (Chapter 1, Section 5.2.4). Evidence also indicates that the Cry1Ac and Cry1F proteins are not toxic to vertebrates or microorganisms (Chapter 1, Section 5.5.4 and Chapter 2, Event 1).

401. The toxicity of the Cry1Ac(synpro) and Cry1F(synpro) proteins for insect pests of cultivated cotton is not considered to be an adverse outcome but rather the intent of the genetic modification. Therefore, this Chapter will be limited to assessing the risk of toxicity for non-target invertebrates as a result of direct and indirect ingestion of the Cry1Ac(synpro) and Cry1F(synpro) proteins in the WideStrike™ cotton. Non-target or beneficial insects may consume the Cry proteins produced in WideStrike™ cotton either directly through feeding on pollen, leaves and other plant parts as well as plant waste, or indirectly through feeding on target insects or through contact with the proteins when present in soil or water. Toxicity of Cry proteins is highly specific due to their mode of action. Susceptible organisms must have the correct combination of gut conditions and suitable binding sites on the midgut cells (see Chapter 1 for details).

402. An increase in the exposure of organisms other than humans to the GM plant material may give rise to adverse impacts such as toxicity to non-target invertebrates leading to lower abundance of beneficial species compared to the current baseline for commercial GM and non-GM cotton. This could occur if the expression of the introduced genes for insect resistance was to provide WideStrike™ cotton with greater toxicity and/or with a significant selective advantage for improved survival in favourable natural environments than the currently available cottons.

Section 2 Consequence and likelihood assessments

403. Consideration is given to Identified Risk 1 from Chapter 2 (Hazard identification) that exposure to WideStrike™ cotton may give rise to toxicity in non-target invertebrates. For this identified risk, the level of risk must be estimated through assessment of the seriousness of harm (the consequence – ranging from marginal to major) and the chance of harm (the likelihood – ranging from highly unlikely to highly likely).

404. The Regulator is required to consider risks to human health and safety or the environment posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMO is considered relative to the baselines of toxicity of the non-GM parent to invertebrates and in the context of existing commercial GM cotton

crops in the environment in which the GM cotton plants are proposed for release. The commercial plantings of Bollgard II® GM cotton in Australia are also relevant to the risk estimate, as are other sources of the introduced genes or similar genes in the environment, such as naturally occurring soil bacteria.

2.1 Toxicity of non-GM cotton to non-target invertebrates

405. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton line being considered in this risk assessment. Cotton is a well established field crop with a long history of use. A comprehensive review of the biology of non-GM cotton is provided in the document *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008).

406. Cultivated cotton naturally contains a compound, gossypol, known to be toxic to insects (Percival et al. 1999). However, high levels of gossypol, even in combination with morphological characteristics that discourage insect infestations, are not sufficient to provide protection against the pink bollworm (*Pectinophora gossypiella*) (Percival et al. 1999) as well as other known pests of cotton such as *Helicoverpa armigera* and *H. punctigera*. Generally, broad spectrum chemical insecticides are used to control these pests (see below for comparison of toxicity of the chemical to adverse impacts of GM cotton).

2.2 Toxicity of other commercial GM cottons to non-target invertebrates

407. Information on Bollgard II® GM cotton is included here to establish a baseline for comparison with the GM cotton being considered in this risk assessment. Bollgard II® cotton currently represents the majority of cotton grown in Australia (OGTR 2008). In the 2008-09 season 95% of the cotton grown in Australia was GM, with the vast majority, 83%, being Bollgard II® (alone or in combination with GM herbicide tolerance traits; pers comm. Cotton Australia, 2009). The current licence application proposes a commercial release of WideStrike™ cotton in all cotton growing areas of Australia south of latitude 22º South, with products derived from the GM cotton entering general commerce.

408. A risk was identified for non-target invertebrates as a result of growing Bollgard II® GM cotton, which expresses Cry1Ac and Cry2Ab proteins, in the RARMPs of DIR 012/2002 and DIR 066/2006. However, the risks to non-target invertebrates were considered in detail and were found to be negligible. The likelihood of toxicity for non-target invertebrates as a result of direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins, expressed in Bollgard II®, was estimated to be highly unlikely and the consequence of toxicity as a result of direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates was assessed as minor (DIR 066/2006).

409. Bollgard II® cotton contains the genes that encode modified Cry1Ac and Cry2Ab proteins. The previously grown INGARD® cotton contained a gene that encoded the same modified Cry1Ac protein as Bollgard II®. Therefore, target and non-target invertebrates have been exposed to a form of the Cry1Ac protein in the cotton crops in the Australian cotton growing regions south of latitude 22º South since the release of INGARD® cotton in 1996. No adverse affects of this exposure have been reported to date. However, whilst both Bollgard II® and WideStrike™ cotton contain genes that encode Cry1Ac proteins, the genes in both cotton lines are synthetic *cry1Ac* genes and have similar, but not identical gene or protein sequences. Target and non-target invertebrates in the Australian cotton growing regions have only a very limited exposure to the Cry1F(synpro) protein as a result of two previous limited and controlled releases of WideStrike™ cotton under licences DIR 040/2003 and DIR 044/2003.

2.3 Identified Risk 1: Direct or indirect exposure of non-target invertebrates to GM plant material containing proteins encoded by the introduced *cry* genes

410. The target species for WideStrike™ cotton relevant to the Australian cotton cropping regions are *H. armigera* and *H. punctigera* south of latitude 22º South. The applicant has stated that WideStrike™ cotton is also effective against a number of other lepidopteran species (Chapter 1, Section 5.1.1) including *S. litura* and *P. gossypiella* which are cotton pests in northern Australia.

411. Toxicity studies of native Cry1Fa1 from *B. thuringiensis* were performed on *H. armigera* and all of the concentrations tested led to growth inhibition, but not mortality (Avilla et al. 2005). Encapsulated Cry1Fa protein was tested for toxicity to *H. armigera* and *H. punctigera* and was found more toxic to *H. punctigera* than to *H. armigera* (Liao et al. 2002).

412. The invertebrate fauna of cultivated cotton consists of a wide range of species including a number of beneficial species that parasitise or prey on various cotton pests, including lepidopteran pests. Unlike several parasitoid species, none of the known predators which attack Lepidoptera in cotton are specialists and can consequently feed on a range of other species (Fitt & Wilson 2002).

413. The risk of toxicity for non-target invertebrates from direct or indirect ingestion of the Cry1Ac(synpro) and Cry1F(synpro) proteins would depend upon the level of toxicity of these proteins both individually and in combination (consequence assessment) and the probability of exposure to the proteins resulting in harm during the release (likelihood assessment). The risk is assessed against the baseline toxicity of the non-GM parent organism for insects and the agronomic management practices for non-GM cotton, particularly the use of broad spectrum insecticides. The risk is also assessed against the baseline toxicity of current commercial plantings of Bollgard II® GM cotton (containing Cry1Ac and Cry2Ab) in Australia and the toxicity due to the presence of the Cry proteins naturally present in the environment. The assessment is also conducted in the context of the large scale of the proposed release and the receiving environment.

2.3.1 Consequence assessment

414. The Cry1F(synpro) and Cry1Ac(synpro) proteins are toxic to a range of lepidopteran insect larvae including pest species of cotton. Bioassays using proteins encoded by cloned *cry* genes were conducted to study their spectrum of insecticidal activity (van Frankenhuyzen 1993); and the references therein). In this study, the Cry1 family of proteins (Cry1Ac(a), Cry1Ac(b), Cry1A(c), Cry1B, Cry1C, Cry1D, Cry1E and Cry1F) indicated specificity to lepidopteran insects. Potential non-target toxicity of a Cry1Ac protein has been assessed in detail in the risk assessments for the commercial release of Bollgard II® GM cotton in Australia (DIR 012/2002, DIR 059/2005, DIR 066/2006), for the continued commercial release of INGARD® GM cotton (DIR 022/2002 and DIR 023/2003), and for two limited and controlled releases of WideStrike™ GM cotton (DIR 040/2003 and DIR 044/2003). These risk assessments are available on OGTR Website.

415. The potential for non-target toxicity of the Cry1F protein has only been considered before in the context of the two previous limited and controlled releases of WideStrike™ cotton (DIR 040/2003 and DIR 044/2003).

416. Toxicity of WideStrike™ cotton and exposure to the Cry1Ac(synpro) and Cry1F(synpro) proteins were not considered a risk to non-target invertebrates in the context of a limited and controlled release as there was limited exposure of non-target invertebrates

(and humans and other vertebrates) due to the small scale and short duration of the releases. The potential for non-target toxicity of the Cry1Ac(synpro) and Cry1F(synpro) proteins is considered here in the context of the current proposed commercial release.

417. A range of broad spectrum insecticides are registered for use on cotton by the APVMA to protect the plants from insect attack. Application of these insecticides impacts negatively on both target and non-target invertebrates in the cotton field (see later in section for comparison of sprayed cotton with GM cottons).

418. Bt insecticides are used on many crops but are highly selective and tend to show virtually no adverse or indirect effects on non-target populations (Federici 2003).

419. The risk assessment of Bollgard II® cotton, which also expresses a form of modified Cry1Ac protein, concluded that the consequence of toxicity to non-target invertebrates as a result of exposure was minor. This assessment was made on the basis of laboratory dietary toxicity studies that showed Cry1Ac to be toxic only to lepidopteran insects, and field studies undertaken in Australia that provided sufficient evidence that growing Bollgard II® had no significant effect on non-target invertebrate populations (DIR 066/2006).

Laboratory studies with Cry1Ac(synpro) and Cry1F(synpro)

420. The applicant has performed a number of toxicity studies on representative non-target invertebrate species from a range of orders (summarised in Chapter 1, Section 5.2.3). The applicant defines the term non-target as organisms incidentally exposed to plant residues or organisms consuming plants or plant parts as an occasional or supplementary food source (Wolt 2002).

421. The laboratory dietary toxicity studies were performed using direct ingestion of either the GM cotton plant tissue or the unpurified Cry protein preparations produced in a microbial expression system, either individually or in combination. The level of expression of Cry1Ac(synpro) in WideStrike™ GM cotton grown in the Australian environment was determined to be up to 3.5 ng/mg. This maximum value was obtained in terminal leaves (see Table 16). For Cry1F(synpro) the expression level was up to 197.5 ng/mg. This value was obtained for Cry1F(synpro) expression in 4th node leaves (see Table 15). The assessment uses those values to gauge the relevance of the concentrations of the Cry proteins used in the laboratory studies.

422. The laboratory studies were performed on selected indicator species of a number of taxonomic groups listed in Table 19. The appropriateness of some of the study methodologies and conclusions drawn are discussed below.

Table 19 A list of non-target and beneficial invertebrate laboratory studies provided by the applicant

Common Name	Scientific Name	Species present in Australia	Same or similar invertebrates present in Australian cotton fields	Reference
Parasitic Hymenoptera	*Nasonia vitripennis*	No#	*Ichneumon promissorius*, *Heteropelma scaposum*, *Netelia producta* and others*	(Sindermann et al. 2002b)
Green lacewing	*Chrysoperla carnea*	No#	Genus *Mallada**	(Sindermann et al. 2002a)

Common Name	Scientific Name	Species present in Australia	Same or similar invertebrates present in Australian cotton fields	Reference
Honey bee	*Apis mellifera*	Yes[#]	Yes (responsible for cross-pollination)[$]	(Maggi 2001)
Water flea	*Daphnia magna*			(Marino & Yaroch 2002a)
Ladybird beetle	*Hippodamia convergens*	No[#]	*Hippodamia variegata*[#]	(Porch & Krueger 2001)
Earthworm	*Eisenia fetida*	Yes[¢]	*Aporrectodera trapezoids*, *A. rosea* and others[§]	(Sindermann et al. 2001)
Collembola	*Folsomia candida*	Yes[^]	*Proisotoma minuta*[&]	(Teixeira 2002)

[#] CSIRO (2009); * Cotton Catchment Communities CRC (2007); ^ http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/taxa/Folsomia_candida; [¢] Blakemore (1999); [$] Thomson (1966), Mungomery & Glassop (1969), OGTR (2008); [§] Lawrence & Baker (2005); [&] Nursita et al. (2004).

423. Several species of parasitic wasp (parasites of *Helicoverpa*) occur as beneficial insects on cotton crops in Australia (Cotton Catchment Communities CRC 2007). A parasitic Hymenoptera, *Nasonia vitripennis*, was used to test the effects of exposure of the Cry proteins (refer to Chapter 1, Section 5.2.3 for details) (Sindermann et al. 2002b). The report concludes that there was no significant difference in mean mortality between the treatment groups and the control, and the high mortality seen on day 9 of the test for both groups was considered a result of the age of the wasps.

424. However, the results of this study are not directly transferable to a field situation for detecting the potential for harm as this study used the full length microbially produced Cry1Ac(synpro) and Cry1F(synpro) proteins presented to adults of *N. vitripennis*. The parasitic wasps considered beneficial species in the Australian cropping situation would potentially ingest the Cry proteins indirectly as a result of ingestion of their caterpillar host. Therefore, the Cry proteins they ingested would contain at least some fraction of the core toxin. The wasp's biology would suggest that exposure to the proteins through parasitisation of *Helicoverpa* larvae would occur at early (larval) developmental stages rather than as adults. This exposure through parasitisation is considered to occur more readily and at a greater level than exposure of adults and therefore to be of greater importance for consideration in an environmental risk assessment.

425. The *Mallada* genus of green lacewing is present in the Australian cotton cropping environment. It consists of important beneficial insects that predate on both the eggs and small larvae of lepidopteran pests (Cotton Catchment Communities CRC 2007). A study was conducted on the green lacewing, *Chrysoperla carnea*, using the microbial full length Cry1Ac(synpro) and Cry1F(synpro) proteins separately and in combination. In one of the tests, this study reported that mortality in the combined Cry1Ac(synpro) and Cry1F(synpro) group was significantly different from the control group. There were no other significant differences in mean mortality between any of the treatment groups and control groups (see Chapter 1, Section 5.2.3 for details) (Sindermann et al. 2002a). As green lacewings predate small larvae of lepidopteran pests it is reasonable to consider that they may come into contact with the Cry proteins produced in WideStrike™ cotton in a similar way to wasps that parasitise larvae of *Helicoverpa* discussed above.

426. Negative effects on invertebrates due to their interaction or dependence on the target lepidopteran pests are a consequence of an intended effect (control of a pest) and are common to all pest control methods including broad spectrum insecticides, biological control and conventional host-plant resistance (Boethel & Eikenbarry 1986; Croft 1990).

427. In Australia, honeybees (*Apis mellifera*) are thought to be responsible for long-distance cross-pollination in cotton crops (OGTR 2008). The effect of exposure of honeybee larvae to the microbially produced Cry1Ac(synpro) and Cry1F(synpro) proteins, was tested using a one dose toxicity determination (Maggi 2001). Mean time to emergence of adult bees was measured and found not to be significantly different between the treatment groups and the sucrose control group. Bees were exposed to the single Cry proteins in cotton pollen from the two parental GM cotton lines of WideStrike™, and as a combination of the two microbially expressed Cry proteins (for details see Chapter 1, Section 5.2.3). While dead bees were found in the emergence cages, there was no significant difference in the number of dead bees between the treatments or the sucrose fed control bees. The author indicates that the level of dead bees found in the emergence cage is unusual for this type of experiment, however as there was no statistical evidence for a treatment effect, the author did not consider it significant and no toxicity to bees was identified (Maggi 2001). The bees were not exposed to GM pollen expressing both the Cry1Ac(synpro) and Cry1F(synpro) proteins, which would be the case if bees were exposed to WideStrike™ cotton pollen in the field.

428. The honeybee is used as a model non-target invertebrate due to the dependence that modern agriculture has on the honeybee for crop pollination and the potential losses that would occur if harm occurred to this insect. In a meta-analysis of Cry protein dietary toxicity studies on honey bees, no significant effects were found on either larval or adult survival. The study consisted of 39 independent assessments of a number of Cry proteins produced in either GM plants or bacteria. Cry1Ac protein was included in the analysis as modified protein produced in GM cotton pollen or microbial expression systems. Cry1F protein was included in the analysis as produced in GM maize pollen and in GM *Pseudomonas fluorescens* (Duan et al. 2008).

429. *Daphnia magna* were used to examine the potential effect on aquatic invertebrates of exposure to the Cry proteins through presence of the proteins in water (Marino & Yaroch 2002a). No effects on mobility or other sublethal effects were observed when the test *D. magna* were exposed to the individual microbially produced Cry1Ac(synpro) or Cry1F(synpro), or a combination of both the proteins, in their water (for details see Chapter 1, Section 5.2.3).

430. Possible effects of the Cry proteins on soil organisms and invertebrates that have a life stage in soil also need to be considered in the context of their potential persistence and accumulation in the soil. The effect of the full length microbially produced Cry1Ac(synpro) and Cry1F(synpro) proteins in soil on the earthworm *Eisenia fetida* has been examined and no difference in mortality or average body weight was detected as a result of exposure to the proteins either in combination or singly (for details see Chapter 1, Section 5.2.3) (Sindermann et al. 2001).

431. Collembola (*Folsomia candida*) are leaf litter feeders that would come in to contact with the Cry1Ac(synpro) and Cry1F(synpro) proteins in WideStrike™ through trash left in the field after harvest. While reproductive activity was reduced by up to 50% when *F. candida* were fed microbially produced Cry1Ac(synpro) (at 22.6 µg/ml diet), feeding microbially produced Cry1Ac(synpro) with Cry1F(synpro), Cry1F(synpro) alone, or lyophilised GM cotton tissue expressing only Cry1Ac(synpro) did not affect reproduction (Teixeira 2002). Although each test and corresponding control group used Collembola of the

same age, the age differed by two days between different tests, and this may have had an impact on the results. No significant differences in mortality were detected in this study. The concentrations of Cry proteins included in the study were well above the concentrations found in WideStrike™ GM cotton tissues (see Chapter 1, Section 5.5.3). Overall, this study suggests that WideStrike™ is unlikely to adversely affect Collembola in the field.

432. When considering the applicability of laboratory studies results, the choice of feeding substrate and concentration of protein must be considered. While elevated levels of protein are normally used in studies there is a possibility of negative interactions between proteins and that elevated levels of one protein in a combined protein study may mask the effect of another protein. However, the studies described here included treatment groups presented with the microbially produced Cry proteins separately and, with the exception of one treatment group in the collembolan, no adverse affects were observed.

433. A number of the invertebrate laboratory studies discussed here used microbially produced Cry1Ac(synpro) and Cry1F(synpro) proteins with a purity of only 14 and 15%, respectively (data supplied by the applicant). This level of protein impurity in the dietary toxicity studies raises an additional level of uncertainty about the cause of any observed effects. Furthermore, the full-length form of the Cry proteins fed to the test organisms does not always relate directly to the form of the proteins that the organisms would potentially be in contact with in the environment. In a study comparing the sensitivity of the American lepidopteran target species, *Heliothis virescens*, to Cry1Ac the insect appeared more sensitive to the trypsin digested protein than the full length protein (Sims 1995). Therefore, non-target studies that rely on the full length protein may also be masking potential sensitivities to the truncated proteins that may be available to non-target invertebrates due to their mode of feeding. For example, parasitic Hymenoptera are more likely to ingest cleaved toxin by consuming insects that have fed on the cotton material as opposed to directly feeding on the cotton material themselves. It has been suggested that using Bt resistant host caterpillars to determine possible effects of Cry proteins on their parasites would help address host-mediated effects (Shelton et al. 2009). However, these caterpillars were not used in these studies.

434. Synergistic, additive or antagonistic effects can occur when different Cry proteins are ingested by an insect at the same time (Schnepf et al. 1998; del Rincon-Castro et al. 1999). As reviewed in Chapter 1, Section 5.2.3, different studies have indicated that ingestion of Cry1Ac and Cry1F together can potentially lead to either additive or synergistic effects in target species. The mode of action responsible for this potential synergism, and any implications for the spectrum of susceptible species, is currently unknown. However, the data presented above suggest that there are no increased toxic effects for the non-target species studied when the two Cry proteins are combined.

Field studies with other commercial GM Bt cotton

435. Field studies of Bt cotton, expressing the modified *cry1Ac* and *cry2Ab* genes (Bollgard II®) or the modified *cry1Ac* gene (INGARD®) but not the *cry1F* gene, both in Australia and overseas have found no significant effect on non-target invertebrate populations. However, small declines in the abundance of different generalist predators have been reported and are generally considered to be associated with the reduced availability of lepidopteran prey.

436. A study conducted on cotton grown at Kununurra, WA, compared invertebrates in non-GM cotton, INGARD® cotton and Bollgard II® cotton (Strickland & Annells 2005). Although some beneficial (mainly predatory) arthropods were more abundant mid season on non-GM

cotton and INGARD® cotton than on Bollgard II®, probably due to a lower availability of lepidopteran prey in the cotton lines expressing two *cry* genes, overall results indicated that non-target invertebrates were unaffected by the Cry1Ac and Cry2Ab proteins present in the GM cotton lines.

437. A study conducted in NSW compared the species diversity between unsprayed non-GM cotton, sprayed non-GM cotton and two unsprayed Bt cotton lines (INGARD® cotton and a GM cotton line expressing the Cry1Ac and Cry2Aa proteins) (Whitehouse et al. 2005). Only small differences in the non-target invertebrate communities were observed between unsprayed non-GM and Bt cotton. These differences were slightly decreased numbers of dipteran (frit and fruit flies) and hemipteran species (damsel bugs and jassids). Bioassays with the Cry2A subfamily of proteins indicated specificity to both lepidopteran and dipteran species (van Frankenhuyzen 1993). Therefore, the slight decrease in the number of Dipterans is not unexpected.

439. A three year field trial conducted in a cotton growing region of Australia found that the abundance of insects was similar in unsprayed non-GM and Bt (INGARD®) fields and the abundance was usually greater than in fully sprayed fields (Fitt & Wilson 2002). The only significant difference was a lower abundance of parasitic Hymenoptera at one site in one year on the GM cotton. The authors suggest that this result is not unexpected as the GM cotton has a lower abundance of *Helicoverpa* larvae, which are hosts of the parasitic Hymenoptera (Fitt & Wilson 2002).

438. A review article has evaluated all published peer-reviewed studies on the effects of various Bt proteins on beneficial invertebrates in different crops species (including cotton) based on laboratory and field studies (Romeis et al. 2006). When compared with insecticide-treated non-Bt crops, Bt crops were found to support higher populations of beneficial species.

Field studies with WideStrike™ cotton

439. Field studies of WideStrike™ cotton have been undertaken in several cotton growing states in the USA. The first year of a two year study, over two states, with different pest insect pressures (*Heliothis virescens* and *Helicoverpa zea* or *Pectinophora gossypiella*) showed no consistently negative effects on non-target arthropods (Mahill & Storer 2002). For one state in the second year of the study there were significantly fewer Hemiptera (other than aphids, brown stink bug, tarnish plant bug or cotton flea hopper) seen in pitfall trap samples in the fields planted with WideStrike™ cotton than the sprayed or unsprayed control plots (Storer 2003). However, the combined analysis of data from both trial years did not find any significant differences in non-target invertebrate abundance between the unsprayed WideStrike™ and unsprayed non-GM parental cotton fields (Storer 2003).

440. The applicant has performed an ecological risk assessment, for the USA, on the impact of cotton expressing the Cry1Ac(synpro) and Cry1F(synpro) proteins to non-target, beneficial and endangered invertebrates (Wolt 2002). The author concludes that, based on their analysis, there are no ecological concerns arising from cropping WideStrike™ cotton. This study takes into account the USA cropping experience and is based on Cry protein expression data from the USA. Field expression data generated under Australian conditions show that the protein expression levels are higher at the end of the season compared to data obtained in the USA (Chapter 1, Section 5.5.2).

441. The applicant has provided a single comparative study of arthropod census data from unsprayed non-GM and WideStrike™ cotton in Australia (see Chapter 1, Section 5.5.4). Although it was only conducted at one site in QLD, for one cotton growing season and used an unreplicated plot set up of a small size (40 m by 40 m), the author concluded that there

was no direct effect from the WideStrike™ cotton on the observed abundance and diversity of non-target arthropods as a result of the WideStrike™ cotton (Murray 2005). The report states that genus and species could not be assigned to most of the collected specimens, although some of the conclusions are based on the number of species identified. The study measured a lower abundance of Diptera in both the pooled data and species diversity in the WideStrike™ plot. Overall, the unsprayed WideStrike™ plot had 19.3% more arthropods, 5.4% fewer species and 2.5% fewer families. The author suggests that the observation regarding the Diptera may be due to a chance occurrence as the data was collected from an unreplicated plot (Murray 2005). It should be noted that no laboratory data on toxicity of Cry1Ac(synpro) and Cry1F(synpro) proteins to Dipteran insects has been provided by the applicant. Therefore, toxicity of WideStrike™ cotton to Diptera can not be discounted as a reason for the lower abundance. As indicated in Section 2.3.1, the Cry1 family are not expected to be toxic to dipteran species (van Frankenhuyzen 1993). However, there are other Bt toxins such as members of the Cry4 protein family and Cyt proteins that specifically target dipteran species.

442. Several field studies have been undertaken to assess the persistence and accumulation of the Cry1Ac(synpro) and Cry1F(synpro) proteins in the soil and the effect of continuous cropping of WideStrike™ cotton on subsequent crops and potentially on invertebrate populations in the soil (Dow AgroSciences 2006a; Shan et al. 2007). In the USA, three different areas that represent different soil types and crop management practices were planted to WideStrike™ cotton for three consecutive crops (Shan et al. 2007). Neither the Cry1Ac(synpro) nor the Cry1F(synpro) protein were detected in the bulk or rhizosphere soil samples by ELISA (enzyme linked immunosorbent assay).

443. In a single Australian study, the effect that continuous cropping of WideStrike™ might have on subsequent crops was examined at one location in Liverpool Plain, NSW (Dow AgroSciences 2006a). The same plot was planted with WideStrike™ cotton in two consecutive seasons. After the second harvest the site was cultivated and planted to wheat. The number of wheat seedlings that emerged at day 16 after planting and the height of the plants at day 30 were measured. No differences were observed between the treatment groups and the control groups. Although this study used five replicate plots for each treatment and control, the plots were small in size (2 x 10 m).

Uncertainty

444. For toxicity to non-target invertebrates, uncertainty in the consequence assessment exists as a result of:

- knowledge gaps in the effects of WideStrike™ cotton on non-target invertebrates in the Australian cotton growing regions, in particular Dipterans and other beneficial arthropods commonly present in Australian cotton fields.
- uncertainty around the methodology of some of the non-target dietary toxicity studies.

445. While no significant adverse effects have been detected on non-target invertebrates in field trial studies in the USA with WideStrike™ cotton or in field trial studies in Australia with other GM cotton expressing Cry proteins, these results may not be directly applicable to WideStrike™ cotton growing on a commercial scale in the Australian environment. Uncertainty arises from differences between Australia and the USA relating to several issues, including the methodology of the studies, the climatic conditions and the spectrum of non-target insects.

446. The applicant has provided data from an Australian field trial study on the effects of growing WideStrike™ cotton on non-target invertebrates (Murray 2005), however, as discussed previously, this study was conducted for only one growing season over a small area. Uncertainty in this study relates to a lesser abundance of Diptera. The size of the trial raises uncertainty over the transferability of the non-target invertebrate abundance data to a full scale commercial cotton field. Different abundances of non-target invertebrates may occur over a large commercial field, for example as a result of predator or other non-target species sheltering in refuge crops and moving only a small distance into the cotton field. For example, a difference in the abundance of predatory beetles has been seen between the refuge crops and within Bollgard II® crops close to the refuge (50 to 200 m from the crop edge) and closer to the centre of the crop (more than 200 m from the crop edge) and this trend was supported over several seasons (Lawrence et al. 2007). It has also been shown that the occurrence of pest species can vary spatially over a commercial cotton field due to the presence of environment effects and soil factors (eg Willers et al. 2005). No concessions to these effects have been made in the determination of non-target invertebrate abundance in the Australian field trial. The small size of the unreplicated trial plots (40 m by 40 m) would mask any potential differences in abundance.

447. Some additional uncertainty is also raised in the laboratory dietary toxicity studies, including uncertainty regarding the methodology used in the experiments on parasitic wasps, green lacewings and honey bees. The microbially produced Cry1Ac(synpro) and Cry1F(synpro) proteins used in a number of the non-target dietary toxicity studies were between 14-15% pure (data supplied by the applicant) and therefore contain other proteins or impurities that may affect the results of the non-target dietary toxicity studies. This does not seem to have been taken into account in the controls used in the analysis. Neither has it been considered that the full-length form of the Cry proteins fed to the test organisms may not always relate directly to the form of the proteins that the organisms would most likely be in contact with in the GM cotton field. There is also uncertainty surrounding the sequence of the Cry1F protein produced by the microbial expression system. While equivalence has been claimed for the microbial and plant produced proteins, for Cry1F the sequence of the protein expressed in bacteria differs by four amino acids from the plant expressed protein. Two of the amino acid differences are in the region thought to be involved in species specificity (refer to Chapter 1, Section 5.2.3), limiting the conclusions that can be drawn from this work.

448. However, the introduced genes encode Cry1 proteins, which as a group have been shown to have a high degree of target species specificity. Cry1 proteins are generally specific to species in the insect order Lepidoptera and a different range of Lepidoptera is susceptible to each individual Cry1 protein.

Conclusion

449. The consequence of toxicity to non-target invertebrates as a result of growing WideStrike™ cotton needs to be considered against the baseline of sprayed non-GM cotton in which non-target invertebrates will be adversely impacted upon by the insecticides used. Laboratory and field studies conducted with Bollgard II® cotton and its history of safe use in Australia indicate minimal or no impact on non-target invertebrates. The field trial studies performed on WideStrike™ cotton in the USA and the history of safe use of WideStrike™ cotton in the USA indicates that non-target invertebrates are not adversely impacted in the USA.

450. However, some uncertainty exists due to the limitations of laboratory and Australian field data on the toxicity of WideStrike™ cotton to non-target invertebrates provided by the applicant.

451. Therefore, consequences of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes causing toxicity in non-target invertebrates are assessed as **minor**.

2.3.2 Likelihood assessment

452. The commercial release of WideStrike™ cotton in all cotton growing regions south of latitude 22º South could result in a large number of non-target invertebrates being exposed to the Cry1Ac(synpro) and Cry1F(synpro) proteins, especially in cotton fields. Exposure to a similar Cry1Ac protein has occurred through the commercial release of GM cottons containing a similar Cry1Ac protein. Non-target invertebrates have also been exposed to the Cry1F protein through field trials of WideStrike™ cotton in Australia, which comprised approximately 13 ha in total. Some exposure to similar proteins would already exist through the natural presence of similar proteins in the environment (see Chapter 1, Section 5.2) and the use of Bt based insecticide products on a range of crops, including cotton.

453. Non-target invertebrates may be directly exposed to the Cry1Ac(synpro) and Cry1F(synpro) proteins, through feeding on the GM cotton plants. Indirect exposure may occur through eating other organisms, including the lepidopteran target pests, which have previously fed on the GM cotton plants. Exposure may also occur in the soil either when cotton tissues break down following incorporation into the soil or as a result of exudation of the introduced proteins through the roots.

454. If non-target invertebrates feed on the GM plants, they would be directly exposed to the Cry1Ac(synpro) and Cry1F(synpro) proteins. As discussed in Chapter 1, Section 5.5.3, the average expression level of Cry1F(synpro) in various tissues at 69-86 DAP was notably higher in the Australian study than for USA plant tissue at an equivalent stage indicating that levels of exposure to the Cry1F(synpro) protein can differ depending on where the GM cotton is grown and levels of exposure may be higher in Australia compared to the USA. Data on expression levels in pollen of WideStrike™ cotton plants grown in Australia were not provided. Values for Cry1Ac(synpro) expression in the Australian and USA studies are not markedly different.

455. Non-target invertebrates that may be indirectly exposed to the Cry1Ac(synpro) and Cry1F(synpro) as a result of parasitism or predation of lepidopteran larvae may be exposed to higher amounts of activated core toxin than expected. There is some evidence that growth inhibition and not mortality occurs in some target invertebrates as a result of ingesting the Cry proteins (Chapter 1, Section 5.2.3).

456. Exposure of non-target invertebrates to the Cry1Ac(synpro) and Cry1F(synpro) proteins in cotton growing areas south of latitude 22º South would be mainly due to commercial crop production. The potential for exposure outside cotton cropping areas in southern Australia is considered low due to limited opportunities for establishment and the environmental conditions that limit cotton persistence in these areas (Chapter 2, Events 4 and 5). Exposure north of this latitude would only be due to volunteers as the GM cotton will not be cultivated in these areas and there are limited opportunities for dispersal. Therefore, the potential for exposure is also expected to be low (Chapter 2, Events 6, 7 and Identified Risk 2 in Chapter 4).

457. The half life of the introduced Cry proteins in soil has been calculated as 1.3 days (Herman & Collins 2001). Persistence of a similar Cry protein (Cry1Ab) in soil has been

demonstrated for several weeks without loss of insecticidal activity (Stotzky 2004). Several studies have investigated accumulation of the introduced Cry proteins in representative soils. The applicant was required to submit a soil/terrestrial expression study for long range soil persistence to the US Environmental Protection Agency as part of their registration requirements for WideStrike™ cotton (US EPA 2005). A study was conducted in the USA using growth inhibition bioassays of tobacco budworm. The study indicated no presence of the introduced proteins after WideStrike™ cropping (Shan et al. 2007).

458. The potential for the introduced proteins to accumulate in the soil under Australian field conditions was also a research requirement under licence DIR 044/2003 for the limited and controlled release of WideStrike™ cotton. An Australian study was conducted to determine if there was an adverse impact on wheat seedling emergence and early growth when fields cropped to WideStrike™ or non-GM cotton for two years were planted to wheat (Dow AgroSciences 2006a). This type of study can potentially give an indirect indication of the impact of protein accumulation on soil functional properties. No differences in wheat seedling emergence and early growth were found, which suggests there were no changes in the soil biota that impacted on the growth of wheat.

459. Many lepidopteran species including pests of cotton have a life stage in the soil. Whilst accumulation of the Cry1Ac(synpro) and Cry1F(synpro) proteins in the soil over several cropping seasons seems unlikely (Dow AgroSciences 2006a; Shan et al. 2007) potential exudation of the proteins from plant roots may have an immediate effect on soil organisms or non-target invertebrates present in the soil. This could lead to tritrophic effects on predator or parasite species such as parasitic Hymenoptera that lay eggs directly into pupae in the soil. However, bioassays with soil from the rhizosphere of a continuously cropped WideStrike™ field trial in the USA did not find any significant difference in tobacco budworm larval growth compared to larvae assayed on control soil samples (Shan et al. 2007).

Uncertainty

460. Uncertainty regarding the likelihood for toxicity of WideStrike™ cotton to non-target invertebrates in the Australian cotton growing regions south of latitude 22º South is identified. This uncertainty relates to knowledge gaps regarding the likelihood of indirect exposure of non-target invertebrates to the proteins encoded by the introduced *cry* genes and the potential for an increase in an increase in exposure as the Cry proteins may cause only growth inhibition, but not death in target invertebrates.

Conclusion

461. The evidence presented in the consequence assessment suggests that the non-target invertebrates tested with the Cry proteins singly or in combination appear insensitive to the levels of these proteins that would be expected to be expressed in WideStrike™ cotton. However, this conclusion can not be directly transferred to the likelihood of harm under Australian field conditions due to data and associated uncertainty. The greatest exposure of non-target invertebrates to the introduced proteins will be where WideStrike™ cotton is cultivated in the field.

462. Therefore, the likelihood of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes causing toxicity in non-target invertebrates are assessed as **unlikely**.

Section 3 Risk estimates

463. Risk estimates (which can range from negligible to high) are based on a combination of the consequences and likelihood assessments, using the *Risk Estimate Matrix* (see Chapter 2) (OGTR 2007).

464. The risk estimates for the adverse outcome of toxicity for non-target invertebrates as a result of the proposed release of these GM cotton plants have been made relative to the baseline of the toxicity of non-GM cotton, and current commercial plantings of other GM cotton events; agronomic management practices for non-GM cotton, particularly the use of broad spectrum insecticides and in the context of the widespread use of commercially released GM cotton plants in Australia without evidence of significant adverse effects on non-target invertebrates.

465. The consequences of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes causing toxicity in non-target invertebrates are assessed as **minor**. The likelihood of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes causing toxicity in non-target invertebrates are assessed as **unlikely**. Therefore, the risk of exposure to WideStrike™ cotton leading to toxicity to non-target invertebrates is estimated to be **low**.

Table 20 Summary of risk assessment

Event that may give rise to toxicity in non-target species	Consequence assessment	Likelihood assessment	Risk estimate	Does risk require treatment?
Identified Risk 1 Direct or indirect ingestion of the introduced Cry1Ac(synpro) and Cry1F(synpro) proteins by non-target invertebrates	**Minor** • Non-target dietary toxicity studies suggest Cry1Ac(synpro) and Cry1F(synpro) proteins are toxic or growth inhibitory only to a limited range of insects. • A field study suggests that growing WideStrike™ cotton plants has no significant effect on non-target invertebrate populations when compared to unsprayed non-GM cotton. • Non-GM cotton is sprayed with insecticides which impact on non-target insects.	**Unlikely** • Exposure to the GM cotton lines and the Cry proteins would occur mostly to those non-target invertebrates directly/indirectly consuming the GM cotton within the cotton field. • Non-target invertebrates appear insensitive to the levels of Cry1Ac(synpro) and Cry1F(synpro) proteins expressed in the WideStrike™ plants.	**Low**	**No**, however PRR conditions are imposed.

Chapter 4 Risk estimate for weediness

466. This Chapter estimates the risks (Identified Risks 2 and 3 from Chapter 2) associated with events that could lead to the adverse outcome of increased weediness arising from this proposed release. The risk estimate is based on the consequence and likelihood assessment for the events.

Section 1 Background

467. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas, such as farms or gardens, and give rise to negative impacts for people or the environment.

468. Negative characteristics of weeds may include competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. The spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to negative impacts such as reduced establishment of desired organisms, reduced quality of products or services obtained from the land use, reduced access to land, toxicity or increased ill-health of people or other desired organisms and increased degradation of the landscape or ecosystems (National Weed Prioritisation Working Group 2006).

469. The spread and persistence (invasiveness), is determined by complex interactions between a plant and its environment (including availability of water, nutrients and light). A number of measurable properties of plants that may influence spread and persistence include the ability to establish among existing plants, reproductive ability such as time to seeding, amount of seed set and ability for vegetative spread, mode of dispersal, likelihood of long-distance dispersal and tolerance to existing weed management practices (National Weed Prioritisation Working Group 2006).

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

471. The GM WideStrike™ cotton proposed for release expresses two insecticidal proteins and a herbicide tolerance protein as a result of the genetic modification. Events that may give rise to weediness were considered in Chapter 2. Potential weediness of the GM cotton in cotton fields, or dispersed outside of these areas south of latitude 22º South was discussed in Chapter 2, Events 4 and 5 and no risk was identified. Expression of the PAT protein is not expected to have any impact on the weediness of the GM cotton (Event 6). Potential weediness resulting from gene transfer and expression of the introduced genes in non-GM cotton or herbicide tolerant GM cottons, was discussed in Chapter 2 (Events 8 and 9) and no risk was identified.

472. The risk of increased weediness as a result of expression of the cry genes in WideStrike™ GM cotton has previously been assessed in the RARMPs prepared for limited and controlled releases under DIR 040/2003 and DIR 044/2003. These documents are available on OGTR Website. The risk assessments, taking into account the limits and controls proposed for the releases, concluded that the potential for the expression of the proteins to enhance the weediness potential of GM cotton plants (in comparison to non-GM cotton plants) during the trials posed a **low risk**.

Section 2 Consequence and likelihood assessments

473. Consideration is given to the identified risks in Chapter 2 (hazard identification) that may give rise to weediness. For each of the identified risks the level of risk is estimated from assessments of the seriousness of harm (consequence – ranging from marginal to major) and the chance of harm (likelihood – ranging from highly unlikely to highly likely).

474. The Regulator is required to consider risks to human health or the environment posed by, or resulting from, gene technology. For this reason, the level of risk from the proposed dealings with the GMO is considered relative to the baseline of weediness of non-GM cotton, other commercially released GM cotton lines and the environment in which the GM WideStrike™ cotton is proposed for release. Therefore, other sources of the introduced genes or similar genes in the environment and the agronomic practices proposed by the applicant are relevant to the risk estimate.

2.1 Weediness of non-GM cotton

475. Information on non-GM cotton is included here to establish a baseline for comparison with the GM cotton being considered in this risk assessment. Attributes of non-GM cotton associated with potential weediness are discussed in the document '*The Biology of* G. hirsutum *L. and* G. barbadense *L. (cotton)*' (OGTR 2008). This document concludes that non-GM cotton is not a serious weed in Australia, because environmental factors including temperature, soil moisture, nutrient limitation and roadside management practices limit the establishment and/or persistence of cotton outside of agricultural and other disturbed environments.

476. Small, persistent cotton populations have been observed, mainly in northern Australia. It has been noted by scientists over many years that the morphology of many of these naturalised cotton populations is distinct from that of the cultivated cotton varieties. It seems likely that many of the naturalised cotton populations resulted from attempts in the early 19th century to establish cotton industries in northern QLD and the NT (Curt Brubaker and Lyn Craven, CSIRO, pers. comm. 2002). Other cotton plants appear to be of more recent origin (eg Eastick 2002) but these are confined to areas of disturbed land with at least a seasonal water supply; typical locations are above the high tide mark on beaches and near river banks.

477. Modelling has been used to predict the areas that are climatically suitable for the long-term survival of cotton in Australia (Rogers et al. 2007). This model indicates that cold stress is the major limiting factor for potential distribution of cotton in southern Australia and dry stress is the major limiting factor in northern Australia. The model predicted that, in the absence of supplementary water, the coastal and sub-coastal areas of the east coast from Cape York to just south of the QLD/NSW border, but excluding the dry tropics, were the only climatically suitable areas for long term survival of cotton populations. When overall soil fertility was considered in addition to climatic data, the area suitable for cotton was further restricted, ie even more closely limited to coastal areas.

478. Roadside surveys in traditional cotton growing regions south of latitude 22º South as well as between Emerald (in the cotton growing region in central QLD) and the Atherton Tablelands (north of latitude 22º South in QLD) have shown that cotton is not a significant roadside weed in any of the regions surveyed (Addison et al. 2007). Survival of cotton volunteer plants seemed to be limited by competition from already established vegetation, low quantity of seed escapes, high disturbance in areas subject to frequent maintenance (such as slashing and herbicide treatment), high rate of seed desiccation and predation.

479. Small quantities of *G. barbadense* (pima cotton) are also commercially grown in Australia. Herbarium records for *G. barbadense* suggest that naturalised populations may occur, or may have occurred in the past, in northern, central and south eastern QLD and in the northern regions of the NT and WA (OGTR 2008). The presence of remnants of some of these populations has not been confirmed.

480. Cotton is not considered to be a serious weed in Australia (Groves et al. 2000; Groves et al. 2002; Groves et al. 2003). It has been grown for centuries throughout the world without any reports that it is a serious weed. Worldwide, there are about 50 species of *Gossypium* (Fryxell 1992; Craven et al. 1994) none of which is listed as a serious weed anywhere in the world (Holm et al. 1979; Holm et al. 1997; Randall 2002; Groves et al. 2003).

481. The weed status of cotton has been considered previously in many of the RARMPs produced during the assessment of a variety of GM cotton lines including commercial releases (eg DIRs 012/2002, 022/2002, 023/2002, 059/2005, 062/2005 and 066/2006). In addition to the information in the *Biology of* G. hirsutum *L. and* G. barbadense *L. (cotton)* (OGTR 2008), these RARMPs have considered new data that has been collected during previous releases of GM cotton lines in Australia.

2.2 Weediness of other GM insect resistant cottons

482. The potential for weediness of GM insect resistant cottons currently approved for commercial release has been considered previously for INGARD® cotton containing a modified *cry1Ac* gene (DIR 022/2002 and 023/2002) and Bollgard II® cotton containing both the modified *cry1Ac* and a modified *cry2Ab* gene (DIRs 012/2002, 059 and 066/2006). INGARD® cotton is no longer cultivated. It was concluded that the risk of the Bollgard II® cotton expressing two *cry* genes establishing as a weed in Australia was low or negligible (DIR 012/2002 and DIR 059/2005 for areas south of latitude 22º South and DIR 066/2006 for areas north of latitude 22º South).

483. Bollgard II® cotton has been grown in areas south of latitude 22º South since 2002 (DIR 012/2002) and was approved for use north of latitude 22º South in 2006 (DIR 066/2006). In 2007/2008, approximately 60,590 ha of Bollgard II® cotton was grown in areas south of latitude 22º South and 795 ha in areas north of latitude 22ºSouth. There have been no reports of any problems controlling GM cotton volunteer plants as a result of these releases.

2.3 Identified Risk 2: Expression of the introduced genes for insect resistance improving the survival of GM cotton plants and leading to increased spread and persistence north of latitude 22º South

484. As discussed in Chapters 1 and 2, the applicant is seeking approval for a number of dealings including commercial scale planting of the WideStrike™ GM cotton in cotton growing areas south of latitude 22º South without specific containment measures. Thus, GM cotton plants could potentially persist in the agricultural environment where grown and/or in the wider environment as a result of seed dispersal.

485. Events that may give rise to weediness were considered in Chapter 2. Potential weediness on cotton farms, or dispersal into other areas south of latitude 22º South has been discussed previously (Chapter 2, Events 4 and 5). This event will therefore relate to the risk of weediness in areas north of latitude 22º South.

486. The risk of weediness of the WideStrike™ GM cotton plants as a result of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in combination would depend on

the weediness of non-GM cotton plants, the importance of lepidopteran herbivory in limiting the spread and persistence of cotton (consequence assessment), the scale of the release and the chance of progeny establishing as weeds (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism, the commercial release of other GM cotton lines and in the context of the large scale of the proposed release and the receiving environment in Australia.

2.3.1 Consequence assessment

487. The *cry1Ac(synpro)* and *cry1F(synpro)* genes in combination could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in increased spread and persistence of the GM cotton in the environment north of latitude 22º South.

488. As discussed in the introduction of this Chapter, a weed could have a number of negative impacts including adversely affecting the health of people, animals or the environment, restricting movement, or reducing the establishment or the yield or amount of desired vegetation.

489. WideStrike™ cotton expressing the *cry* genes is not expected to adversely impact the health of people, other vertebrates or microorganisms compared to non-GM cotton (refer to Event 1). The impact of WideStrike™ on non-target invertebrates is considered in Chapter 3 (Identified Risk 1) and is considered to be a low risk. With regards to environmental health effects, the GM cotton is not expected to have an adverse impact on the fire regime of an area, soil salinity or stability, or water table levels. The GM cotton is not expected to restrict the movement of people, animals, vehicles, machinery or water.

490. However, resistance to lepidopteran insects as a result of expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in combination could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton and could potentially result in spread and persistence of the GM cotton in the environment north of latitude 22º South. This could then reduce the establishment of native vegetation, giving rise to lower abundance of desirable species, reduced species richness, or lead to undesirable changes in species composition. This could in turn have secondary impacts such as adversely changing animal or microorganism species composition due to altered food or shelter availability.

491. The impact of WideStrike™ cotton in managed areas such as cattle yards and disturbed environments such as roadsides is expected to be minimal as control options for cotton are readily available and relatively easily applied in these situations. For example, herbicides such as glyphosate, bromoxynil, carfentrazone and a combination of paraquat and diquat (Roberts et al. 2002) can be used to control seedling cotton volunteer plants. Cultivation is also a very effective method to control seedling cotton volunteer plants (Australian Cotton Cooperative Research Centre 2002). Established or ratoon cotton plants are most effectively controlled by mechanical methods involving mulching, root cutting and cultivation (Roberts et al. 2002). Hence, any increased fitness advantage would have to be very large to have a serious adverse impact in these areas.

492. Cotton is present outside of the cropping system but is limited by environmental factors including temperature, soil moisture, nutrient limitation and roadside management practices and tends only to exist in areas that have been disturbed or have minimal competition from other plants. The GM cotton is not expected to expand beyond the current distribution of non-GM cotton as the genetic modification is not expected to increase the plant's ability to withstand these abiotic stresses. No alterations have been seen to basic physiological or

phenotypic traits in WideStrike™ cotton compared to the non-GM parent (Chapter 1, Section 5.5.2). This indicates that any potential adverse impact of WideStrike™ cotton, like non-GM cotton, will be limited mainly to disturbed areas with suitable environmental conditions such as those that occur in more northern parts of Australia and will only have a marginal effect on the overall plant biodiversity of an area unless the cotton plants reached a high density.

493. In environments where there is no pressure of lepidopteran insect herbivory, the GM cotton plants will behave similarly to non-GM cotton plants. Lepidopteran insect pressure in cultivated cotton will be highly variable across different regions and seasons, or throughout an individual season. For example, studies on the ecology of *Heliothis* (*Helicoverpa*) species in Australia showed that seasonal abundance was directly influenced by temperature, host sequence and host suitability (Fitt 1989) and indirectly by rainfall, which influences the abundance and suitability of host plants. Other physical factors thought to affect numbers and local movement of *Helicoverpa* pest species are temperature, evaporation, prevailing wind systems and changes in vegetation and soil type (Zalucki et al. 1994; Oertel et al. 1999; Gregg & Wilson 2008). The variability observed for insect numbers in cultivated regions is also likely to be seen in natural ecosystems. If there is high insect pressure and if lepidopteran insect herbivory does play a role in controlling cotton abundance, it is possible that WideStrike™ could become more abundant than the non-GM cotton over a long period of time in some areas.

494. The potential weediness of other GM cottons with resistance to insects due to expression of one or more Cry proteins has been considered for commercial releases (DIRs 012/2002, 022/2002, 023/2002, 059/2005 and 066/2006). The introduced insect resistance genes in the commercially approved GM cotton lines include the *cry1Ac* and *cry2Ab* genes in Bollgard II® which confers resistance to lepidopteran insect herbivory. Taking into account the information in the RARMPs prepared for DIR 012/2002, 059/2005 and 066/2006, it was concluded that GM cotton with the Bollgard II® trait would have a minor consequence on the Australian environment. Currently there have been no reports that suggest that Bollgard II® is weedier than non-GM cotton.

495. WideStrike™ also contains two Cry proteins, including a *cry1Ac(synpro)* gene. The *cry1Ac* gene in Bollgard II® is also a synthetic gene but is comprised of sections of *cry1Ac* and *cry1Ab* and thus is different to *cry1Ac(synpro)* present in WideStrike™. Additionally, WideStrike™ and Bollgard II® differ in toxicity to certain insects (Chapter 1, Section 5.5.4 and RARMPs for DIR 012/2002, DIR 059/2005, DIR 066/2006). Therefore, although WideStrike™ and Bollgard II® are both Bt cottons, there may be some differences in how they behave in the presence of lepidopteran insects, and possibly other invertebrates (see Chapter 3) in northern Australia.

Conclusion

496. The consequences of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes increasing the potential for spread and persistence north of latitude 22º South of the GM cotton plants proposed for release through reduced lepidopteran herbivory are assessed as **minor**.

2.3.2 Likelihood assessment

497. The adverse outcome of weediness and an increase in the spread and persistence of GM WideStrike™ cotton plants in the environment is contingent on a number of steps occurring including:

- dispersal of viable seed into favourable habitats to germinate

- GM cotton plants need surviving the main limiting factors for cotton to reach flowering
- repetition of the cycle of fertilisation, survival and dispersal, allowing the population to persist
- WideStrike™ cotton plants having a fitness advantage compared to other plants including other cotton plants, due to relief from otherwise limiting lepidopteran insect herbivory.

Dispersal of seed

498. As cotton does not generally reproduce vegetatively (Serdy et al. 1995), spread within the environment occurs by seed dispersal (OGTR 2008). Dispersal of cotton seeds is a physical process. Basic morphological characteristics of WideStrike™ cotton are unaffected by the inserted insect resistance genes (see Chapter 1, Section 5.5.2). It is unlikely, therefore, that dispersal of WideStrike™ cotton would differ from the dispersal of the other baseline cottons.

499. Volunteer GM plants in northern Australia may arise from unintended seed dispersal during transportation, use as stockfeed, via animals or adverse weather conditions such as flooding.

500. Some GM cotton seed may be dispersed during transport of seed for storage, planting, ginning, processing and stockfeed and therefore GM cotton volunteer plants may establish on roadsides. Roadside surveys have shown the existence of cotton volunteers indicating that some viable seed is occasionally dispersed during transport (Addison et al. 2007). However, it is expected that the industry standard of transporting ginned cotton seed in covered containers/vehicles will be used when transporting the GM cotton material. Only a low quantity of seed escapes during transportation (Eastick 2002; Farrell & Roberts 2002). Therefore, spillage of seed during transport would be rare and any incident involving spillage of GM seed is expected to be in areas subject to management, such as roadside verges.

501. Seed could be dispersed when used as stockfeed. It has been estimated that in 2006/07, around 75,000 tonnes of cottonseed meal was used in feed in Australia (AOF 2007), and 30,000 tonnes of cottonseed (as quoted in Ansell & McGinn 2009). However, the amount of cotton seed being used in stockfeed each year can be highly variable. For example, the use of cotton seed as stockfeed increases significantly during drought. The presence of gossypol and cyclopropenoid fatty acids in cotton seed limits the use of whole cotton seed as a protein supplement in animal feed, except for cattle which are less affected by these components. Cottonseed is also used in as a supplementary feed for sheep (Knights & Dunlop 2007). Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually to avoid potential toxic effects. As part of research required under licences for DIR 023/2002 and DIR 022/2002, it was determined that very little cotton seed is used as stockfeed in northern QLD and it had not been used for stockfeed in NT and northern WA. However, drought has increased in recent years and current practices are unknown.

502. Seed may be spilt when fed out to cattle. A survey of nine dairy farms which used cotton seed to feed cattle identified instances of spilled cotton seed in seed storage areas, along paths, in feed lots and in grazing paddocks (Farrell & Roberts 2002).

503. In addition to seed dispersal during feeding, a small percentage of cotton seed consumed by stock can pass through the digestive system intact and is able to germinate (Eastick 2002). It has been estimated that more than 5% of the cotton seed that is fed to cattle are excreted whole (Sullivan et al. 1993a; Sullivan et al. 1993b), while other studies have

indicated that as much as 347 g/day/cow of whole or unlinted seed can be excreted (Coppock et al. 1985). Whole seed may be defecated in a cattle yard, or in a field where animals graze after being fed, under conditions which may be suitable for germination.

504. Seed could potentially be dispersed via other animals. However, mature cotton bolls are large, covered with thick fibres and enclosed in a tough boll that retains most of the seeds on the plant (Llewellyn & Fitt 1996). There are no reports of mammals, including rodents, feeding on mature cotton bolls or carrying seed cotton any great distance from the cotton fields. Similarly there is no evidence of avian species transporting cotton seeds (OGTR 2008).

505. Dispersal via flooding or other extreme environmental conditions is also possible. Areas that get flooded regularly may not be favourable for commercial production, as cotton plants are poorly adapted to waterlogging (Hodgson & Chan 1982). Irrigation practices (Good Management Practice of cotton industry) used by cotton growers retain irrigation water run off, as well as the first 15 mm of storm water run-off, on-farm to minimise the entry of pesticide residues into natural waterways. This would minimise seed dispersal from the fields where WideStrike™ is grown. Following seed dispersal from the cotton fields by flooding or extreme weather conditions, there would still need to be further dispersal for the seed to reach areas north of latitude 22º South.

Germination

506. No field experiments have been carried out in the natural Australian environment comparing the germination of WideStrike™ cotton and the baseline cottons.

507. However, laboratory studies carried out in the USA do not suggest that the germination of WideStrike™ cotton was changed compared to non-GM cotton (Pellow 2003). In these studies, seeds were sampled to investigate their germination and dormancy characteristics. The results suggested that the introduced genes generally had no statistically significant effect on germination or the induced dormancy of cotton seeds.

Establishment and persistence outside the agricultural environment

508. If viable cotton seed was dispersed away from the cotton fields, it must fall into a suitable habitat to germinate and survive. As discussed in the document *The Biology of* Gossypium hirsutum *L. and* Gossypium barbadense *L. (cotton)* (OGTR 2008), non-GM cotton is not a serious weed in Australia because environmental factors, including temperature, soil moisture, nutrient limitation and roadside management practices, limit the establishment and/or persistence of cotton outside of agricultural and other disturbed environments.

509. Dispersal of WideStrike™ cotton seed is most likely to occur around the regions where it is grown, ie regions south of latitude 22º South. Dispersal into northern areas of Australia is less likely to occur from this proposed release, with transport for stockfeed being the most likely route. However, cotton seed that is dispersed into northern areas is more likely to establish than in southern areas, as evidenced by the existence of naturalised populations that have existed long term (refer to Section 1 of the Chapter). However, reliable availability of water is still a major limiting factor in these areas, thus naturalised cotton populations mainly occur in areas with a sustained fresh water supply (eg coastal habitats or on the banks of permanent water courses) (Eastick 2002; Hnatiuk 1990; OGTR 2008).

510. If seed was dispersed during transport, volunteer establishment is mainly expected in disturbed, favourable habitats such as ditches and roadside drains. A survey of the transport

routes between Emerald (in the cotton growing region in central QLD) and the Atherton Tablelands (north of latitude 22º South in QLD, conducted in 2002, indicated that cotton plants had established in the roadside environment only infrequently, despite 12 years of use of these routes for transporting ginned seed (including GM cotton varieties since their respective commercial releases) for stockfeed (Farrell & Roberts 2002). The study concluded that cotton volunteer plants tend to establish in highly and regularly disturbed environments and appear to have negligible ability to invade non-disturbed habitats, eg native bush. The following factors that limit survival of cotton volunteer plants in the roadside environment were identified: competition from already established vegetation, low quantity of seed escapes, high disturbance in areas subject to frequent maintenance and high rate of seed desiccation. Similarly, follow up surveys carried out in 2004 and 2005 found that transient feral cotton populations may occur along cotton transportation routes but weed competition and roadside slashing prevent the establishment of stable populations in areas with otherwise suitable climates (Addison et al. 2007).

511. The type of habitat that the cotton seed is dispersed into has also been shown to affect germination. A study on the spread and persistence of cotton seed showed germination was highest in disturbed habitats, especially if the seed was buried (Eastick & Hearnden 2006). There were clear trends indicating that the habitat into which seeds were sown affected survival. Survival at sites located near cattleyards or adjacent to water bodies was consistently high, probably because of high soil nutrients and/or soil moisture (Eastick & Hearnden 2006). The result is in agreement with field observations that the occurrence of naturalised and volunteer cotton plants appears to be limited by the availability of adequate soil moisture (Addison et al. 2007).

512. Cotton volunteer plants could establish in areas where livestock is fed cotton seed or where stock graze after being fed. A survey of dairy farms in the Atherton Tablelands which regularly feed stock with cotton seed found volunteer plants at seven of the nine farms (Farrell & Roberts 2002). However, volunteer plants were all close to dairy infrastructure, suggesting that their ability to invade is negligible. Although cotton growing in cattle yards may reach reproductive maturity, persistence and seed dispersal from these areas is limited by trampling and grazing. No cotton volunteer plants were found in the undisturbed bush habitats surrounding these areas (Eastick 2002; Eastick & Hearnden 2006).

513. If some seed from the GM cotton plants is dispersed via flooding or other extreme environmental conditions, cotton volunteer plants are most likely to establish along waterways, eg drains, creeks and rivers, or in flood prone areas. However, much of this dispersed seed is not expected to survive as modern cotton varieties have been bred to be soft-seeded (Mauncy 1986; Hopper & McDaniel 1999) and the viability of cotton seed is affected by moisture (Stephens 1958; Halloin 1975). Extended soaking in water generally reduces cotton seedling emergence and results in smaller seedlings (Buxton et al. 1977). In the event of cotton seed reaching the sea, experiments using seawater showed that the viability of modern cultivated cottons with thin seed coats decreased markedly after one week, probably due to the thin seed coat enabling rapid water uptake (Stephens 1958).

514. Cotton seed in commercial trade must be handled properly to preserve germination quality. In humid environments, seed left in the field will not usually survive until the next season (Jenkins 1993).The existence of a soil seed bank seems unlikely because dispersed seeds that do not germinate are rapidly weathered, leading to significant decreases in their viability (Halloin 1975; Woodstock et al. 1985). However, it is widely accepted that dormancy can be induced in cotton seeds by low soil temperature and/or soil moisture.

515. In addition to induced dormancy, cotton seeds collected immediately following fruit maturation can display 'innate dormancy' (Taylor & Lankford 1972) – an inherent condition of the mature seed/embryo that prevents the seed from germinating, even when exposed to appropriate environmental conditions. The duration of innate dormancy varies between varieties and timing of maturity (Hsi & Reeder 1953; Christidis 1955) and it can depend on when in the season the boll opened. Cotton seed stored for two years showed higher germination than seed stored for one year, or seed planted the season following harvest (Taylor & Lankford 1972). The positive effect of seed age on germination ability could reduce the negative impact of factors that may induce dormancy, such as cold temperature or salinity.

516. There are abiotic and biotic factors that determine whether cotton will persist in the environment, including soil type, fire, competition from other plants, herbivory (insects and other animals) and physical destruction such as trampling (Farrell & Roberts 2002; Eastick & Hearnden 2006). The relative impact of each of these factors is dependent on whether the cotton plants are in coastal or inlands areas, as well as whether they are in northern or southern areas of Australia.

517. Even though cotton has been grown previously in a number of places in northern Australia, only isolated cotton populations have been able to naturalise. For example, cotton has not persisted in the environment in the Ord River Irrigation Area following the abandonment of farms, with actively growing cotton plants in the fields, in the 1960s and 70s (Eastick 2002). However, in northern Australia, cotton volunteer plants have been observed in areas that have not been cultivated for cotton in many years (Williams 2002). Many of these volunteer plants appear to benefit from water and nutrients that may run off other areas that are tended regularly and are within metres of the volunteer plants.

Selective pressure and weediness

518. The *cry1Ac(synpro)* and *cry1F(synpro)* genes in combination could confer a selective advantage in areas where lepidopteran insect predation limits one or more of the key life stages of cotton. This could result in spread and persistence of the GM WideStrike™ cotton in the environment.

519. Although lepidopteran pests (mainly *H. armigera* and *H. punctigera*) are the main insect pests in cultivated cotton, they do not seem to be a major limiting factor in naturalised cotton populations. The *cry* genes have been introduced into WideStrike™ cotton to protect the plants against damage of reproductive tissues, ie flower buds and bolls, by lepidopteran pests. Monitoring of seven naturalised cotton populations in the NT revealed abundant seed production, suggesting that these cotton plants were not significantly affected by lepidopteran pests (Eastick 2002). The major insect herbivores observed, particularly over the wet season, were grasshoppers.

520. Similarly, insect exclusion studies in northern Australia showed no difference between seedling survivorship and fruit production between caged and non-caged plants during the dry season but during the wet season, uncaged plants were attacked by grasshoppers (order: Orthoptera) and other leaf eating insects (Eastick 2002). Indeed, grasshoppers are considered to be the most important grazing insects in tropical savannah ecosystems (Andersen & Lonsdale 1991). No data has been provided as to whether WideStrike™ has any effects on grasshoppers. If there is an effect then this may provide a selective advantage to WideStrike™ cotton plants in the north of Australia.

521. As discussed in detail in the RARMP prepared for DIR 066/2006, sampling of insects from naturalised cotton populations in the NT found the dominant insect order was

Hemiptera (28% of total insects) and only 16% were from the order Lepidoptera of which none were confirmed to be a Noctuid (Eastick 2002), the insect family to which *H. armigera* and *H. punctigera* belong.

522. Results from a study on the potential weediness of Bollgard II® in northern Australia conducted over four years indicates that expression of the *cry1Ac* and *cry2Ab* genes in combination does not confer a significant selective advantage and it was concluded that lepidopteran insect pressure is not the critical factor limiting establishment and growth of cotton populations (Eastick 2002; Eastick & Hearnden 2006). Factors that influenced cotton plant survival during this four year study were: nutrient and water availability, plant competition, herbivory by non-lepidopteran insects, grazing and trampling by cattle, and fire.

523. INGARD® cotton (containing a modified *cry1Ac* gene) was in commercial cultivation since 1996 (DIR 022/2002), and Bollgard II® cotton since 2002 (DIR 012/2002). Since their commercial release, seed from these GM cotton lines has been used as stockfeed in Australia, including in northern Australia. Over this period there has been no evidence that these GM cotton lines have become problematic weeds.

524. However, the target pest range indicated by the applicant for WideStrike™ cotton (see Chapter 1, Section 5.1.1 for details) is much broader than the previously released Bollgard II® cotton and therefore may have a larger impact on plant survival and only some detail of how these species are impacted on by WideStrike™ has been provided. It is likely that the following indicated target species would actually be present as pests in Australian cotton fields: cotton bollworm (*Helicoverpa armigera*), native budworm (*H. punctigera*), pink bollworm (*Pectinophora gossypiella*), beet armyworm (*Spodoptera exigua*), other unspecified armyworms (*Spodoptera* spp., including cluster caterpillar *S. litura*), and cutworm (*Agrotis ipsilon* and other spp.). These would also exist in the natural environment.

525. For Bollgard II® it was stated that insecticides may be needed to control heavy infestations of the lepidopteran pest *S. litura* as it is only moderately susceptible to these Bt toxins. INGARD® cotton, which contains only the Cry1Ac insecticidal protein, has been shown to poorly control *S. litura* (Strickland et al. 2003). The applicant has stated that *S. litura* is a target of WideStrike™ cotton and provided efficacy data indicating toxicity (Dow AgroSciences 2006b). This is an important difference between Bollgard II® and WideStrike™ cotton which may have the potential to alter the likelihood of spread and persistence in the environment north of latitude 22º South.

Uncertainty

526. For the potential weediness of WideStrike™ in areas north of latitude 22º South, uncertainty in the assessment exists as a result of:

- data uncertainty around the toxicity to target and non-target insects
- knowledge gaps in the potential survival of WideStrike™ cotton north of latitude 22º South due to;
    - the absence of field studies on survival of WideStrike™ in areas north of latitude 22º South
    - no laboratory- or field-based studies on effects of some invertebrates which are important pests in northern Australia.

527. While the applicant has not proposed to intentionally grow WideStrike™ cotton in areas north of latitude 22º South, they have not proposed containment measures to restrict the dealings other than growing the GMO to areas south of latitude 22º South. Therefore, the

applicant is proposing that seed be able to be transported and used in northern Australia, and thus volunteer plants may occur there. No data has been provided on the survival of seedlings in the natural environment in northern Australia or whether the *cry* genes in WideStrike™ cotton will provide any selective advantage. In northern Australia, the survival of cotton plants is likely to be affected by insect herbivory, although factors such as water availability, soil nutrients, grazing by vertebrates, fire and plant competition are likely to also affect seedling survival.

528. Bollgard II® cotton was approved for commercial release in southern Australia in 2002 (DIR 012/2002), but limits were placed on its use in northern Australia until data was available on characteristics likely to lead to weediness. Although WideStrike™ also contains *cry* genes, these are different synthetic genes (see Chapter 1, Section 5.2.2). There is some uncertainty as to what insects they may impact on (see Identified Risk 1). A wider target range has been listed than for Bollgard II® cotton and this may have a greater impact on reducing lepidopteran herbivory in natural situations.

529. Grasshoppers 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 volunteer plants in areas north of latitude 22º South. No information is available on the effect of WideStrike™ cotton on grasshopper populations, although it is acknowledged that Cry1 proteins are generally considered lepidopteran specific.

530. *S. litura* and *P. gossypiella* are thought to be major pests of cotton in northern Australia and therefore may be important limiting factors for cotton outside of cotton fields as well. These are listed as target species for WideStrike™ cotton, and thus volunteer WideStrike™ cotton plants may have a selective advantage in the presence of lepidopteran herbivory. Other *Spodoptera* spp are adversely affected by the Cry1Ac protein and leaf assays have shown toxicity of WideStrike™ to *S. litura*. The impact of *S. litura* predation on limiting the presence of cotton in areas north of latitude 22º South is unclear. Therefore, expression of the introduced genes may confer a selective advantage on WideStrike™ cotton in these environments. Similarly, if WideStrike™ cotton has a negative impact on the target species *P. gossypiella* (pink bollworm), and if this insect has a role in limiting cotton in areas north of latitude 22º South, this may confer a selective advantage. LC_{50} data presented suggests that neither Cry1Ac(synpro) nor Cry1F(synpro) are effective against pink bollworm (Chapter 1, Section 5.2.2). However, field experiments from the USA concluded that the Cry1Ac(synpro) parental line and WideStrike™ cotton gave excellent control of pink bollworm, although the Cry1F(synpro) parental line did not (Pellow 2001). It is therefore unclear if WideStrike™ cotton would have a selective advantage. This uncertainty would be reduced by Australian field experiments on WideStrike™ cotton.

Conclusion

531. Some GM cotton seed may spread from where it is cultivated into areas north of latitude 22º South and germinate and persist in the wider environment. As cotton does not compete well with other plants and has high water and nutrient requirements, volunteer plant establishment is mainly expected in disturbed, favourable habitats. WideStrike™ cotton volunteer plants can be effectively controlled by mechanical means or, if still in the seedling stage, by the use of herbicides other than glufosinate ammonium. Although lepidopteran insects are the main insect pests of cultivated cotton, herbivory by other non-lepidopteran insects, eg grasshoppers (Order: Orthoptera) and sucking insects (Order: Hemiptera), is also important in naturalised cotton populations. No data is available on whether WideStrike™ affects these insects and there is uncertainty about which insects it does impact on. However,

the expression of the insecticidal genes is not expected to alter susceptibility to many of the factors that are known to limit the spread and persistence of cotton in northern Australia, eg reliable water and nutrient availability (see Section 2.1 of this Chapter).

532. Therefore, the chance of WideStrike™ plants establishing as weeds by finding suitable ecological niches is expected to be no greater than for the non-GM parent organism, however the introduced genes may have an effect on their survival. Therefore, the likelihood of weediness as a result of Identified Risk 2 is assessed as **unlikely**.

2.4 Identified Risk 3: Expression of the introduced cry genes in other insect resistant GM cotton plants as a result of gene transfer leading to increased spread and persistence

533. Events that may give rise to weediness were considered in Chapter 2. Potential weediness due to expression of the introduced gene for herbicide tolerance was considered in Events 4, 5 and 6 and no risk was identified. Potential weediness due to gene transfer to non-GM cotton or other herbicide tolerant GM cotton lines has been discussed previously (Chapter 2, Events 9 and 11) and no risk was identified. The presence of the *pat* gene in other GM cottons is not expected to give a selective advantage anywhere in Australia. This section will therefore focus on the risk of weediness from transfer of the introduced *cry* genes to other insect resistant GM cotton plants.

534. The risk of weediness as a result of transfer of the *cry1Ac(synpro)* and *cry1F(synpro)* genes to other insect resistant GM cotton plants would depend on the importance of lepidopteran herbivory in limiting the spread and persistence of cotton and the impact of the combination of the Cry proteins on toxicity of the GM cotton to the susceptible insects (consequence assessment), the chance of gene transfer occurring and the chance of progeny establishing as weeds following gene transfer (likelihood assessment). The risk is assessed against the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release which includes the commercial release of other GM cotton lines.

535. It should be noted that WideStrike™ cotton was generated from two GM lines and, so the introduced genes have inserted into different regions of the plant genome and segregate independently of one another. This means that after any initial outcrossing of WideStrike™ cotton to other cotton, any subsequent generations of cotton volunteer plants may contain either both *cry* and *pat* genes, one *cry* and *pat* gene or no *cry* or *pat* genes from WideStrike™ cotton. However, this does not impact on the assessment for weediness as a result of gene transfer of the introduced genes to other cottons because any GM cotton produced from outcrossing containing either one *cry* gene or no *cry* gene will have equivalent or less insecticidal efficacy than a GM cotton volunteer plant with both *cry* genes. Therefore, segregation of the *cry* genes will not be considered further.

536. The following insect resistant GM cotton lines are currently approved for commercial release in Australia:

- insect resistant INGARD® cotton (DIR 022/2002)
- insect resistant Bollgard II® cotton (DIR 012/2002, DIR 066/2006)
- glyphosate tolerant/insect resistant (Roundup Ready®/INGARD®) cotton (DIR 023/2002)
- insect resistant/glyphosate tolerant (Bollgard II®/Roundup Ready®) cotton (DIR 012/2002, DIR 066/2006).

- glyphosate tolerant/insect resistant (Roundup Ready Flex®/ Bollgard II®) cotton (DIR 059/2005, DIR 066/2006)

537. INGARD® cotton was withdrawn from the market in favour of Bollgard II® cotton in 2004. Roundup Ready® cotton will be withdrawn next season in favour of Roundup Ready Flex®. Therefore, INGARD® and Roundup Ready® cotton will not be considered further.

538. The introduced insect resistance genes in the commercially approved and currently grown GM cotton lines are modified *cry1Ac* and *cry2Ab* genes in Bollgard II® which confers resistance to lepidopteran insect herbivory. The *cry1Ac* gene in Bollgard II® is a synthetic gene, comprised of sections of *cry1Ac* and *cry1Ab*, and so is different to *cry1Ac(synpro)* present in WideStrike™ cotton.

2.4.1 Consequence assessment

539. As discussed in the introduction to this Chapter, a weed could have a number of negative impacts including adversely affecting the health of people, animals or the environment, restricting movement, or reducing the establishment or yield/amount of desired vegetation. The impact of WideStrike™ cotton is discussed in Identified Risks 1 and 2 and similar impacts are expected if the genes from WideStrike™ cotton were transferred to other insect resistant GM cottons.

540. Transfer of the *cry1Ac(synpro)* and *cry1F(synpro)* genes to other insect resistant GM cottons that are currently approved for commercial release could result in the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in these plants in addition to their own introduced genes.

541. Cotton containing the introduced genes in combination with other genes conferring insect resistance may have adverse impacts on non-target invertebrates, or spread and persist in the environment which could reduce the establishment of native vegetation, giving rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition.

Transfer of the introduced genes into other insect resistant GM cottons in cotton fields

542. As discussed in Event 4, the impact of volunteer WideStrike™ cotton in the agricultural setting is expected to be minimal as control options for cotton are readily available and relatively easily applied in these situations. Similarly, the impact of cotton plants containing the introduced *cry* genes in addition to those present in other insect resistant GM cottons is also expected to be minimal.

Transfer of the introduced genes into other GM cottons outside of cotton fields

543. The impact of WideStrike™ cotton in managed areas such as cattle yards and roadsides is expected to be minimal as control options for cotton are readily available and relatively easily applied in these situations. Hence, any increased fitness advantage would have to be very large to have an adverse impact in these areas. Similar minimal impacts would be expected from cotton plants containing the introduced *cry* genes in addition to those present in other insect resistant GM cottons.

544. Cotton plants present outside of the cropping system may be limited by environmental factors including temperature, soil moisture, nutrient limitation, plant competition, herbivory, grazing and trampling by cattle, and fire. The relative impact of each of these factors is dependent on whether the cotton plants are in coastal or inlands areas, as well as whether they are in northern or southern areas of Australia. For example, frost is a major limiting factor in

southern areas of Australia, whereas the reliable availability of water is a limiting factor in most areas of Australia.

545. WideStrike™ cotton and any progeny resulting from transfer of the *cry* genes to other insect resistant cottons are not expected to expand beyond the current distribution of non-GM cotton as the genetic modification is not expected to increase the plant's ability to withstand abiotic stresses. This indicates that any potential adverse impact of the hybrid containing multiple *cry* genes, like non-GM cotton, will be limited mainly to disturbed areas with suitable environmental conditions such as those that occur in more northern parts of Australia and will only have a marginal effect on the overall plant biodiversity of an area unless the cotton plants reached a high density.

546. For GM plants expressing more than one *cry* gene, synergistic, additive or antagonistic interactions between the expressed toxins may be a possibility after ingestion by susceptible insect species. A number of studies have shown synergistic effects between Cry proteins (some combinations showed greater activity than would be expected from the activity of the individual fractions; Schnepf et al. 1998; Glare & O'Callaghan 2000). Additive and antagonistic effects have also been noted for some Cry protein combinations (reviewed in del Rincon-Castro et al. 1999). The mechanism of such interactions is unclear, but a number of factors appear to be involved, including the particular protein combinations and the target insects.

547. Therefore it is possible that the combination of *cry* genes from WideStrike™ with the *cry* genes in Bollgard II® (*cry1Ac*, *cry1Ac(synpro)*, *cry1F(synpro)* and *cry2Ab*) may result in cotton plants with greater toxicity to target invertebrates or may have an adverse impact on some non-target invertebrates. The interactions between the Cry proteins in the individual GM cottons are generally understood; however there is little published information on possible effects from the combination of the proteins expressed in the two GM cottons.

548. There are a number of abiotic and biotic factors that limit the spread and persistence of cotton plants and cotton does not possess certain innate characteristics typically associated with problematic weeds (see Section 2.1 of this Chapter).

549. If gene transfer occurred from WideStrike™ to other insect resistant cotton plants outside of managed environments then this would not be expected to alter the susceptibility of cotton to the abiotic environmental factors that normally limit cotton. However, in a suitable environment (in particular in northern Australia) the presence of multiple *cry* genes with unknown combination effects may give the plants a selective advantage over non-GM cotton plants and other commercially approved insect resistant GM plants.

Uncertainty

550. Uncertainty exists as to the behaviour of the combination of *cry* genes that may occur in a hybrid between WideStrike™ and Bollgard®. There is uncertainty surrounding the combination of *cry1F(synpro)* and *cry2Ab* as there is no data available on how these two proteins behave in combination, either in the laboratory or in GM plants. Additionally, if the combination of these *cry* genes resulted in toxicity to a greater number of insect species and these insects were limiting cotton then this could potentially result in increased spread and persistence. As the Cry1Ac proteins in the two GM cottons are slightly different, there is also some uncertainty about their behaviour in combination with the *cry* proteins in the other GM cotton.

Conclusion

551. The consequences of expression of the introduced cry genes in combination with the Cry proteins from other in insect resistant GM cotton plants increasing the potential for spread and persistence in cotton is assessed as **minor**.

2.4.2 Likelihood assessment

552. The adverse outcome of weediness resulting from gene transfer from WideStrike™ to other insect resistant GM cotton plants leading an increase in the spread and persistence of cotton plants is contingent on a number of steps occurring, including:

- pollen transfer to other cotton plants
- successful pollination and setting of viable seed
- dispersal of viable seed into favourable habitats to germinate
- GM cotton plants need surviving the main limiting factors for cotton to reach flowering
- repetition of the cycle of fertilisation, survival and dispersal, allowing the population to persist
- cotton plants having a fitness advantage compared to other plants, including other cotton plants, due to relief from otherwise limiting insect herbivory.

Gene Transfer

553. Transfer of the introduced genes present in WideStrike™ to other insect resistant GM cotton plants could occur between cultivated cottons adjacent to each other, cultivated cotton and nearby volunteer plants, or between volunteer/naturalised plants that are growing outside of cultivation. WideStrike™ will not be planted in areas north of latitude 22º South, however some volunteer plants may occur there from seed dispersed during transport, use as stockfeed or flooding. Limited quantities of Bollgard II® cotton are currently grown in areas north of latitude 22º South, although this may increase in the future. There is therefore very limited opportunity to produce a hybrid plant containing multiple *cry* genes north of latitude 22º South.

554. The likelihood of gene transfer to other cotton plants is dependent on the rate of successful cross-pollination. Cotton is primarily self-pollinating, having pollen that is large, sticky, heavy and not easily dispersed by wind (Jenkins 1992; OGTR 2008). In Australia, honeybees and native bees are the most likely insects responsible for any cross-pollination in cotton (OGTR 2008). Cotton pollen dispersal studies conducted in Australia consistently show that outcrossing is localised around the pollen source and decreases significantly with distance (OGTR 2008 and references therein). For example, levels of outcrossing between cotton plants in adjacent rows is in the order of 1-2% (Thomson 1966; Mungomery & Glassop 1969; Llewellyn & Fitt 1996). Therefore, gene transfer from the GM cotton to other insect resistant GM cotton plants is only expected to occur in close proximity and at low frequencies. This situation would occur if WideStrike™ cotton was cultivated immediately adjacent to another insect resistant GM cotton field. Gene flow is most likely to occur in this circumstance.

555. However, information on outcrossing rates above is from the southern cotton growing areas of Australia. Tropical northern regions have higher insect numbers and different environmental conditions (Llewellyn et al. 2007). In Kununurra, WA, outcrossing rates were

higher than seen in southern Australia, with 7.9% at 1 m, falling to 0.79% at 50 m. A similar, earlier experiment had recorded much higher outcrossing rates of 30% at 1 m then down to 0.76% at 50 m, thought to be due to the presence of beehives in an adjacent field (Llewellyn et al. 2007). Thomson (1966) looked at out crossing in the Ord River valley, WA, over two growing seasons. Cross pollination between adjacent plants, was in the range of 0 to 5%, with mean values of 1.6% and 1.0%, in the first and second seasons, respectively. Very little cross-pollination was detected at a distance of more than 3 m (average less than 0.01%) and none was detected at distances between 3 and 8 m. However, insecticides were applied at least weekly to control insect pests as without the sprays it was not possible to obtain seed. As bees are sensitive to insecticides, the extensive use of insecticides for control of insect pests will limit the extent of cross-pollination due to repellence as well as bee mortality (Jenkins 1993; Rhodes 2002).

556. Other than for adjacent rows, the studies cited above measured out-crossing across 'buffer rows' of cotton. The out-crossing rate outside of cotton fields, between cotton plants separated by bare ground, might be expected to be higher. In an Australian study, out-crossing occurred over 50 m of bare ground to give an average level of 1.9% in the first row of cotton plants (Llewellyn et al. 2007). The out-crossing level dropped to 0.19% at 5 m into the cotton field, suggesting that pollinators did not carry viable pollen far into the field but remained at the edges. In northern Australia, the out-crossing rate over 50 m of bare ground was 0.3% (Llewellyn et al. 2007), lower than in the southern regions study, possibly due to insecticide use. However, as cotton grown in Australia are mainly Bollgard II® varieties, fewer insecticides will be used. Insecticides are unlikely to be used in volunteer WideStrike™ plants, so pollination rates are likely to be higher than for conventionally managed non-GM cotton.

Weediness of the recipient plants as a result of expression of the introduced genes

557. Transfer of the *cry1Ac(synpro)* and *cry1F(synpro)* genes to other insect resistant GM cottons that are currently approved for commercial release could result in the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in these plants in addition to their own introduced genes (refer to the introduction to this Section for details). This could confer a selective advantage in situations where lepidopteran insect herbivory is limiting cotton.

558. As discussed in Chapter 1, Section 5.2.3, synergistic, additive and antagonistic effects between Cry proteins might occur after ingestion by susceptible insect species. Synergistic effects when combining Cry proteins have been reported (Chakrabarti et al. 1998; Ibargutxi et al. 2008), showing a greater toxicity to the same insects targeted by the individual proteins. No literature has been identified that shows combining Cry proteins results in an increase in the range of insects affected compared to the range of insects affected by the individual Cry proteins by themselves. No literature has been found to suggest that the specificity of individual Cry proteins change in the presence of another Cry protein.

559. If these plants did indeed have greater toxicity to target organisms or had a broader spectrum of activity, it is unlikely to give the plants a selective advantage in most areas south of latitude 22º South due to the other factors limiting cotton. It is therefore expected that any gene transfer from WideStrike™ cotton to Bollgard II® cotton which resulted in plants entering the natural environment south of latitude 22º South would only result in ephemeral populations similar to the baseline cottons.

560. If these plants did have a broader spectrum of activity towards insects this could give them an advantage in areas north of latitude 22º South, where cotton can persist, but it would depend on the degree of increased activity. However, WideStrike™ and Bollgard II® cottons

would still have to cross with each other (noting that no intentional crossing is intended) and these plants would have to reach these areas before any adverse impact could occur (noting that WideStrike™ cotton is not proposed to be grown in areas north of latitude 22º South).

Uncertainty

561. As a result of data uncertainty around the toxicity to target and non-target insects; the role of lepidopteran insects in limiting cotton in areas north of latitude 22º South; and knowledge gaps due to the absence of relevant field studies on survival of WideStrike™ in these areas, uncertainty exists for the potential weediness of WideStrike™ in areas north of latitude 22º South. This has been discussed in Identified Risk 2.

Conclusion

562. Some gene transfer may occur between WideStrike™ cotton and Bollgard II® cotton. This is most likely to occur in the cotton fields south of latitude 22º South where both may be commercially planted. However, these hybrid plants are unlikely to persist in agricultural areas due to management or in other areas south of latitude 22º South due to abiotic factors limiting cotton. Limited gene flow between WideStrike™ and Bollgard II® cotton is expected but gene transfer from WideStrike™ cotton to Bollgard II® cotton could result in plants which had tolerance to a wider range of insects than either of these GM cottons, individually, and therefore had a selective advantage in areas north of latitude 22º South. As discussed in Identified Risk 2, the chance of a volunteer insect resistant plant establishing as a weed by finding a suitable ecological niche is no greater than for the non-GM parent organism, however resistance to lepidopteran herbivory may offer a selective advantage to survival. Although a causal pathway from gene flow to increased weediness can be identified, the chance of a cotton plant containing *cry* genes from both WideStrike™ and Bollgard II® cottons establishing in northern Australia would be limited.

563. Therefore, the likelihood of weediness as a result of the expression of the introduced *cry* genes in other insect resistant GM cotton plants is assessed as **highly unlikely**.

Section 3 Risk estimates

564. Risk estimates (which can range from negligible to high) are based on a combination of the consequences and likelihood assessments, using the *Risk Estimate Matrix* (see Chapter 2) (OGTR 2007).

565. The risk estimates for the adverse outcome of weediness of the GM cotton as a result of the expression of the *cry1Ac(synpro)* and *cry1F(synpro)* genes, or the transfer of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in other insect resistant cottons, have been made relative to the baseline of the low weediness potential in the non-GM parent organism and in the context of the large scale of the proposed release and the receiving environment for this proposed release. Consideration has also been given to the current widespread use of Bollgard II® cotton (containing modified *cry1Ac* and *cry2Ab* genes) in commercial cotton crops in Australia.

566. The consequences of increased spread and persistence of cotton north of latitude 22º South resulting from the presence of the *cry1Ac(synpro)* and *cry1F(synpro)* genes (Identified Risk 2) have been assessed as **minor**, and the likelihood of this resulting in weediness as **unlikely**. Therefore, the risk estimate is **low**.

567. The consequences of increased spread and persistence resulting from the presence of the *cry1Ac(synpro)* and *cry1F(synpro)* genes in other insect resistant GM cotton plants, as a

result of gene transfer (Identified Risk 3), have been assessed as **minor**, and the likelihood of this resulting in weediness as **highly unlikely**. Therefore, the risk estimate is **negligible**.

Table 21 Summary of risk assessment

Event that may give rise to weediness	Consequence assessment	Likelihood	Risk estimate	Does risk require treatment?
Identified risk 2 Expression of the introduced genes for insect resistance improving the survival of GM cotton plants and leading to increased spread and persistence north of latitude 22º South.	**Minor** • The expressed genes for insect resistance are not expected to impact on health of humans, other vertebrates or microorganisms. • The expression of *cry* genes will not extend the range of GM cotton compared to non-GM cotton.	**Unlikely** • WideStrike™ cotton will not be grown north of latitude 22º South. • WideStrike™ cotton volunteer plants can be effectively controlled by mechanical means, or if still at the seedling stage by the use of alternative herbicides. • The chance of volunteer GM plants arising from unintended seed dispersal finding suitable ecological niches and establishing as weeds would be no greater than for non-GM cotton. • The expressed genes for insect resistance would only confer a selective advantage in areas where insect predation limits cotton.	**Low**	**Yes**
Identified risk 3 Expression of the introduced *cry* genes in other insect resistant GM cotton plants as a result of gene transfer leading to increased spread and persistence.	**Minor** • The expressed genes for insect resistance are not expected to impact on health of humans, other vertebrates or microorganisms. • The expression of *cry* genes will not extend the range of GM cotton compared to non-GM cotton. • Although the effects of combining the *cry* genes from WideStrike™ and Bollgard® cotton could provide unexpected protection from herbivory, if the GM cottons were to spread and persist it is expected to have a limited impact on native vegetation and only in areas with suitable environmental conditions.	**Highly unlikely** • Cotton is primarily self-pollinating and gene transfer to other insect resistant GM cotton plants would only occur over short distances and at low frequencies. • The GM cotton will not be grown north of latitude 22º South. • The chance of volunteer GM plants arising from seed dispersal finding suitable conditions to establish as weeds would be no greater than for non-GM cotton plants. • Although reduced lepidopteran insect herbivory may offer a small competitive advantage, abiotic and biotic factors, are likely to be more important in limiting the spread and persistence of cotton, especially in southern Australia • Insect resistant cotton volunteers can be effectively controlled by mechanical means, or if still at the seedling stage by the use of alternative herbicides.	**Negligible**	**No**

Chapter 5 Risk management

568. Risk management includes evaluation of risks identified in Chapters 2, 3 and 4 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or to the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter, and post release review activities are discussed. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

569. 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. All licences are required to be subject to three conditions prescribed in the Act.

570. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, 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.

571. 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 and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

572. 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.

573. 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.

574. FSANZ has approved the oil and linters derived from this GM cotton event for use in human food (FSANZ 2004). FSANZ has reviewed their decision and reaffirmed their

previous conclusion that oil and linters derived from the GM cotton are fit for human consumption (FSANZ 2005c).

575. An AQIS permit has been granted to allow the importation of seed.

576. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. The GM cotton proposed for release meets the definition of an agricultural chemical product under the *Agricultural and Veterinary Chemicals Code Act 1994*, due to its production of insecticidal substances. Therefore, WideStrike™ cotton is also subject to regulation by the APVMA. The APVMA is currently assessing an application from Dow for WideStrike™ cotton.

577. Although the GM cotton has also been modified to be tolerant to glufosinate ammonium, the applicant does not intend this herbicide to be applied to the GM cotton and therefore is not seeking approval from the APVMA. If glufosinate ammonium were to be applied to the GM cotton, approval from the APVMA would be required.

578. The Regulator has liaised closely with the APVMA during the assessment of this licence application and will continue to liaise with the APVMA regarding their assessment for commercial release of GM WideStrike™ cotton.

Section 3 Risk treatment measures for identified risks

579. The risk assessment of events listed in Chapter 2 and the identified risks in Chapters 3 and 4 concluded that there is a low risk to the environment for two of the events from the proposed dealings with the GM WideStrike™ cotton, ie its commercial release in areas south of latitude 22º South and products entering general commerce. For the other events, it was considered that these pose negligible risks. All events were considered in the context of the large scale of the proposed release and the receiving environment, including other commercially approved GM cotton lines.

580. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines low risks as minimal, but may invoke actions for mitigation beyond normal practices. A negligible risk is one that is insubstantial and there is no present need to invoke actions for mitigation.

581. The risks of the following two events that may lead to harm, ie toxicity to non-target invertebrates and weediness, were estimated to be low:

- direct or indirect exposure of non-target invertebrates to GM plant material containing proteins encoded by the introduced *cry* genes
- expression of the introduced genes for insect resistance improving the survival of GM cotton plants and leading to increased spread and persistence north of latitude 22º South.

582. Risk treatment measures are imposed by the Regulator relating to Identified Risk 2 (Event 7 – Expression of the introduced genes for insect resistance improving the survival of the GM cotton plants in areas north of latitude 22º South). In addition, the Regulator requires further research under Post Release Review (PRR; see Section 5, below).

583. Taking all the available and relevant scientific evidence into account, risk treatment measures are not imposed by the Regulator for potential toxicity to non-target invertebrates (Identified Risk 1). However, the Regulator requires further research under PRR to verify the findings of the RARMP (see Section 5, below).

3.1 Summary of imposed specific licence conditions

584. A number of licence conditions are imposed by the Regulator to mitigate the risk of the increased survival of WideStrike™ cotton north of latitude 22º South (refer to Chapter 4, Identified Risk 2). Taking into account the considerable uncertainty identified during the assessment of this event, the risk has been estimated as low.

585. These licence conditions are with regard to dealings with the GMO in areas north of latitude 22º South and include requirements to:

- transport viable seed derived from the GMO in covered vehicles
- only feed GM cotton seed to livestock inside stockyards, feedlots or dairies.

586. These requirements are intended to limit dissemination of viable GM cotton material. Dissemination is the first step in a credible causal pathway through which dealings with the GMO in areas north of latitude 22º South may potentially lead to harm, ie increased spread and persistence with associated toxicity to non-target invertebrates and / or other detrimental effects of weediness.

587. Limiting the likelihood of dissemination is considered to decrease the chance of the GMO establishing populations along transport routes and in other areas in the natural environment where it may have a selective advantage compared to the baseline cottons.

588. In addition to imposing conditions relating to the restrictions of the release, the Regulator has also imposed conditions specifying actions the licence holder must take to inform persons covered by the licence to whom the above specific conditions apply.

589. Similar licence conditions have been imposed to manage previous commercial releases of other GM cotton lines in which planting was restricted to areas south of latitude 22º South, ie DIR 012/2002 and DIR 59/2005.

Section 4 General risk management

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

- applicant suitability
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the licence holder becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the licence holder, or a person covered by the licence, allows access to areas where dealings with the GMO are being undertaken (eg are being grown or fed to livestock), by the Regulator or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.1 Applicant suitability

591. 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.

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

593. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

594. Dow also must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2 Testing methodology

595. Dow is required to provide a method to the Regulator for the reliable detection of the presence of the GMO and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

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

596. Any person, including the licence holder, may conduct any permitted dealing(s) with the GMO.

4.4 Reporting requirements

597. 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 release.

598. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

599. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 5, below).

4.5 Monitoring for Compliance

600. 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. For this proposed licence this would include areas where the GMO is being grown or where GM cotton seed has been fed to livestock or where livestock has grazed or was housed after being fed GM cotton seed.

601. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the

provision for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Post release review

602. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

603. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through the following post release review (PRR)[14] activities:

- adverse effects reporting system (Section 5.1)
- requirement to monitor specific indicators of harm (Section 5.2)
- review of the RARMP (Section 5.3).

603. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

5.1 Adverse effects reporting system

604. Any member of the public can report adverse experiences/effects resulting from an intentional release to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform the review of the RARMP (see 5.3 below) as well as the risk assessment of future applications involving similar GMO(s).

5.2 Requirement to monitor specific indicators of harm

605. The triggers for this component of PRR are risk estimates greater than negligible and uncertainty in the risk assessment. As discussed in Chapters 3 and 4 of the RARMP, the risk estimates for two events, ie 'Direct or indirect exposure of non-target invertebrates leading to toxicity' and 'Expression of the introduced insect resistance genes leading to increased spread and persistence north of latitude 22º South', are low because of uncertainty regarding the effects of WideStrike™ GM cotton on certain non-target invertebrates and the methodology used in some experiments relating to testing toxicity to non-target organisms. Therefore, the Regulator considers it appropriate to impose licence conditions regarding PRR research activities and surveys relating to these identified risks.

606. The additional / new information obtained by the licence holder through the research activities and surveys are required to be submitted to the Regulator for review as specified in the licence. This will inform the progress of the release with the potential to reduce the level of uncertainty and to provide a mechanism for closing the loop in the risk analysis process.

[14] Details of the PRR concept is provided in the *Risk Analysis Framework*

607. The licence holder is required to design and conduct a research project in consultation with the Regulator to collect further information on the potential for toxicity of WideStrike™ GM cotton to key non-target invertebrates present in the Australian environment, including studies investigating the presence and abundance of these key species in WideStrike™ GM cotton fields.

608. The applicant is also required to conduct a survey, after designing the project in consultation with the Regulator, to collect further information on the potential for improved spread and persistence of WideStrike™ cotton volunteer plants in areas north of latitude 22º South, where livestock has been fed GM cotton seed or has been housed after feeding WideStrike™ GM cotton seed. The information gained from this survey will addresses the uncertainty as to whether WideStrike™ will have any selective advantage in areas north of latitude 22º South.

609. Data on these issues were are also identified as research requirements (refer to this Chapter, Section 6).

5.3 Review of the RARMP

610. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would be desktop-based and take into account any relevant new information or may be triggered by findings from either of the other components of PRR. The purpose of the review would be to ensure the findings of the RARMP remained current, and the timing of the review would be determined on a case-by-case basis. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to review of the risk management plan and changes to the licence conditions.

Section 6 Issues to be addressed for future releases

611. Additional information has been identified that may be required to assess an application for reduced containment measures. This would include:

- characteristics, type and abundance of beneficial/non-target invertebrates in crops of the GM cotton grown north of latitude 22º South
- information on the potential for WideStrike™ cotton to have increased survival in the natural environment north of latitude 22º South compared to other commercial GM and non-GM cottons as a result of the introduced genes for insect resistance
- information on any potential synergistic effects of the introduced genetic material when stacked with Bollgard II® cotton either as individual genes or in combination.

612. A new licence application and subsequent authorisation from the Regulator would be required to undertake plantings of GM WideStrike™ cotton north of latitude 22º South.

Section 7 Conclusions of the RARMP

613. The risk assessment concludes that this commercial release of WideStrike™ cotton, to be grown in areas south of latitude 22º South, and the entry of products derived from the GM cotton into general commerce Australia wide, poses **negligible** risks to the health and safety of people, and **negligible** to **low** risks to the environment as a result of gene technology.

614. The risk management plan concludes that one of the low risks requires specific risk treatment measures which are imposed through conditions of the licence. General licence conditions are also imposed to ensure that there is ongoing oversight of the release.

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Appendix A Definitions of terms in the Risk Analysis Framework used by the Regulator

Term	Definition
Consequence	outcome or impact of an adverse event • Marginal: there is minimal negative impact • Minor: there is some negative impact • Major: the negative impact is severe
Event*	occurrence of a particular set of circumstances
Hazard*	source of potential harm
Hazard identification	the process of analysing hazards and the events that may give rise to harm
Intermediate	the negative impact is substantial
Likelihood	chance of something happening • Highly unlikely: may occur only in very rare circumstances • Unlikely: could occur in some circumstances • Likely: could occur in many circumstances • Highly likely: is expected to occur in most circumstances
Quality control	to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement
Risk	the chance of something happening that will have an undesired impact • Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation • Low: risk is minimal but may invoke actions for mitigation beyond normal practices • Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective • High: risk is unacceptable unless actions for mitigation are highly feasible and effective
Risk analysis	the overall process of risk assessment, risk management and risk communication
Risk analysis framework	systematic application of legislation, policies, procedures and practices to analyse risks
Risk assessment	the overall process of hazard identification and risk estimation
Risk communication	the culture, processes and structures to communicate and consult with stakeholders about risks
Risk context	parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process
Risk estimate	a measure of risk in terms of a combination of consequence and likelihood assessments
Risk evaluation	the process of determining risks that require treatment
Risk management	the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts
Risk management plan	integrates risk evaluation and risk treatment with the decision making process
Risk treatment*	the process of selection and implementation of measures to reduce risk
Stakeholders*	those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk
States	includes all State governments, the Australian Capital Territory and the Northern Territory governments

Term	Definition
Uncertainty	imperfect ability to assign a character state to a thing or process; a form or source of doubt

* Terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004.

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities on any matters considered relevant to the preparation of a Risk Assessment and Risk Management Plan for DIR 091

The Acting Regulator received a number of submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and where they are addressed in the consultation RARMP, are grouped and summarised below.

Summary of issues raised	Comment/Where considered
The application is poorly presented and lacks proof-reading, and suggests that the applicant be asked to resubmit a corrected version.	Noted. The applicant was asked to resubmit their application with a number of changes and additional information supplied.
The specificity of the combination of the two Cry proteins in the GM cotton and potential toxicity to non-target organisms under Australian conditions should be considered.	The specificity of the combination of the two Cry proteins has been discussed in Chapter 1, Section 5.2.3 and the potential toxicity to non-target organisms under Australian conditions has been considered in Chapter 2, Event 2 and as Identified Risk 1 in Chapter 3.
The tolerance to glufosinate ammonium, conferred by the presence of two full length copies of the *pat* gene and risks that may be associated with this trait should be considered.	Risks that may associated with presence of the two full length copies of the *pat* gene have been considered in Chapter 2, Events 1, 3, 4, 5, 6, 8, 9 and 10. The applicant is not intending to apply glufosinate ammonium to the cotton line in the field.
Emergence of insects resistant to the expressed Cry toxins should be considered.	Insect resistance is the responsibility of the APVMA but see also Event 13 for a brief discussion.
The potential for unintended presence of the GM cotton in areas north of latitude 22º South and the possible impacts of any unintended presence north of this latitude should be considered.	The potential for unintended presence of the GM cotton in areas north of latitude 22º South has been considered in Chapter 2, and Identified Risks 2 and 3 in Chapter 4.
The impact of stacking this GM cotton with previously approved commercialised GM cotton lines which have insect resistance and herbicide tolerance traits (DIR 062/2005 and DIR 066/2006) should be considered.	The impact of stacking this GM cotton with previously approved GM cotton lines which have insect resistance and herbicide tolerance traits has been considered in Chapter 2, Events 9 and 10, and Identified Risk 3 in Chapter 4.
Unintended transfer of the introduced genes to non-GM cotton crops, naturalised populations of cultivated cotton, native cotton (*Gossypium* spp) and to other species, including microbes, and the potential for ecological impacts should be considered.	Event 8 considers the potential for gene transfer to non-GM cotton and Section 2.3 considers the potential of gene transfer to native cotton species. Event 11 considers the potential for gene transfer to unrelated species.
Persistence and/or accumulation of Cry toxins in soils or water (ie environmental chemistry and fate of the 'plant pesticide') should be considered.	Considered in Chapter 1, Section 5.5.2.

Summary of issues raised	Comment/Where considered
Consideration should be given to properties of the expressed Cry1Ac and Cry1F and PAT proteins including • mode of action and molecular basis for function and specificity of the expressed Cry1Ac, Cry1F and PAT proteins • species specificity of the expressed Cry1Ac, Cry1F and PAT proteins • expression levels of the Cry toxins in Australian cultivars under Australian conditions.	The properties of the expressed Cry1Ac, Cry1F and PAT proteins are described in Chapter 1 of the RARMP. Some points have been considered further in Chapters 2 and 3 of the RARMP including toxicity to non-target organisms.
Any altered fitness conferred by the introduced genes should be considered.	See Events 4 to 12 and Identified Risks 2 and 3.
Possibility for increased weediness in potentially favourable habitats as a result of transfer of the introduced genes or seed dispersal should be considered.	See Events 8, 9 and 10 for a consideration of gene transfer to other cotton plants, Events 4 to 7 for seed dispersal and Identified Risks 2 and 3.
Survival of GM cotton seed in the soil should be considered.	The introduced genes are not expected to alter the survival of WideStrike™ cotton seeds in soil.
Licence conditions should include requirements to monitor and report on unintended occurrence of the GM cotton and on unpredicted impacts through appropriate level of surveillance.	See the proposed licence at Chapter 6 of the consultation RARMP. These are standard licence conditions.

Appendix C Summary of issues raised in submissions received from prescribed experts, agencies and authorities[15] on the consultation RARMP for DIR 091

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. Several submissions received raised issues relating to risks to the health and safety of people or the environment as summarised below.

Summary of issues raised	Comment/Where considered
Agrees that the risk to non-target organisms is low. However, considers that more information on the impacts on non-target insects under Australian conditions is required and that exposure to the GM cotton should be minimised. Therefore, management conditions to achieve this could include limiting the amount of on-farm area planted and restricting the release to south of latitude 22° South.	The release is restricted to areas south of latitude 22º South. Impacts on non-target invertebrates were considered in detail in IR1 and the risk was estimated to be low. It was concluded that no risk treatment measures were required. However, to verify the findings of the RARMP, a PRR condition requiring the licence holder to collect further information of the potential for toxicity of WideStrike™ to non-target invertebrates in Australian cotton fields has been imposed.
Considers that the conclusion that insect and/or herbicide resistance is not an identified risk is not appropriate in the absence of a completed assessment of the matters.	The Regulator did not identify a risk because the issues of insect resistance and herbicide tolerance are actively managed through the application of the Ag Vet Code Act which is administered by the APVMA. Therefore, the conclusion of Event 13 has been reworded to better reflect this. It was acknowledged that cultivation of WideStrike™ cotton may require the implementation of a resistance management plan and/or other conditions that may be imposed by the APVMA.
Suggests that the Regulator should consider licence conditions for a restriction on the maximum on-farm area planted to WideStrike™ to 10% and that Dow undertake further research and monitoring into cross resistance between Cry1Ac(synpro) and Cry1F(synpro).	The suggested licence conditions relate to insect resistance management, which is addressed by the APVMA. Licence conditions have been imposed to restrict the growing of WideStrike™ to south of latitude 22° South.
The proposed release is unlikely to pose any significant risks in relation to insect resistance development as the two Bt gene approach should reduce this risk.	The issue of insect resistance development is being considered by the APVMA (Event 13). Cultivation of WideStrike™ cotton may require the implementation of a resistance management plan and/or other conditions that may be imposed by the APVMA.
Considers that the risk assessment has adequately assessed potential environmental risks for the proposed release. Agrees with proposed risk management measures. Supports the prescribed research requirements, which would be valuable in verifying the	A requirement has been included in the licence for the licence holder to provide a final analysis of data five years after commencement of the research programme. Research findings will be analysed by the Regulator.

[15] GTTAC, State and Territory Governments, Australian Government agencies, Local Councils and the Minister for the Environment, Heritage & the Arts.

Summary of issues raised	Comment/Where considered
conclusions of the RARMP. Recommends the licence to be altered to include either provisions to limit the duration of the release or a requirement to review the release after a specified time, which would allow Dow sufficient opportunity to gather requested data.	

Appendix D Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 091

The Regulator received one submission from the public on the consultation RARMP. This submission, summarised in the table below, raised issues relating to labelling of the GMO. This was considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Position (general tone): n = neutral; **x** = do not support; **y** = support
Issue raised: EN: Environmental issues.
Other abbreviations: GM: Genetically modified; **Sub. no.**: submission number;
Type: I: individual

Sub. no.	Type	Position	Issue	Summary of issues raised	Consideration in RARMP	Comment
1	I	X	EN	Believes that it is impossible to identify risks to ecological balance as there are too many unknown environmental factors, eg to do with soil microbiology and insect life.	Ch 2, 3, 4 and 5	Risks to the environment were subject to rigorous analysis using the available relevant scientific information. As outlined in the RARMP, risks to the environment, including soil organisms and insects were considered to be negligible to low. The licence imposes conditions to manage the low risks and includes conditions for the ongoing oversight of the release.
			EN	Believes the environment is threatened by extending monoculture, especially cotton culture, and GM crops encourage this process.	-	The release is limited to areas south of latitude 22º South. Cotton only grows in areas with suitable environmental conditions. The genetic modification would not extend the range of areas within which cotton can grow.